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Associations between prenatal and early postnatal heat stress exposure and first-lactation milk production, age at first insemination, and age at first calving in Holstein Friesian cows

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ABSTRACT

This study investigated associations between prenatal and early postnatal heat stress exposure and first-lactation performance in 3182 Holstein Friesian cows born between 2016 and 2022. Early-life periods were defined as the first (T1), second (T2), and third (T3) trimesters of gestation and the postnatal period (0–3 months). Heat stress was quantified as the number of days with THI ≥68 and cumulative THI load. Linear mixed-effects models assessed associations with 305-day milk, fat and protein yield, age at first insemination (AFI), age at first calving (AFC), services per conception (SPC), and first-service conception rate (FSCR). Heat stress during T1 was significantly associated with lower 305-day milk yield (−6.65 kg per additional day with THI ≥68; 95% CI [−10.44, −2.87], $p < 0.001$), protein yield (−0.22 kg; 95% CI [−0.34, −0.10], $p < 0.001$), and fat yield (−0.16 kg; 95% CI [−0.31, −0.01], $p = 0.040$). No significant associations with production traits were found for T2, T3, or postnatal exposure. Prenatal heat stress during T2 and T3 was associated with later AFI and AFC. Each additional day with THI ≥68 increased AFI and AFC by 0.20 and 0.19 days in T2 ($p < 0.001$) and by 0.12–0.13 days in T3 ($p \leq 0.032$). No associations were observed for T1 or postnatal exposure. SPC and FSCR showed no associations with early-life heat stress exposure. These findings indicate that prenatal heat stress at different stages of gestation was differentially associated with first-lactation milk yield, AFI and AFC.

1. Introduction

Heat stress is one of the most significant environmental challenges facing the global dairy industry, with well-documented negative effects on cow welfare, milk production, and reproductive efficiency [1,2]. Rising ambient temperatures and increased frequency of heat waves, driven by climate change, have intensified the magnitude and duration of thermal stress experienced by dairy cattle, particularly in continental regions of Europe [3]. Heat stress disrupts thermoregulation, feed intake, endocrine balance, and metabolic homeostasis, leading to immediate reductions in milk yield and fertility during lactation or around insemination [4–6]. Consequently, substantial research has focused on mitigating short-term effects through cooling systems, nutritional strategies, and management interventions. Beyond these acute impacts, growing evidence suggests that heat stress experienced during early life may induce persistent carry-over effects that extend into adulthood [7]. The concept of developmental programming proposes that environmental conditions during sensitive periods of prenatal and early postnatal development can permanently shape organ structure, physiological capacity, and long-term performance [8]. In dairy cattle, prenatal heat stress has been associated with altered growth trajectories, compromised immune function, reduced milk yield, and impaired thermotolerance later in life [9–12]. However, most studies have focused on late gestation or the dry period, with limited attention to the full gestational timeline or cumulative heat stress across distinct developmental windows. Gestation comprises a sequence of tightly regulated phases, including implantation, organogenesis, tissue differentiation, and rapid foetal growth, each characterised by distinct physiological priorities and sensitivities to environmental stressors [13–16]. Thermal stress during these phases may disrupt placental

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function, alter maternal–foetal nutrient exchange, and modify endocrine signalling [17–20], potentially influencing developmental trajectories long after birth. The extent to which such prenatal alterations persist into productive life remains incompletely understood. Beyond gestation, early postnatal life is another critical period during which environmental conditions can influence growth, metabolic adaptation, and immune development. Exposure to thermal stress during this stage may alter nutrient utilisation and growth efficiency [21], potentially affecting later-life performance. Whether disruptions during this window have lasting effects on productive and reproductive outcomes remains unclear. Although developmental programming has gained attention in dairy science, several gaps persist. Most studies examine isolated periods, often late gestation. Additionally, research has largely focused on either production or reproduction outcomes, limiting understanding of how early-life heat stress shapes overall performance in the first lactation. Moreover, the majority of these studies have been conducted in arid or semi-arid regions, with relatively few investigations under continental climates, where temperature fluctuations and seasonal extremes may present distinct challenges [22,23]. Consequently, it remains uncertain whether early thermal exposure imposes permanent constraints or whether animals retain sufficient developmental plasticity to adapt later. Addressing these uncertainties is particularly relevant under current climatic conditions, as rising temperatures will increasingly expose pregnant cows and young calves to heat stress. A clearer understanding of heat stress history effects is essential for informing management strategies, refining mitigation efforts during sensitive periods, and supporting the long-term sustainability of dairy production systems. Based on the developmental programming framework and existing evidence of period-specific sensitivity to thermal stress in utero, we hypothesised that heat stress exposure during distinct early-life developmental windows would be differentially associated with first-lactation milk production and reproductive performance. Accordingly, the main objective of this study is to investigate the associations between heat stress exposure during prenatal trimesters and the early postnatal period and first-lactation productive and reproductive outcomes in Holstein Friesian cows.

2. Material and methods

2.1. Animals and data

The study was conducted in southern Hungary under continental climate conditions. A total of 3182 Holstein Friesian cows with complete first-lactation records were included in the present study. These cows were born between 2016 and 2022 and originated from 2396 dams to 184 sires, enabling the linkage of both maternal heat stress exposure during gestation and individual heat stress exposure during postnatal development to first-lactation production and reproductive performance. The production traits analysed were 305-day milk yield (kg) (Milk 305), 305-day fat yield (kg) (Fat 305), and 305-day protein yield (kg) (Protein 305). Reproductive traits included services per conception (SPC), first service conception rate (FSCR), age at first insemination (AFI), and age at first calving (AFC).

Calves were housed in individual polyethylene hutches situated in an open outdoor area from birth until weaning at 3 months of age. Lactating cows were housed in a free-stall barn without any cooling system. The farm management protocol included feeding colostrum within the first 24 h of life, followed by a standard milk replacer regimen. Calf starter concentrate, hay, and water were introduced ad libitum during the first weeks of life to stimulate rumen development, with weaning occurring at approximately three months of age. Lactating dairy cows were similarly fed a total mixed ration (TMR) ad libitum, consisting of alfalfa silage, corn silage, alfalfa hay, molasses, concentrate, and a mineral–vitamin premix formulated to support maintenance and high milk production. According to farm protocols, the farm operated a non-seasonal, year-round calving and breeding system with no fixed seasonal breeding window or batch calving. Heifers were inseminated individually upon reaching a target body weight. Consequently, birth dates, insemination dates, and first-calving dates were distributed across all months of the year. This continuous management generated variation in the timing of gestation, birth, and first lactation across seasons, which helps the statistical models distinguish associations with early-life heat stress exposure from seasonal variation during the outcome period.

The early-life period was categorised into four major developmental windows for analysis, based on established physiological and developmental processes. The prenatal period, extending from conception to birth and encompassing critical windows of embryonic differentiation, placental establishment, and foetal organogenesis [24,25]. This period was divided into three consecutive 90-day trimesters to reflect temporally distinct stages of foetal growth and development. The first trimester (T1; days 0–90) corresponds to maternal recognition of pregnancy, embryo implantation, placental establishment, and early organogenesis, during which foundational tissue differentiation occurs [26,27]. The second trimester (T2; days 91–180) is characterised by myogenesis, oogenesis, skeletal development, and continued placental maturation, representing a critical period for structural and functional development of foetal tissues [28–31]. The third trimester (T3; days 181–birth) represents the phase of exponential foetal growth, with pronounced increases in foetal body weight, adipose tissue accretion, and mammary gland development, processes that are highly sensitive to maternal environmental conditions [32,33]. The postnatal period, extending from birth to 3 months of age, was also included as a distinct window in the analysis. This period encompasses biologically meaningful developmental stages, including the neonatal, pre-weaning, and weaning phases, which are critical for metabolic programming, immune system development, and rumen maturation [34,35].

2.2. Meteorological data

Daily temperature and relative humidity data were obtained from a meteorological station of the Hungarian Meteorological Service located 2 km from the farm. The calculation of a temperature-humidity index (THI) was performed using the method reported by Dikmen et al. [36]:

$$THI = (1.8 \text{ x } T + 32) - (0.55 - 0.0055 \text{ x } RH) \text{ x } (1.8 \text{ x } T - 26.8)$$

Where:

T = air temperature (°C)

RH = relative humidity (%)

Heat stress conditions were defined as THI ≥68, a threshold widely reported as the point at which lactating dairy cows begin to experience physiological and production-related heat stress responses [37,38].

Two metrics of heat stress were used in the study;

Number of days above the threshold (Days ≥68): the total number of days during which THI ≥68.

$$\mathbf{Days}_{\geq 68} = \sum (\mathbf{THI} \geq 68)$$

Cumulative heat load (THIc): the sum of daily THI values above the threshold (THI ≥68), providing a measure of both intensity and duration of heat stress. This was calculated as:

$$\mathbf{THIc} = \sum \max(\mathbf{THI} - 68, 0)$$

These measures were computed separately for each developmental window (first, second, and third trimesters of gestation, and the postnatal period) to assess the effect of both the duration and intensity of heat stress on subsequent productive and reproductive performance.

2.3. Statistical analysis

Linear mixed-effects models were used to evaluate the association between early-life heat stress exposure and milk production and reproductive performance in Holstein Friesian cows. All analyses were

conducted in R software (version 4.4.1; R Core [39]) using the lmer function from the lme4 package [40]. Model selection was guided by the Akaike Information Criterion (AIC), with the model exhibiting the lowest AIC value selected, which also satisfied all model assumptions.

2.3.1. Milk production traits

For milk production traits (305-day milk yield, 305-day protein yield, and 305-day fat yield), the following linear mixed-effects model was fitted:

$$Y_{ijklmn} = \beta_0 + \beta_1 HS_{T1,i} + \beta_2 HS_{T2,i} + \beta_3 HS_{T3,i} + \beta_4 HS_{Post,i} + u_j + v_k + w_l + x_m + \epsilon_{ijklmn}$$

Where:

Y_{ijklmn} is the response variable (305-day milk yield, 305-day fat yield, or 305-day protein yield) of cow i;

$HS_{T1,i}$, $HS_{T2,i}$, $HS_{T3,i}$, and $HS_{Post,i}$ represent the heat stress exposure of cow i during the first trimester of gestation, second trimester of gestation, third trimester of gestation, and the postnatal period (0–3 months of age), respectively, expressed either as the number of days with THI ≥68 or as cumulative heat stress load (THIc);

β_0 is the overall intercept of the model;

β_1, β_2, β_3, and β_4 are fixed-effect regression coefficients representing the change in the response variable associated with a one-unit increase in heat stress exposure during the first trimester (T1), second trimester (T2), third trimester (T3), and postnatal period, respectively;

u_j is the random effect of dam j;

v_k is the random effect of sire k;

w_l is the random effect of year of calving l;

x_m is the random effect of month of calving m;

ϵ_{ijklmn} is the residual error term, assumed to be independently and normally distributed with mean zero and constant variance.

Year and month of calving were included to account for environmental and management conditions affecting milk production during lactation.

2.3.2. Reproductive traits

For reproductive traits (AFI, AFC), a similar linear mixed-effects modelling framework was applied:

$$Y_{ijklmnqr} = \beta_0 + \beta_1 HS_{T1,i} + \beta_2 HS_{T2,i} + \beta_3 HS_{T3,i} + \beta_4 HS_{Post,i} + u_j + v_k + w_l + x_m + z_q + s_r + \epsilon_{ijklmnqr}$$

Where:

$Y_{ijklmnqr}$ represents the reproductive trait of cow i (AFI or AFC)

$HS_{T1,i}$, $HS_{T2,i}$, $HS_{T3,i}$, and $HS_{Post,i}$ represent early-life heat stress exposure of cow i during the first, second, and third trimesters of gestation and the postnatal period (0–3 months of age), respectively, expressed either as the number of days with THI ≥68 or as cumulative heat stress load (THIc);

β_0 is the overall intercept of the model;

β_1, β_2, β_3, and β_4 are fixed-effect regression coefficients representing the change in the reproductive trait associated with a one-unit increase in heat stress exposure during the first trimester (T1), second trimester (T2), third trimester (T3), and postnatal period, respectively;

u_j is the random effect of dam j;

v_k is the random effect of sire k;

w_l is the random effect of year of insemination or calving, depending on the trait;

x_m is the random effect of month of insemination or calving;

z_q is the random effect of inseminator q;

s_r is the random effect of semen r;

$\epsilon_{ijklmnqr}$ is the residual error term, assumed to be independently and normally distributed with mean zero and constant variance.

By including year and month of calving (for production traits) and year and month of insemination or calving (for reproductive traits) as random effects, the models account for potential confounding in environmental and management conditions during the lactation or breeding periods. Because prenatal heat exposure is inherently linked to seasonal temperature patterns, potential confounding with environmental conditions experienced later in life was addressed in several ways. The farm operates a continuous, non-seasonal breeding system, resulting in gestation, birth, and first lactation occurring throughout the year rather than within fixed seasonal cohorts. Additionally, the dataset spans six years, providing substantial interannual variation in temperature patterns and gestational timing. These design features, together with the inclusion of year and month random effects, help reduce potential confounding between early-life heat exposure and environmental conditions experienced during adulthood. Nevertheless, residual confounding from unmeasured seasonal factors cannot be fully excluded, as discussed in the Limitations section.

Service per conception (SPC) was analysed using generalised linear mixed-effects models assuming a Poisson distribution with a log link function. First-service conception rate (FSCR), a binary outcome, was analysed using mixed-effects logistic regression assuming a binomial distribution with a logit link function. The same fixed and random effects structure described above was applied to these models.

Potential multicollinearity among heat exposure variables during the different gestational windows (T1, T2, T3) and the postnatal period was assessed using variance inflation factors (VIFs). All VIF values were <5, indicating no evidence of problematic multicollinearity.

To enhance biological interpretability, regression coefficients for Days ≥68 were scaled to represent the estimated effect per 30 accumulated heat stress days, recognising that these days were not necessarily consecutive. Coefficients derived on a per-day basis were multiplied by 30. For THIc-based models, coefficients represent the effect per 30-unit increase in cumulative THI above the threshold. 95% confidence intervals (95% CI) were calculated for both raw and per-30-day to assess the precision of effect size estimates. Fixed effects were considered statistically significant at $P < 0.05$.

3. Results

The analysis of first-lactation 305-day milk yield revealed a significant negative association with heat stress exposure during early foetal development. Specifically, each additional day with THI ≥68 during the first trimester was associated with a reduction of 6.65 kg/305-day yield (95% CI [-10.44, −2.87], $p < 0.001$), which corresponds to a decrease of 199.63 kg of milk over a 30-day exposure period (95% CI [-313.18, −86.07] kg). In contrast, heat stress during the second trimester was not significantly associated with milk yield (0.246 kg/305-day yield; 95% CI [-2.60, 3.09], $p = 0.865$), nor was exposure during the third trimester (−1.940 kg/305-day yield; 95% CI [-5.43, 1.55], $p = 0.277$). Postnatal heat stress was also not significantly associated with milk yield (-7.97 kg/305-day yield; 95% CI [-15.97, −0.04], $p = 0.083$) (Fig. 1).

The analysis of first-lactation 305-day milk protein yield indicated that heat stress exposure during early foetal development (T1) had a significant negative association. Specifically, each additional day with THI ≥68 during T1 was associated with a reduction of 0.22 kg/305-day yield (95% CI [-0.34, −0.10], $p < 0.001$), which corresponds to a decrease of 6.63 kg of protein over a 30-day exposure period (95% CI [-10.18, −3.08] kg). In contrast, heat stress during the second (T2) and third (T3) trimesters of gestation, as well as postnatal heat stress, was not significantly associated with milk protein yield (T2: 0.04 kg/305-day yield, 95% CI [-0.13, 0.05], $p = 0.34$; T3: 0.02 kg/305-day yield, 95% CI [-0.13, 0.09], $p = 0.73$; postnatal: 0.13 kg/305-day yield, 95% CI [-0.30, 0.04], $p = 0.370$) (Fig. 1).

The analysis of first-lactation 305-day milk fat yield indicated that heat stress exposure during early foetal development (T1) had a significant negative association. Specifically, each additional day with THI ≥68 during T1 was associated with a reduction of 0.16 kg/305-day yield (95% CI [-0.31, −0.01], $p = 0.040$), which corresponds to a decrease of

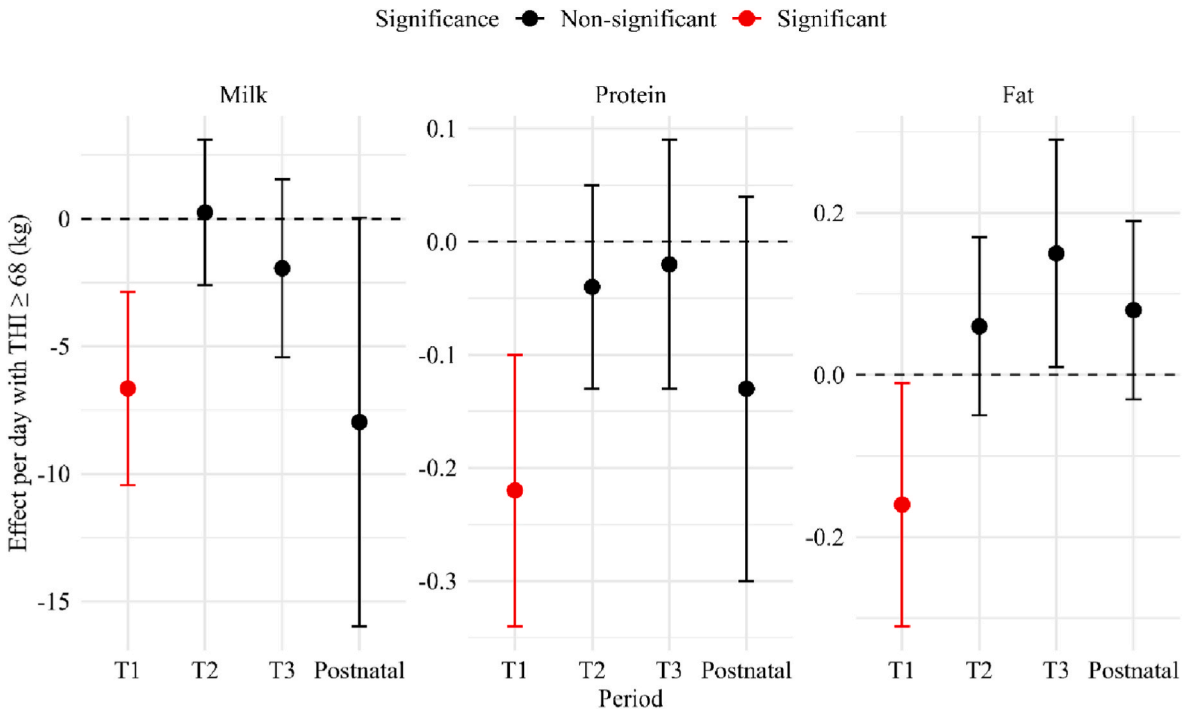

Fig. 1. Estimated associations of heat stress exposure, expressed as the number of days with a temperature–humidity index (THI) ≥ 68 during distinct early-life periods, first trimester of pregnancy (T1), second trimester (T2), third trimester (T3), and postnatal period (0–3 months), with 305-day milk, fat, and protein yield in Holstein Friesian cows. Associations are presented as the change in total 305-day yield (kg) per additional day with THI ≥68, along with corresponding 95% confidence intervals (CI).

4.73 kg of fat over a 30-day exposure period (95% CI [-9.23, −0.23] kg). In contrast, heat stress during the second (T2) and third (T3) trimesters of gestation, as well as postnatal heat stress, was not significantly associated with milk fat yield (T2: 0.06 kg/305-day yield, 95% CI [-0.05, 0.17], p = 0.307; (T3: 0.15 kg/305-day yield, 95% CI [0.01, 0.29], p = 0.35; postnatal: 0.08 kg/305-day yield, 95% CI [-0.03, 0.19], p = 0.622) (Fig. 1).

Consistent with these findings, analyses based on cumulative heat stress load confirmed that only first-trimester foetal exposure was significantly associated with reduced 305-day milk yield, 305-day milk protein, 305-day milk fat, whereas cumulative heat stress during second- and third-trimester gestation and the postnatal period showed no significant association (Table 1).

The analysis of AFI showed that prenatal heat stress exposure had period-specific associations. Heat stress during the second trimester of foetal development (T2) was significantly associated with delayed AFI. Specifically, each additional day with THI ≥68 during foetal T2 increased AFI by 0.20 days (95% CI [0.10, 0.30], p < 0.001), corresponding to a delay of 6.01 days per 30 days of exposure (95% CI [3.12, 8.89]). Similarly, heat stress exposure during the third trimester (T3) had a significant but smaller association, with each additional day with THI ≥68 increased AFI by 0.12 days (95% CI [0.01, 0.24], p = 0.032), equivalent to 3.70 days per 30 days of exposure (95% CI [0.32, 7.08]). In contrast, heat stress during the first trimester of gestation (T1) was not significantly associated with AFI (0.04 days/day; 95% CI [-0.08, 0.16], p = 0.503), nor was postnatal heat stress (-0.11 days/day; 95% CI [-0.24, 0.02], p = 0.463) (Fig. 2).

The analysis of AFC revealed clear period-specific associations of prenatal heat stress exposure. Heat stress during the second trimester of foetal development (T2) was significantly associated with a delay in AFC. Each additional day with THI ≥68 during foetal T2 increased AFC by 0.19 days (95% CI [0.10, 0.29], p < 0.001), corresponding to an increase of 5.82 days per 30 days of exposure (95% CI [2.86, 8.79]). Similarly, heat stress exposure during the third trimester (T3) had a significant, albeit smaller, association. Each additional day with THI ≥68 during T3 was associated with an increase of 0.13 days in AFC (95%

Table 1
Estimated associations of cumulative heat stress exposure (THIc), defined as the sum of daily temperature–humidity index values exceeding the threshold (THI ≥68), during distinct early-life periods with 305-day milk, fat, and protein yield in Holstein Friesian cows. Associations are expressed as the change in total 305-day yield (kg) per unit increase and per 30-unit increase in cumulative THI above the threshold, together with corresponding 95% confidence intervals (CI).

Parameters	Period	Estimate per unit increase (kg)	95% CI (kg)	Estimate per 30-unit increase (kg)	95% CI (kg)	P-value
Milk 305	Foetal T1	−1.63	[-2.41, −0.84]	−48.7	[-72.4, −25.1]	<0.001
	Foetal T2	0.01	[-0.65, 0.67]	0.4	[-19.4, 20.2]	0.967
	Foetal T3	−0.35	[-1.06, 0.36]	−10.6	[-31.9, 10.8]	0.332
	Postnatal	−1.23	[-2.72, 0.26]	−37.0	[-81.6, 7.7]	0.105
Protein 305	Foetal T1	−0.06	[-0.07, −0.03]	−1.66	[-2.38, −0.94]	<0.001
	Foetal T2	−0.01	[-0.02, 0.01]	−0.26	[-0.86, 0.34]	0.397
	Foetal T3	−0.01	[-0.02, 0.01]	−0.14	[-0.78, 0.51]	0.682
	Postnatal	−0.02	[-0.07, 0.02]	−0.73	[-2.12, 0.65]	0.299
Fat 305	Foetal T1	−0.04	[-0.07, −0.01]	−1.29	[-2.21, −0.38]	<0.001
	Foetal T2	0.01	[-0.02, 0.02]	0.12	[-0.64, 0.87]	0.765
	Foetal T3	0.03	[-0.01, 0.05]	0.81	[-0.02, 1.63]	0.056
	Postnatal	0.01	[-0.04, 0.05]	0.14	[-1.45, 1.73]	0.859

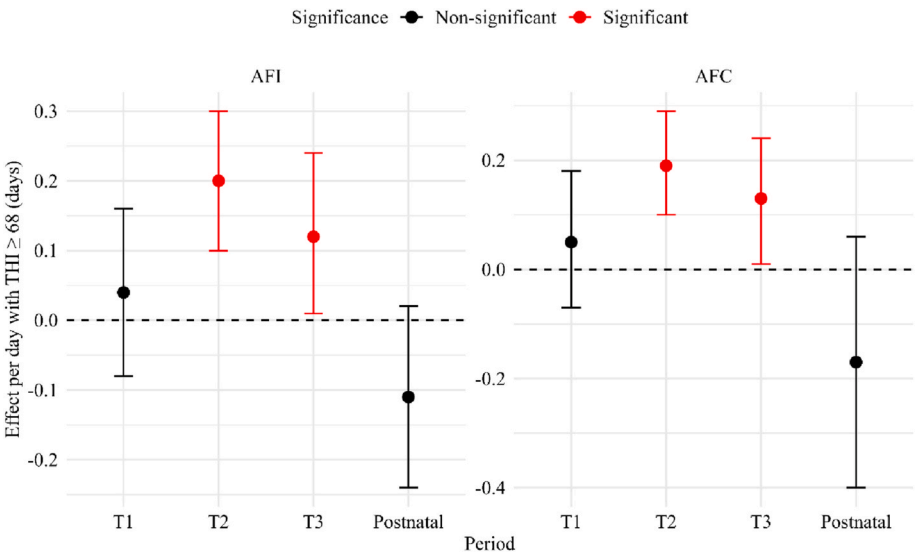

Fig. 2. Estimated associations of heat stress exposure, expressed as the number of days with a temperature–humidity index (THI) ≥ 68 during distinct early-life periods, first trimester of pregnancy (T1), second trimester (T2), third trimester (T3), and postnatal period (0–3 months), with AFC, AFI in Holstein Friesian cows. Associations are expressed as changes in AFI and AFC (days) per additional day with THI ≥68, together with corresponding 95% confidence intervals (CI).

CI [0.01, 0.24], p = 0.030), equivalent to 3.82 days per 30 days of exposure (95% CI [0.37, 7.27]). In contrast, heat stress exposure during the first trimester of gestation (T1) was not significantly associated with AFC (0.05 days/day; 95% CI [-0.07, 0.18], p = 0.393), nor was postnatal heat stress (-0.17 days/day; 95% CI [-0.40, 0.06], p = 0.259) (Fig. 2).

Consistent with these findings, analyses based on cumulative heat stress load confirmed that prenatal heat stress exposure during the second and third trimesters of gestation was significantly associated with increased AFI and AFC (Table 2).

Heat stress exposure during the foetal period, first, second, and third trimesters, as well as during the postnatal period, showed no significant association with SPC or FSCR (all p > 0.05; Incidence Rate Ratios for SPC and Odds Ratios for FSCR were close to 1.00 across all developmental periods). Similarly, analyses based on cumulative heat stress load (THIc) revealed no significant effects on SPC or FSCR.

4. Discussion

In this study, we evaluated the long-term associations of heat stress during specific early-life developmental windows with first-lactation productivity and some reproductive performance in Holstein Friesian cows. As global temperatures continue to rise, understanding the impact of early-life heat stress on lifetime performance is increasingly important, particularly in continental regions such as Central Europe, where extreme summer temperatures are becoming more frequent. Identifying sensitive developmental windows is essential for designing effective mitigation strategies that protect animals during vulnerable stages of physiological programming. Our findings reveal early-life windows during which heat stress has lasting consequences for dairy cow productivity, providing insights for targeted intervention.

Our results indicate that heat stress during the first trimester of foetal development, assessed both as the number of days with THI ≥68 and cumulative heat stress load, was significantly associated with reduced first-lactation milk yield, fat, and protein production. In contrast, heat stress during the second and third trimesters had no significant associations. These findings align with previous research showing that early gestational heat stress can negatively influence productive performance in dairy cattle [8,22,23,41]. Most comparable studies have been conducted in regions characterised by more extreme heat loads, including arid or semi-arid climates such as Iran [22,41], and subtropical environments in the United States (Florida) [8,23], where summer temperatures and THI values frequently exceed those observed in temperate continental regions. In contrast, the present study was conducted under a continental climate characterised by an average summer temperature of approximately 28 °C and moderate relative humidity (59%) [42]. During the study period (2016–2022), the mean number of days with THI ≥68 in the study area was 81.7 per year (range: 70–102), with peak values typically recorded during July and August. Despite these more moderate environmental conditions, we detected similar associations between early gestational heat stress exposure and later milk production. This finding suggests that early gestation may represent a particularly sensitive developmental window, during which even moderate thermal perturbations could be associated with measurable differences in subsequent productive performance. To place the magnitude of this association into context, the mean 305-day milk yield in this herd was 10,333 ± 1909 kg. The observed reduction of 200 kg per 30 days of

Table 2
Estimated associations of cumulative heat stress exposure (THIc), defined as the sum of daily temperature–humidity index values exceeding the threshold (THI ≥68), with distinct early-life periods on age at first insemination (AFI) and age at first calving (AFC) in Holstein Friesian cows. Associations are expressed as changes in AFI and AFC (days) per unit increase and per 30-unit increase in cumulative THI above the threshold, together with corresponding 95% confidence intervals (CI).

Parameters	Period	Estimate per unit increase (days)	95% CI (days)	Estimate per 30-unit increase (days)	95% CI (days)	P-value
AFI	Foetal T1	−0.01	[-0.02, 0.02]	−0.09	[-0.84, 0.66]	0.812
	Foetal T2	0.03	[0.01, 0.05]	1.02	[0.38, 1.67]	0.001
	Foetal T3	0.02	[0.00, 0.04]	0.69	[0.01, 1.37]	0.045
	Postnatal	−0.04	[-0.09, 0.00]	−1.27	[-2.71, 0.17]	0.085
AFC	Foetal T1	0.00	[-0.02, 0.02]	−0.03	[-0.78, 0.73]	0.945
	Foetal T2	0.03	[0.01, 0.05]	0.95	[0.29, 1.61]	0.005
	Foetal T3	0.02	[0.00, 0.05]	0.68	[-0.02, 1.37]	0.006
	Postnatal	−0.04	[-0.01, 0.00]	−1.43	[-2.90, 0.04]	0.564

first-trimester heat stress exposure represents 1.9% of the herd mean and 10.5% of one standard deviation, indicating that while the association is statistically robust, its magnitude is modest relative to the natural variation in milk yield within this population. However, even modest per-animal associations can translate into meaningful economic consequences when aggregated across a commercial herd. Using conservative estimates, the per-animal milk revenue loss associated with 30 days of first-trimester heat stress exposure is $110 per lactation at a current European farm-gate milk price of $0.55/kg [43]. In a herd of 1000 cows where 25% of animals experience significant first-trimester heat stress exposure, this would represent an estimated annual loss of $27,500 from milk production alone. In the context of climate change, where the frequency and intensity of heat stress events are projected to increase [3], even modest per-animal associations may carry meaningful implications for herd productivity and farm profitability when considered across time and animals. One hypothesis that may explain these associations relates to the timing of mammary gland development during early gestation. Mammary gland development begins at approximately 30 days of gestation [44], when alveolar precursor cells undergo proliferation and establish the structural foundation required for milk synthesis and secretion [45]. Thermal stress during this period could hypothetically disrupt cellular proliferation or differentiation processes within mammary epithelial tissue [46], potentially influencing the number or functionality of secretory cells available during the first lactation. In line with this hypothesis, the observed reductions in milk fat and protein may also be linked to altered mammary epithelial differentiation. Major milk protein genes are mammary-specific and developmentally regulated, serving as markers of epithelial cell differentiation [47], while milk fat synthesis depends on coordinated metabolic activity within mammary epithelial cells [48]. If early gestational heat stress interferes with these developmental pathways, it could plausibly influence the functional capacity of the mammary gland during the first lactation. However, because the present study is observational and no direct measurements of mammary development were available, these mechanisms should be considered hypothetical explanations rather than demonstrated causal pathways.

The absence of significant associations between heat stress exposure during the second and third trimesters, as well as during the postnatal period, may be partly related to differences in developmental sensitivity across stages. By mid-to late gestation, the fundamental architecture of the mammary gland and other major organ systems has largely been established [45]. Consequently, foetal development during these later stages is primarily characterised by tissue growth, cellular maturation, and functional refinement rather than by structural or epigenetic programming [29,49,50]. As a result, the potential for heat stress to be associated with permanent alterations in mammary development and subsequent lactational performance may be markedly reduced during these periods.

In contrast to prenatal heat stress during early gestation, which can induce permanent developmental programming effects, postnatal heat stress primarily affects calves through short-term reductions in feed intake and alterations in energy balance and metabolism [8]. These physiological responses may lead to decreases in average daily gain and body weight as a consequence of reduced feed intake [51]. However, such effects are generally reversible and largely dependent on the intensity and duration of heat stress exposure, as well as on the effectiveness of management and nutritional mitigation strategies that can substantially improve growth and subsequent lactation performance [52]. In the present study, the absence of a significant postnatal heat stress association may therefore reflect effective physiological adaptation and the implementation of appropriate management practices that limited its long-term impact on milk yield and composition.

For reproductive parameters, prenatal heat stress exposure, assessed as the number of days with THI $\geq$68, and heat stress intensity, assessed as cumulative heat stress load during the second and third trimesters of pregnancy, were associated with a significant increase in both AFI and AFC. In contrast, heat stress exposure during the first trimester and the postnatal period was not significantly associated with either reproductive trait, consistent with previous studies reporting negative effects of heat stress on reproductive performance following mid-to late-gestational exposure [22,23,53]. To contextualise these associations, it is important to consider the herd's average reproductive timing and potential economic impact. The mean AFI in this herd was 496 $\pm$ 85 days and the mean AFC was 775 $\pm$ 86 days. The observed delay of 6.0 days in AFI per 30 days of second-trimester heat stress exposure represents 1.2% of the herd mean and 7.1% of one standard deviation, while the corresponding delay of 5.8 days in AFC represents 0.75% of the herd mean and 6.7% of one standard deviation. Although these proportional effects are modest relative to the natural variation in reproductive timing, they have direct economic implications. Based on a total heifer rearing cost of $2717 [54] and the mean AFC of 775 days, the estimated daily rearing cost is $3.50 per heifer. Therefore, a delay of 6 days in AFC per 30 days of second-trimester heat stress exposure translates to an additional rearing cost of $21 per exposed heifer. Across a commercial herd, these costs accumulate meaningfully, particularly when combined with the milk yield losses associated with first-trimester exposure. These per-window estimates should be regarded as conservative, as animals exposed during multiple gestational periods or severe seasons may experience cumulative reproductive delays exceeding those reported here. To explain these associations, several biological mechanisms can be hypothesised. In this herd, heifers were inseminated upon reaching a target body weight rather than at a fixed age, suggesting a growth-mediated pathway. Heat stress during the third trimester coincides with the phase of exponential foetal growth, during which approximately 60% of birth weight is accumulated [55]. Furthermore, heat stress during the second and third trimesters has been linked to placental dysfunction, oxidative stress, and impaired nutrient and oxygen transfer [10,17,20] potentially reducing calf birth weight. Consequently, calves born lighter often exhibit slower postnatal growth trajectories [56], delaying attainment of the body weight threshold required for insemination and extending both AFI and AFC. However, birth weight and longitudinal growth data were not available, preventing distinction between growth-mediated and potential direct effects of prenatal heat stress on reproductive development. A secondary, more speculative possibility is that mid-to late gestation coincides with key stages of foetal reproductive development, including oogenesis, early folliculogenesis, and establishment of the primordial follicle pool [57]. In addition, alterations in placental steroidogenesis and foetal exposure to hormones such as insulin-like growth factors, leptin, and glucocorticoids may influence ovarian development or maturation of the hypothalamic–pituitary–gonadal axis [56,58,59]. Nevertheless, in the absence of direct measurements of birth weight, growth, or endocrine indicators, these mechanisms remain hypothetical, and the associations are most conservatively interpreted as primarily growth-mediated.

In contrast to the associations observed during mid-to late gestation, the absence of significant associations following first-trimester heat stress may reflect differences in developmental priorities during early gestation. Although early embryonic development is highly sensitive to environmental insults, reproductive organs and the ovarian reserve are not yet fully differentiated at this stage [60]. Consequently, it is plausible that heat stress exposure during the first trimester has a limited direct association with the programming of reproductive traits that depend on later ovarian and endocrine maturation, though this interpretation remains speculative without direct mechanistic evidence. Interestingly, our results showed no significant association between postnatal heat stress and AFI or AFC, which may be attributed to several hypotheses such as the greater plasticity and compensatory capacity of the postnatal period [61]. Unlike prenatal exposure, postnatal heat stress primarily affects growth and metabolism through transient reductions in feed intake and alterations in energy balance [21,62]. Under adequate nutritional and management conditions, these effects are often reversible, allowing heifers to compensate for early growth delays and

reach reproductive milestones at comparable ages [63]. Supporting this, Dado-Senn et al. [8] reported that postnatal heat-stressed calves show higher average daily gain and significant increases in overall hip height compared with both prenatal heat-stressed and non-heat-stressed calves, suggesting that postnatal heat stress effects may be more readily compensated for than prenatal heat stress effects. Whether this compensatory growth is sufficient to fully normalise the timing of reproductive milestones likely depends on the severity and duration of heat stress exposure and the nutritional management provided during the pre-weaning period. Furthermore, postnatal reproductive development remains responsive to management interventions, such as shading and heat abatement strategies, which may mitigate the long-term impacts of heat stress [64]. These explanations are not mutually exclusive, and their relative contributions cannot be determined from the present data. However, the consistency of the null associations observed across multiple production and reproductive traits, together with evidence from the literature supporting postnatal compensatory capacity [8,63], suggests that under adequate nutritional management the long-term consequences of postnatal heat stress may be partially mitigated, although this interpretation should be considered with caution.

No significant associations of heat stress exposure during the first, second, or third trimester of pregnancy, nor during the postnatal period, were detected on SPC or FSCR. This absence of an association may be explained by fundamental differences between the biological determinants of pubertal timing and those governing conception success. Whereas AFI and AFC are strongly associated with the developmental programming of growth and endocrine maturation in response to early-life heat stress, conception outcomes are more directly determined by the immediate reproductive environment at the time of insemination. These include body temperature, health status, semen quality, insemination technique, and periconceptional management conditions [65–68]. Furthermore, services per conception and first-service conception rate primarily reflect acute reproductive efficiency and are therefore highly sensitive to short-term factors such as heat stress around oestrus, insemination timing, and early embryonic development, rather than to thermal exposure occurring months or years earlier during foetal or early postnatal life [1,4,69]. Consequently, although prenatal heat stress may hypothetically alter reproductive development and delay sexual maturity, such associations may not persist sufficiently to impair fertilisation or early pregnancy establishment once animals reach reproductive age under favourable management conditions.

Taken together, these findings suggest that the gestational period, may represent the primary window during which thermal stress is associated with long-term carry-over influences on productive and reproductive performance, whereas postnatal tissues appear to retain greater compensatory capacity. These timing-specific associations highlight pregnancy as a potentially important target for intervention strategies aimed at safeguarding lifetime performance under increasingly hot climatic conditions. However, given the observational nature of this study, these interpretations should be regarded as strong hypothesis-generating, and future studies incorporating birth weight, growth trajectories, and detailed physiological data are needed to confirm and extend these associations.

This retrospective observational study, conducted on a single commercial dairy farm, identified robust statistical associations using a large dataset of 3182 Holstein Friesian cows. Nevertheless, several limitations should be acknowledged. First, because the data originate from a single farm, the findings may not be fully generalisable to other herds or production systems that differ in management, genetics, or climatic conditions. Second, the study is observational in nature; although year and month of calving/insemination were included as random effects and the herd followed a continuous year-round calving system to minimise seasonal bias, residual confounding from unmeasured factors such as photoperiod, forage quality, disease pressure, or management practices cannot be excluded. Therefore, the reported associations, while statistically robust and biologically plausible, should not be interpreted as definitive evidence of causality or irreversible developmental programming. Third, birth weight and postnatal growth trajectories were not recorded. This limitation restricts our ability to directly evaluate the proposed developmental mechanisms underlying the observed effects of prenatal heat stress. Because heifers in this herd were inseminated when reaching a target body weight rather than at a fixed age, the delays observed in age at first insemination and age at first calving associated with mid-to late-gestation heat stress may partly reflect slower growth rather than direct reproductive programming. Finally, although calves were housed in uniform individual polyethylene hutches and received standardised feeding, no information on micro-environmental heat load within hutches or individual growth rates was available. Additionally, meteorological data were obtained from a weather station located 2 km from the farm, which may introduce minor microclimatic discrepancies. Despite these limitations, the large dataset and consistent management conditions provide valuable insights into the potential long-term consequences of prenatal heat stress in dairy production systems and highlight priorities for future multi-farm and mechanistic studies.

5. Conclusion

This study demonstrates associations between prenatal heat stress exposure and stage-specific outcomes in first-lactation performance of Holstein Friesian cows. Heat stress during the first trimester of gestation was associated with lower 305-day milk, fat, and protein yield, whereas exposure during the second and third trimesters and the early postnatal period showed no significant associations with production traits. In contrast, heat stress during the second and third trimesters was associated with later AFI and AFC. No associations were observed with SPC or FSCR. Importantly, both the duration and intensity of heat stress exposure, as reflected by cumulative THI, contributed to the observed associations. Collectively, these findings suggest that early gestation may represent a sensitive developmental window associated with reduced milk production in the first lactation, while mid-to late-gestation heat stress is linked to delayed reproductive maturation without impairing conception efficiency once animals reach breeding age. Improved cooling of pregnant cows during periods of elevated THI may therefore help reduce potential long-term productivity losses. However, given the observational design of this study, future studies incorporating growth measurements and physiological indicators may help clarify the biological mechanisms underlying these associations.

CRediT authorship contribution statement

Wissem Baccouri: Writing – original draft, Software, Methodology, Conceptualization. **George Wanjala:** Writing – review & editing, Validation, Data curation. **Violetta Tóth:** Writing – review & editing, Validation, Data curation. **István Komlósi:** Writing – review & editing, Validation, Conceptualization. **Edit Mikó:** Writing – review & editing, Supervision, Resources, Methodology.

Data availability statement

The raw/processed data required to reproduce the above findings cannot be shared at this time as the data also forms part of an ongoing study.

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Declaration of competing interest

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