



## Modeling heat stress effects on first service to conception rates in Canadian Holstein dairy cattle

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### ABSTRACT

Heat stress (HS) can result in decreased production and poor fertility performance in dairy cattle. There is limited understanding of the point at which heat load begins to affect fertility, causing a major challenge for the industry. The temperature-humidity index (THI) is a metric commonly used to represent the realized heat load on livestock, as it incorporates both ambient temperature and humidity percentage. The objectives of this study were to estimate the threshold of THI at which the interval of days from first service to conception (FSTC) begins to increase due to HS, evaluate the effect of estrus synchronization on that threshold, and identify geographic regions of concern for Canadian dairy farming. Data comprised 2,033,928 FSTC records on 1,239,053 Canadian Holsteins in parities 1, 2, and 3, collected between November 2008 and April 2022. Hourly ambient temperature and relative humidity were extracted from the NASA Prediction of Worldwide Energy Resources (POWER) database (<https://power.larc.nasa.gov/>; accessed Jan. 7, 2025), and daily THI was calculated by averaging hourly THI across the full 24-h day. Daily THI was averaged across 3 temporal windows relevant for follicular growth and zygotic survival: 3 d before to 2 d after first insemination, 7 d before to 2 d after first insemination, and 10 d before to 2 d after first insemination. The FSTC phenotype was adjusted using a single-trait repeatability model to account for known sources of environmental and genetic effects on the trait. Segmented linear-linear regressions were fit to identify the point at which the residual FSTC began to increase in response to HS effects. Thresholds varied between THI 65 and 68.6 across parities and temporal windows. The window spanning 3 d before to 2 d after first insemination showed the highest overall accuracy,

and the average threshold for this window (THI 66) was selected as the representative onset of HS effects on FSTC. These results suggest that shorter windows may be sufficient to evaluate HS effects on fertility. Timed artificial insemination (AI) protocols were found to have a greater sensitivity to HS than heat detection methods, displaying a difference in thresholds (timed AI: 64; heat detection: 72.8). However, heat detection inseminations were found to have a higher rate of increase in FSTC above group-specific thresholds (timed AI: 0.44; heat detection: 3.27). Analysis of weather data indicated that Ontario is a province of concern, with average summer temperatures exceeding THI 66. Additionally, Ontario had an average of 50% of 24-h day with THI above 66 as well as 50% of summer days with average THI above 66. All provinces, except for British Columbia, had an observed hottest season within the past 4 years within the dataset. Results of this study suggest an increase in FSTC above THI 66 with a significant effect of timed AI protocol on HS response. A genetic analysis is needed to evaluate a possible genotype by environment interaction and the corresponding re-ranking of sires.

**Key words:** heat stress, fertility, estrus synchronization, first service to conception

### INTRODUCTION

Climate change has driven global temperatures to increase by  $\sim 0.85^{\circ}\text{C}$  over the past century, largely due to increased GHG emissions (Bush et al., 2019). Although Canada reduced GHG emissions by 8.5% between 2005 and 2023 (Government of Canada, 2024), temperatures in the country have risen at nearly twice the global average rate (Zhang et al., 2019). Rising temperatures amplify heat stress (HS), posing significant challenges to livestock industries worldwide. Heat stress is a major challenge for the dairy industry, reducing fertility in cattle by lowering conception rates and extending calving intervals. Dairy cows in temperate regions, such as Canada, may exhibit greater sensitivity to acute

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heat exposure than those in tropical climates, due to a lack of acclimatization (Becker et al., 2020). Holsteins, Canada's dominant dairy breed, are especially vulnerable to HS due to additional metabolic heat production associated with high milk yields (Rocha et al., 1998; Chebel et al., 2004; Hansen, 2004). As HS intensifies, dairy cows must undergo behavioral and physiological adjustments to cope, inevitably resulting in diminished reproductive performance.

Heat stress occurs when environmental temperatures exceed the animal's thermoneutral zone, requiring additional energy for thermoregulation and potentially impairing performance (Polsky and von Keyserlingk, 2017; Yan et al., 2021). In addition to temperature, environmental variables such as humidity, wind, and solar intensity can affect physiological responses to environmental challenges by restricting cooling methods such as sweat evaporation (West, 2003; Roy and Collier, 2012). Although metrics of heat load that incorporate these environmental variables into a single index do exist (Mader et al., 2010), dairy cattle in North America are often housed indoors and therefore experience fewer effects of wind and solar intensity. The temperature-humidity index (**THI**) accounts for both ambient temperature and relative humidity in a single measure and has widely been used as a measure of HS in dairy cattle (NRC, 1971). Phenotypic analyses using THI indicate that the onset of HS for dairy cattle may fall between 60 and 72 THI (Ravagnolo and Misztal, 2000; Bohmanova et al., 2007; Hammami et al., 2013; Carabaño et al., 2016; Campos et al., 2022b). When the heat load experienced by the animal exceeds this threshold, performance has been observed to decrease, particularly for energetically demanding processes such as milk production and fertility.

Heat stress negatively alters several critical stages of reproduction in dairy cattle, particularly around conception and embryo implantation. High temperatures reduce the production of key reproductive hormones essential for follicular development, estrus expression, and early embryo survival (Gilad et al., 1993; Howell et al., 1994; Roth et al., 2001; Wolfenson et al., 2002). Additionally, HS can impair reproductive success by reducing follicle size and promoting the ovulation of aged dominant follicles, resulting in decreased oocyte quality and lower conception rates (Roth et al., 2001; Schüller et al., 2017). Reduced estradiol production under HS conditions also leads to shorter or undetected estrus, complicating insemination timing and subsequently lowering conception success (Wolfenson et al., 1988; Schüller et al., 2017; Tippenhauer et al., 2021). Increased THI results in lower conception rates, with studies reporting decreases of up to 19% under HS (López-Gatius, 2003; El-Tarabany and El-Bayoumi, 2015; Schüller et al., 2014).

The interval of days from first service to conception (**FSTC**) is an important fertility indicator trait evaluated in Canada that may be particularly susceptible to the adverse effects of HS due to its direct relationship with conception rates and early embryonic survival. Heritability of FSTC has been estimated to be  $0.03 \pm 0.001$  ( $\pm$  SE), which indicates a large influence of environmental effects on trait performance (Oliveira Junior et al., 2021b). Although many studies have established HS thresholds for production traits, there is limited research on the THI at which fertility traits, such as FSTC, are affected. Estimated thresholds for HS effects on production traits in dairy cattle range from 62 to 79 THI (Hammami et al., 2013; Carabaño et al., 2016; Campos et al., 2022b). Estimates on fertility indicators suggest that this threshold may fall within a similar range, depending on the specific trait analyzed (Ravagnolo and Misztal, 2002; Schüller et al., 2014; Vinet et al., 2024; Ojo et al., 2025).

Given the critical role of fertility in dairy herd sustainability, further investigation into HS thresholds for reproductive traits is needed. The objectives of this study were (1) to identify the THI threshold at which FSTC is negatively affected by HS in Canadian Holsteins; (2) to evaluate the influence of estrus synchronization on the interaction between HS and FSTC; and (3) to analyze weather patterns across Canada to identify geographic regions of concern.

## MATERIALS AND METHODS

No approval by an Institutional Animal Care and Use Review Board was required because data were obtained from an existing database.

### Data

Fertility data were provided by Lactanet Canada (Guelph, ON) and included FSTC records from 1997 to 2022. To ensure adequate density of data, records were filtered to include only first-parity cows born between 2007 and 2018, second-parity cows born between 2006 and 2017, and third-parity cows born between 2005 and 2016. After filtering, data comprised 2,802,889 FSTC records from 1,572,811 Holstein cows across 12,026 Canadian herds. Approximately 55% of cows had FSTC records for multiple parities. Associated information included first and last insemination dates, parity number, herd, and previous calving details. Contemporary groups were generated based on region (1: Quebec, 2: Ontario, 3: Alberta and Saskatchewan, 4: British Columbia), year, and month (**RYM**) of birth; herd and year (**HY**) of birth; and age at previous calving with month of first service (**ApMf**). Pedigree data with a minimum depth of

4 generations from the phenotyped population, including 2,106,814 individuals, were matched to the fertility dataset.

Meteorological data were extracted from the National Aeronautics and Space Administration (NASA) Langley Research Center Prediction of Worldwide Energy Resources (POWER) Project, funded through the NASA Earth Science/Applied Science Program, using the POWER hourly database (<https://power.larc.nasa.gov/>; accessed Jan. 7, 2025). Data were accessed using the “na-sapower” package in R (Sparks, 2018), which provides a programmatic interface to the POWER API. Hourly ambient temperature (AT, °C) and relative humidity (RH, %) were collected across 2,304 postal codes representing 9,086 herds across British Columbia, Alberta, Saskatchewan, Ontario, and Quebec from November 2008 to April 2022. Hourly THI was calculated using the NRC (1971) formula as follows:

$$\text{THI} = (1.8 \times \text{AT}) + 32 - [0.55 - (0.0055 \times \text{RH})] \times [(1.8 \times \text{AT}) - 26],$$

and daily THI values were derived by averaging hourly THI across the full 24-h day and rounded to the tenth decimal place. After merging meteorological and fertility data, the final dataset included 2,505,332 FSTC records from 1,405,765 cows across 9,086 herds, ensuring each record had corresponding weather data.

### Adjustment of Phenotypes

The FSTC phenotype was adjusted for known sources of environmental and genetic effects using a single-trait repeatability model, defined as

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Wu} + \mathbf{Za} + \mathbf{Vo} + \mathbf{e},$$

where  $\mathbf{y}$  is the vector of FSTC phenotypes,  $\mathbf{X}$  is a design matrix for the fixed effects in  $\mathbf{b}$ ,  $\mathbf{W}$  is a design matrix for the random HY effects in  $\mathbf{u}$ ,  $\mathbf{Z}$  is a design matrix for the random animal additive genetic effects in  $\mathbf{a}$ ,  $\mathbf{V}$  is a design matrix for the random permanent environmental effects in  $\mathbf{o}$ , and  $\mathbf{e}$  is a vector of random residuals. Fixed effects included RYM and ApMf effects.

The random HY effects in  $\mathbf{u}$ , random animal additive genetic effects in  $\mathbf{a}$ , random permanent environmental effects in  $\mathbf{o}$ , and the random residual effects in  $\mathbf{e}$  were assumed to be normally distributed with mean zero and variances defined as

$$\text{var} \begin{bmatrix} \mathbf{u} \\ \mathbf{a} \\ \mathbf{o} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{I}\sigma_u^2 & 0 & 0 & 0 \\ 0 & \mathbf{A}\sigma_a^2 & 0 & 0 \\ 0 & 0 & \mathbf{I}_r\sigma_o^2 & 0 \\ 0 & 0 & 0 & \mathbf{I}\sigma_e^2 \end{bmatrix},$$

where  $\mathbf{I}$  is an identity matrix with dimensions equal to the number of animals with records,  $\sigma_u^2$  is the HY variance,  $\mathbf{A}$  is the numerator relationship matrix,  $\sigma_a^2$  is the additive genetic variance,  $\mathbf{I}_r$  is an identity matrix with dimensions equal to the number of animals with multiple records,  $\sigma_o^2$  is the permanent environmental variance, and  $\sigma_e^2$  is the residual variance. Residuals were modeled with homogeneous variance across all observations. This model was run using the BLUPF90+ program in the BLUPF90 family of programs (Misztal et al., 2014).

The residual term captures the phenotypic variation due to unknown sources, including THI, such that  $\hat{\mathbf{e}}_i = \mathbf{y}_i - \hat{\mathbf{y}}_i$  reflects the unexpected deviation in the phenotype of animal  $i$  after accounting for fixed and random effects. This residual term, hereafter referred to as residual FSTC, was filtered to exclude outliers outside the range of 1.5 times the interquartile range below the first quartile and above the third quartile, resulting in a total of 2,033,928 records on 1,239,053 cows with distribution across parity and province (Table 1).

### Identification of Thresholds

Residual FSTC records were merged with weather data by averaging the daily THI across 3 time windows: 3 d before to 2 d after first insemination ( $\mathbf{W1}$ ), 7 d before to 2 d after first insemination ( $\mathbf{W2}$ ), and 10 d before to 2 d after first insemination ( $\mathbf{W3}$ ). These time windows were selected based on key stages in follicular development and early zygotic survival. General consensus indicates that most dairy cows exhibit either 2- or 3-wave fol-

**Table 1.** Distribution of records used for analysis by parity and province (BC = British Columbia, AB = Alberta, SK = Saskatchewan, ON = Ontario, QC = Quebec)

Item	BC	AB	SK	ON	QC	Overall
Parity 1	84,544	98,385	24,256	386,057	389,338	982,580
Parity 2	53,086	63,270	15,270	251,998	273,304	656,928
Parity 3	29,159	36,062	8,643	147,405	173,151	394,420
Overall	166,789	197,717	48,169	785,460	835,793	2,033,928

licular cycles (Taylor and Rajamahendran, 1991; Noseir, 2003), with the timing of the ovulatory wave differing between the 2. In 2-wave cycles, the ovulatory wave typically begins around d 10 (~11 d before ovulation; Knopf et al., 1989), while in 3-wave cycles it begins closer to d 14 or 15 (~6 d before ovulation; Adams et al., 2008). To account for this variation, W2 and W3 encompassed the ovulatory follicle development period for 3- and 2-wave cycles, respectively. The W1 window captures the final stages of follicular maturation and the onset of luteal regression. The corpus luteum begins to regress approximately 3 d before ovulation (Sumiyoshi et al., 2014), marking the start of the transitional period known as proestrus, which is characterized by endocrine shifts and the cessation of follicular growth (Pierson and Ginther, 1984; Driancourt et al., 1991).

During the 4 d after fertilization, the zygote begins the cleavage process as it travels down the oviduct toward the uterus for implantation. All 3 windows included the period of 2 d after insemination, which is a critical window for early embryonic development and zygotic survival. The first 48 h after insemination are especially crucial for zygote viability, cleavage, and oviductal interactions. It has been shown that the initial 2 d of development determine the distinction between heat-susceptible and heat-resistant zygotes, with lower risk of embryonic death due to HS occurring by d 3 (Ealy et al., 1993; García-Ispuerto et al., 2007).

The residual FSTC was plotted against each of the 3 windows to visualize the relationship between THI and FSTC. Visual analysis of the relationship was used to approximate number and location of breakpoints necessary to accurately represent the data. Following visual analysis of the relationship, thresholds were identified using a segmented regression approach where the relationship is described by multiple linear functions. This model considers breakpoints at which the slope of the relationship changes. The segmented regression analysis was performed using the “segmented” package in R (Fasola et al., 2018), which allows for the estimation of breakpoints and the fitting of segmented polynomial regressions. The procedure was applied separately for each parity, and only THI values with sufficient representation ( $n > 500$  records per THI value) across the THI scale were included in the analysis. The breakpoints for each model were identified by assessing the model’s fit, with the optimal breakpoints selected based on the adjusted coefficient of determination ( $R^2$ ) and the visual inspection of plots.

### Estrus Synchronization Effect

Estrus synchronization has previously been demonstrated to bias genetic evaluations, indicating that ferti-

lity under the influence of a timed artificial insemination (AI) protocol may be a different trait than fertility under natural conditions (Lynch et al., 2021; Oliveira Junior et al., 2021a; Haile-Mariam et al., 2023). To evaluate the effects that estrus synchronization protocols may have on response of FSTC to HS, a subset of data containing information on timed AI use was analyzed. Information on whether insemination was based on a timed AI protocol or heat detection through various methods was available for a subset of fertility records ( $n = 245,802$ ; Lynch et al., 2021). This subset was used to estimate the influence of estrus synchronization on response to HS, either on identified threshold or rate of change. Three segmented regressions were generated using the same methodology previously described, (1) on the entire subset, (2) on timed AI records only, and (3) on heat detection records only, to observe any changes in thresholds.

Additionally, a segmented linear-linear regression with group-specific breakpoints and nested slopes and intercepts was fit to compare the differences in slopes between the 2 groups. Breakpoints, denoted as  $th$ , were fixed at the THI thresholds previously identified for each group through the segmented linear regression. The heat load term,  $HL_i$ , was defined as

$$HL_i = \begin{cases} 0 & \text{if } THI_i \leq th_{T_i} \\ THI_i - th_{T_i} & \text{if } THI_i > th_{T_i} \end{cases}$$

for each residual FSTC record  $i$ , where  $th_{T_i}$  refers to the group-specific breakpoint for that record and  $THI_i$  is the THI associated with record  $i$ . The following regression was fit:

$$r_i = \beta_0(T_i) + \beta_1 THI_i(T_i) + \beta_2 HL_i(T_i) + e_i,$$

where  $r_i$  represents the residual FSTC for record  $i$ ,  $T_i$  indicates the insemination protocol group (0 = timed AI; 1 = heat detection) used as nesting factor,  $\beta_0$  is the group-specific intercept,  $\beta_1$  is the group-specific slope before the breakpoint ( $th$ ),  $\beta_2$  is the group-specific slope after the breakpoint, and  $e_i$  is the residual term. Differences in slopes before and after the breakpoint between groups were assessed by contrasts using  $t$ -tests at significance level  $\alpha = 0.05$ .

### Weather Pattern Analysis

All available weather data were analyzed to identify regional patterns in summer climatic conditions across Canada. Due to years with incomplete data, only data from 2009 to 2021 were analyzed. Summer was defined as the months of June through August, and the THI

threshold associated with HS effects on FSTC was identified using the segmented linear regression applied to the full dataset. Average summer THI was calculated and mapped across Canada to illustrate regional variation in thermal conditions. In addition, the average percentage of the 24-h day during which hourly THI exceeded the identified threshold was calculated across all summer days for each province. Finally, the percentage of summer days in which the average daily THI exceeded the threshold was also determined for each province.

## RESULTS AND DISCUSSION

### Summary Statistics

Records were predominantly concentrated in Ontario and Quebec (80%) and in parity 1 (48%; Table 1). It is not surprising that the majority of records were within Ontario and Quebec, given that 80% of Canadian dairy farms are located in those 2 provinces (CDIC, 2024). Similarly, it is expected that a higher density of records would be found in parity 1, as estimates indicate that between 8% and 19% of cows are culled within first lactation (Bach, 2011; Brickell and Wathes, 2011). Involuntary culling is often attributed to fertility challenges (Bascom and Young, 1998; de Souza et al., 2023). Because the trait FSTC does not capture cows that are serviced but fail to conceive, it is possible that lower representation for later parities is indicative of early culling due to poor fertility.

Average FSTC increased with each subsequent parity, whereas average residual FSTC decreased (Table 2). It has previously been shown that fertility tends to decrease with increasing parity, particularly when differentiating between primiparous and multiparous cows, due to the added metabolic demands and increased likelihood of health challenges (Inchaisri et al., 2010; Polsky et al., 2017; Tippenhauer et al., 2021, 2023). The overall mean FSTC in this dataset ( $36.5 \pm 43.4$  d;  $\pm$  SD) was consistent with previous estimates reported for Canadian (Jamrozik et al., 2005; Oliveira Junior et al., 2021b) and Danish (Ojo et al., 2025) Holstein populations. High standard deviations were observed for both raw (43.4 d) and residual (39.1 d) FSTC, indicating substantial biological and environmental variability. Average THI associated with fertility records remained stable across the 3 time windows, with an average of  $47 \pm 15$  ( $\pm$  SD) and a maximum ranging from 76 to 77.

### THI Thresholds

Across all evaluated time windows and parities, thresholds indicating the onset of HS effects ranged from THI 65 to 68.6 (Table 3). Thresholds were generally consistent across parities within each window but showed

more variation between windows within each parity. This finding suggests that window length may have a stronger influence on the estimated onset of HS effects than parity. Slopes tended to increase with longer windows across all parities, indicating larger estimated HS effects when exposure is measured over a broader period. Parity 1 had the lowest degree of variation in thresholds across windows, ranging from THI 65.6 to 66.8. The largest increase in residual phenotype above the threshold was seen in W2 (slope =  $0.84 \pm 0.08$ ;  $\pm$  SE). Parity 2 exhibited greater threshold variation, with thresholds of THI 66.8 (W1), 68.6 (W2), and 67.3 (W3). Slope estimates were greatest for this parity and ranged from  $0.98 \pm 0.07$  ( $\pm$  SE) (W1) to  $1.69 \pm 0.21$  ( $\pm$  SE) (W2), with stronger effects observed in the longer windows. Parity 3 exhibited the greatest variability between windows, with thresholds of THI 65.0 (W1), 65.6 (W2), and 68.5 (W3). Slope estimates for this group increased from  $0.60 \pm 0.06$  ( $\pm$  SE) (W1) to  $1.24 \pm 0.17$  ( $\pm$  SE) (W3). Across all models, adjusted  $R^2$  values ranged from 0.82 to 0.98, indicating strong model fit. The highest model fit was observed for parity 1 across all windows, which is likely due to the greater data availability in this group, resulting in more robust estimates. Additionally, models for W1 performed best across all parities, suggesting that this shorter time window may provide the most stable estimates of HS effects on this fertility trait. Given the better model fit and low degree of threshold variation for W1, the average THI threshold for this window (THI 66) was selected as the representative threshold for further analyses.

Segmented regression plots are shown in Figures 1, 2, and 3. The relationship between residual FSTC and THI was fit as a linear-linear regression with a single breakpoint. Slopes were allowed to vary before and after the estimated threshold, and both slopes and thresholds varied by parity and window. Although slope magnitudes differed between parities, the direction remained consistent across windows. Before the breakpoint, parity 1 consistently had a negative slope, parity 2 a positive slope, and parity 3 no discernible trend. After the breakpoint, all

**Table 2.** Summary statistics of days from first service to conception (FSTC) and FSTC adjusted by known sources of variation (Res. FSTC) by parity

Item	Mean	SD	Minimum	Maximum
FSTC (d)				
Parity 1	33.3	41.6	0	206
Parity 2	38.8	44.4	0	206
Parity 3	40.4	45.3	0	206
Overall	36.5	43.4	0	206
Res. FSTC				
Parity 1	-3.68	37.6	-90.1	110.4
Parity 2	-3.58	40.2	-102.6	110.4
Parity 3	-4.19	40.9	-107.8	110.4
Overall	-3.75	39.1	-107.8	110.4

**Table 3.** Identified THI thresholds for 3 evaluated windows of 3 d before to 2 d after first insemination (W1), 7 d before to 2 d after first insemination (W2), and 10 d before to 2 d after first insemination (W3) across parities 1, 2, and 3 with associated slope effects and adjusted (adj.)  $R^2$  values for each model (values represent parameter estimates  $\pm$  SE)

Item	Threshold	Slope	Adj. $R^2$
Parity 1			
W1	65.6 $\pm$ 0.35	0.52 $\pm$ 0.03	0.98
W2	66.5 $\pm$ 0.50	0.84 $\pm$ 0.08	0.93
W3	66.8 $\pm$ 0.42	0.83 $\pm$ 0.08	0.94
Parity 2			
W1	66.8 $\pm$ 0.36	0.98 $\pm$ 0.07	0.97
W2	68.6 $\pm$ 0.39	1.69 $\pm$ 0.21	0.93
W3	67.3 $\pm$ 0.42	1.23 $\pm$ 0.13	0.95
Parity 3			
W1	65.0 $\pm$ 0.66	0.60 $\pm$ 0.07	0.93
W2	65.6 $\pm$ 0.68	0.70 $\pm$ 0.09	0.92
W3	68.5 $\pm$ 0.72	1.24 $\pm$ 0.28	0.82

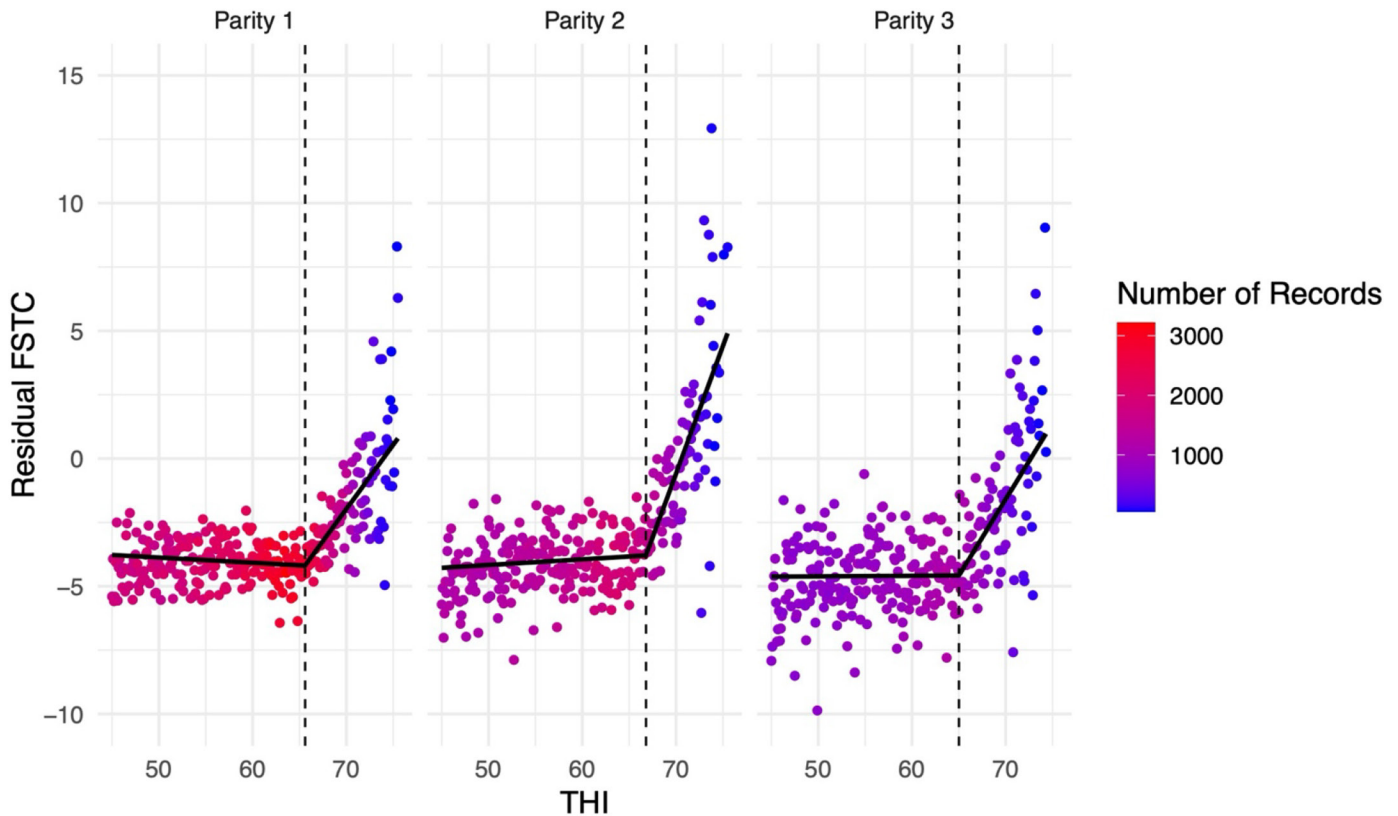
parities had steep increases, with W2 showing the greatest post-threshold slopes for parities 1 and 2. In contrast, W1 consistently showed the smallest slopes across all parities. The consistent patterns support the robustness of the identified relationship, indicating that regardless of window length, HS begins to affect this fertility trait at a similar THI and produces comparable effects on FSTC. The greater spread observed in parity 3, particularly above the threshold, may reflect increased biological variation in response to HS. However, this group had the fewest records, and this pattern may change as more data become available.

Despite our finding little difference in HS response between parity groups, other studies have reported parity-specific sensitivity to HS. With increasing parity, greater metabolic demands are associated with higher production and risk of disease (Lee and Kim, 2006; Wathes et al., 2007). Greater metabolic challenges often coincide with greater susceptibility to HS, particularly for production traits. Previous studies have found slower declines in production of milk and milk components for first-parity cows in response to HS (Aguilar et al., 2009; Campos et al., 2022b). In addition to changes in production, increasing parity is often associated with decreasing reproductive performance (Inchaisri et al., 2010; Polsky et al., 2017; Tippenhauer et al., 2021, 2023). However, a study on 45-d nonreturn rate found that first-parity cows were more susceptible to HS effects than those in later parities (Ravagnolo and Misztal, 2002). It is possible that the low parity differences observed in this study are due to the trait analyzed. The FSTC reflects the time taken for dairy cows to conceive, meaning results may be biased by the exclusion of cows that never conceive, as well as by those able to conceive but exhibiting delayed time between inseminations.

The relationship found between THI and FSTC in this study was similar to previous analyses on comparable fertility indicator traits. Studies in Danish Holsteins identified a threshold of response in the interval from first to last insemination to HS slightly lower (THI = 60) than what was identified in this study (Ojo et al., 2025). Differences in thresholds may be attributable to differences in population, environment, and methodological approaches. In particular, Ojo et al. (2025) calculated THI using average daily temperature and relative humidity, which may underestimate HS conditions compared with the average hourly-based THI used in this study. Despite different thresholds, Ojo et al. (2025) similarly found a linear relationship between interval from first to last insemination and THI within the thermoneutral zone, and an increase above the threshold. Ojo et al. (2025) reported minimal variation in the relationship between the fertility indicator trait and THI across different time windows around first insemination. Similar thresholds observed across windows in both studies suggest that narrower time windows may be sufficient to detect HS effects on FSTC. However, this consistency may be affected by autocorrelation in THI during hot periods, which may make it difficult to isolate specific temporal effects. Testing longer windows may clarify the period that FSTC is most impacted, though this would likely require alternative approaches to defining the environmental scale beyond window averages, such as averaging THI around each insemination within the FSTC window.

Reported thresholds for other fertility indicator traits tend to be higher than those we identified for FSTC. Using 45-d nonreturn rate, Ravagnolo and Misztal (2002) reported a threshold of THI 68 across Georgia, Florida, and Tennessee in the United States. In northern Germany, Schüller et al. (2014) found a continuous decline in conception rate across the THI scale, with steeper losses after THI 73. Variation in thresholds across traits despite their similarity is unsurprising, as a similar trend has been found in production traits where thresholds for milk, fat, and protein production vary (Carabaño et al., 2016; Campos et al., 2022b).

Comparing THI thresholds across studies is challenging due to differences in both data sources and calculation methods. In this study, daily THI was calculated from hourly temperature and humidity data collected via satellites. This approach attempts capture daily variation, avoid oversimplification, and offer broader geographic coverage compared with weather stations (Rockett et al., 2023). Schüller et al. (2014) similarly derived daily THI from hourly values but used on-site sensors, better reflecting conditions within barns. In contrast, Ojo et al. (2025) and Ravagnolo and Misztal (2002) used data from nearby weather stations and daily summary statis-



**Figure 1.** Segmented polynomial representing the relationship between the days from first service to conception adjusted by known sources of variation (residual FSTC) and average temperature-humidity index (THI) across the window of 3 d before to 2 d after first insemination (W1). Dotted line indicates identified THI threshold and solid line indicates the identified polynomial.

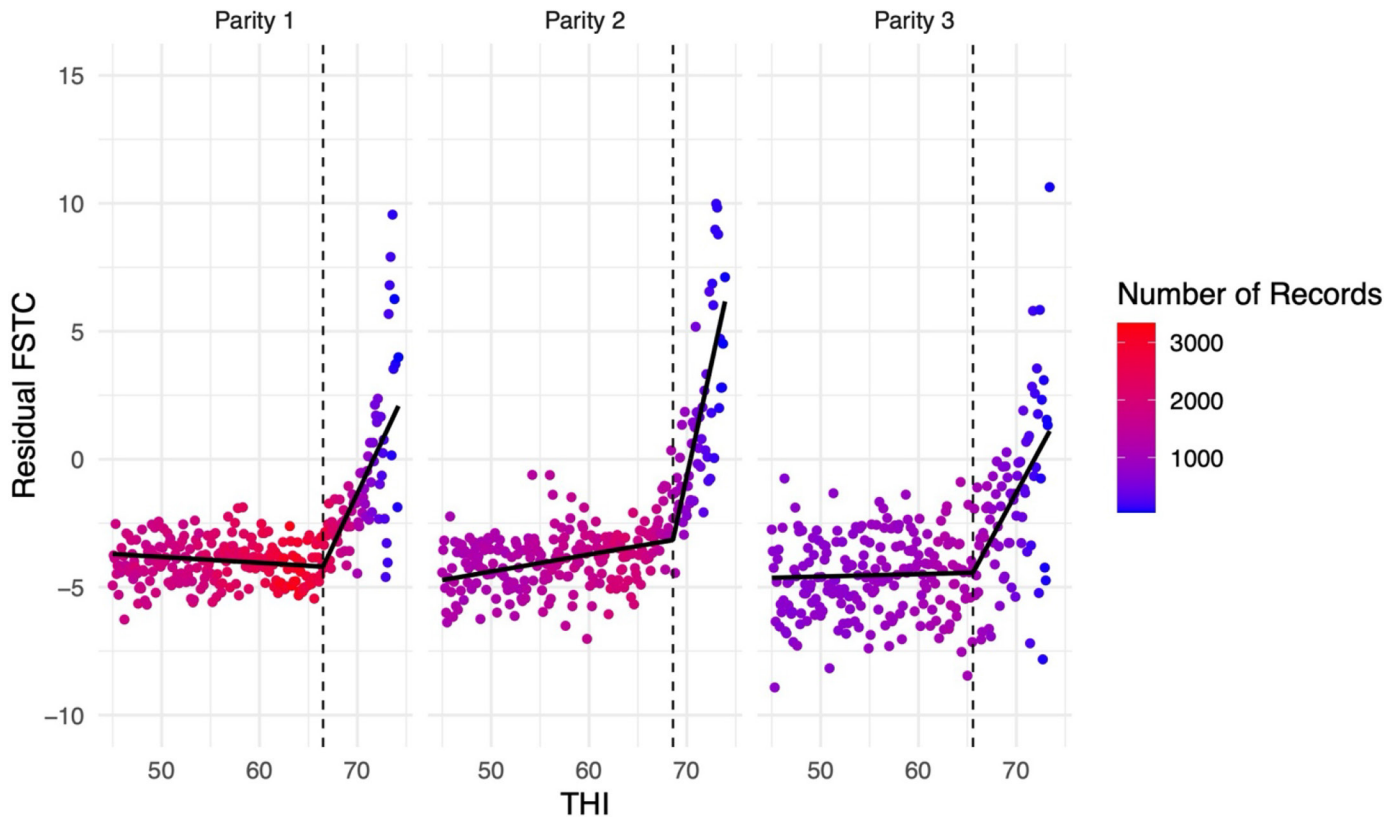
tics, though the methods between studies for calculating daily THI differed. Ojo et al. (2025) used daily average temperature and humidity, whereas Ravagnolo and Misztal (2002) calculated daily THI using the maximum temperature and minimum humidity observed for that day. These differences in how weather data are collected and how daily THI likely contributed to variation in THI thresholds and should be considered when comparing across studies.

Because W1 demonstrated the best model fit and most consistent results across parities, the average of raw and residual FSTC were evaluated for W1 only across all parities for THI 55, 60, 65, 70, and 75 (Figure 4). Average residual FSTC was consistently 4 d shorter ( $-4.1 \pm 0.001$ ;  $\pm$  SD) for THI 55, 60, and 65. This indicates that under thermoneutral conditions, animals tend to conceive sooner than the baseline, even after accounting for other known sources of variation. As THI exceeded the identified threshold at THI 66, the negative effects of HS start and the average residual FSTC increased to about 2 d shorter ( $-1.8 \pm 0.001$ ;  $\pm$  SD) at THI 70. Under more severe HS, residual FSTC lengthens further to 3 d longer ( $3.1 \pm 0.031$ ;  $\pm$  SD) at THI 75, indicating that conception

is significantly delayed, even after accounting for the known sources of variation. Average raw FSTC increased by 1 d for each of THI 55, 60, 65, and 70, with a more pronounced increase of 5 d for THI 75 (from  $35 \pm 0.002$  to  $40 \pm 0.034$ ; mean  $\pm$  SD). This pattern further supports the identified threshold at THI 66, as both raw and residual FSTC begin to notably increase beyond this point. It is also important to note that the estimated effects of HS on FSTC may be underestimated in this study, given the focus on windows surrounding first insemination only. As a result, the observed effects may reflect the difference between successful (FSTC = 0) and unsuccessful (FSTC > 0) first inseminations, without fully capturing the intricacies of the interaction when conception does not occur at first service.

### Estrus Synchronization Effect

Analysis of the effect of estrus synchronization on HS effects was conducted using average THI over W1 only, without separating by parity. The relationships between residual FSTC and THI in this subset were slightly higher than those identified in the full dataset (Figure 5). All 3



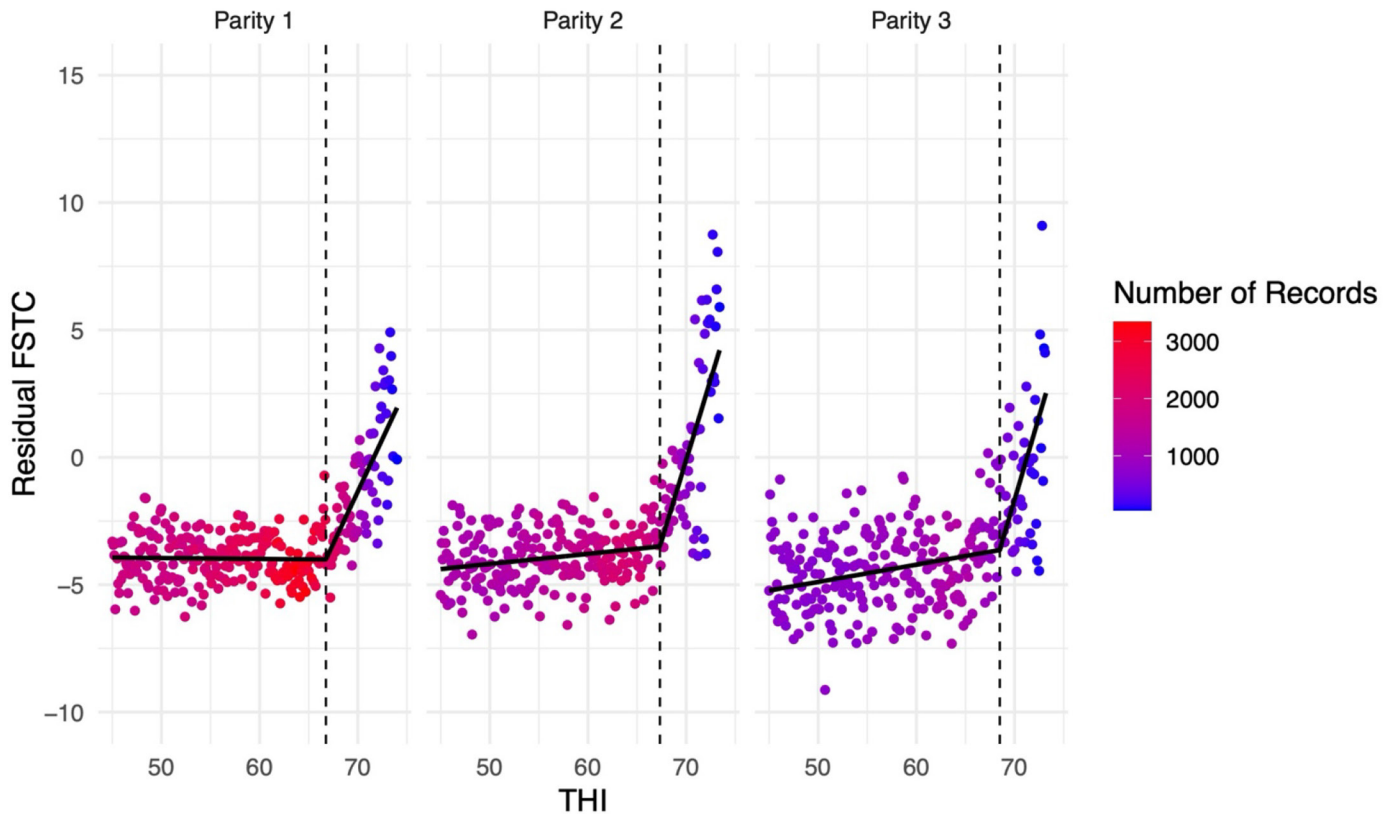
**Figure 2.** Segmented polynomial representing the relationship between the days from first service to conception adjusted by known sources of variation (residual FSTC) and average temperature-humidity index (THI) across the window of 7 d before to 2 d after first insemination (W2). Dotted line indicates identified THI threshold and solid line indicates the identified polynomial.

segmented regressions had slightly positive slopes within the thermoneutral zone and steeper increases under HS; however, thresholds for HS varied by insemination protocol. A threshold of 68 was identified for all records in the subset, slightly higher than what was expected based on the full dataset. When broken up by insemination protocol, a threshold of 64 was identified for timed AI records, and a threshold of 72.8 was identified for heat detection records. This result suggests that FSTC for cows under a timed AI protocol increases at lower levels of environmental stress compared with cows bred after display of estrus.

Different responses to HS between timed AI and heat detection were also observed through the nested regression (Table 4). Assuming group-specific thresholds (timed AI: 64; heat detection: 72.8) allowing independent intercepts and slopes, the heat detection group showed a significantly ( $P < 0.0001$ ) steeper rate of change under HS (3.28) compared with the timed AI group (0.44). Although post-threshold responses differed significantly, pre-threshold slopes were similarly shallow and did not differ by insemination protocol ( $P = 0.7132$ ), indicating comparable performance in thermoneutral conditions.

Although the steeper slope for heat detection records after the breakpoint suggests a more abrupt decline in performance for these inseminations under extreme HS, it is important to note that the threshold for this group was substantially higher. As a result, fertility performance for heat detection inseminations remained relatively stable over a broader range of THI values. In contrast, the lower threshold and earlier onset of decline observed in the timed AI group indicated greater overall sensitivity to HS.

Timed AI protocols are often implemented during the summer to mitigate the negative effects of HS on estrus detection and guarantee insemination. However, studies indicate that while insemination rates increase, conception rates do not improve and early pregnancy losses increase under the use of timed AI when HS is present (de la Sota et al., 1998; Cartmill et al., 2001). This pattern may indicate that many cows enrolled in timed AI are not physiologically prepared for insemination, particularly if there is no display of estrus behavior. Estrus behavior is strongly associated with reproductive performance in both timed AI and heat detection inseminations. Tippenhauer et al. (2023) found that 41.2% to 65.1% of timed-



**Figure 3.** Segmented polynomial representing the relationship between the days from first service to conception adjusted by known sources of variation (residual FSTC) and average temperature-humidity index (THI) across the window of 10 d before to 2 d after first insemination (W3). Dotted line indicates identified THI threshold and solid line indicates the identified polynomial.

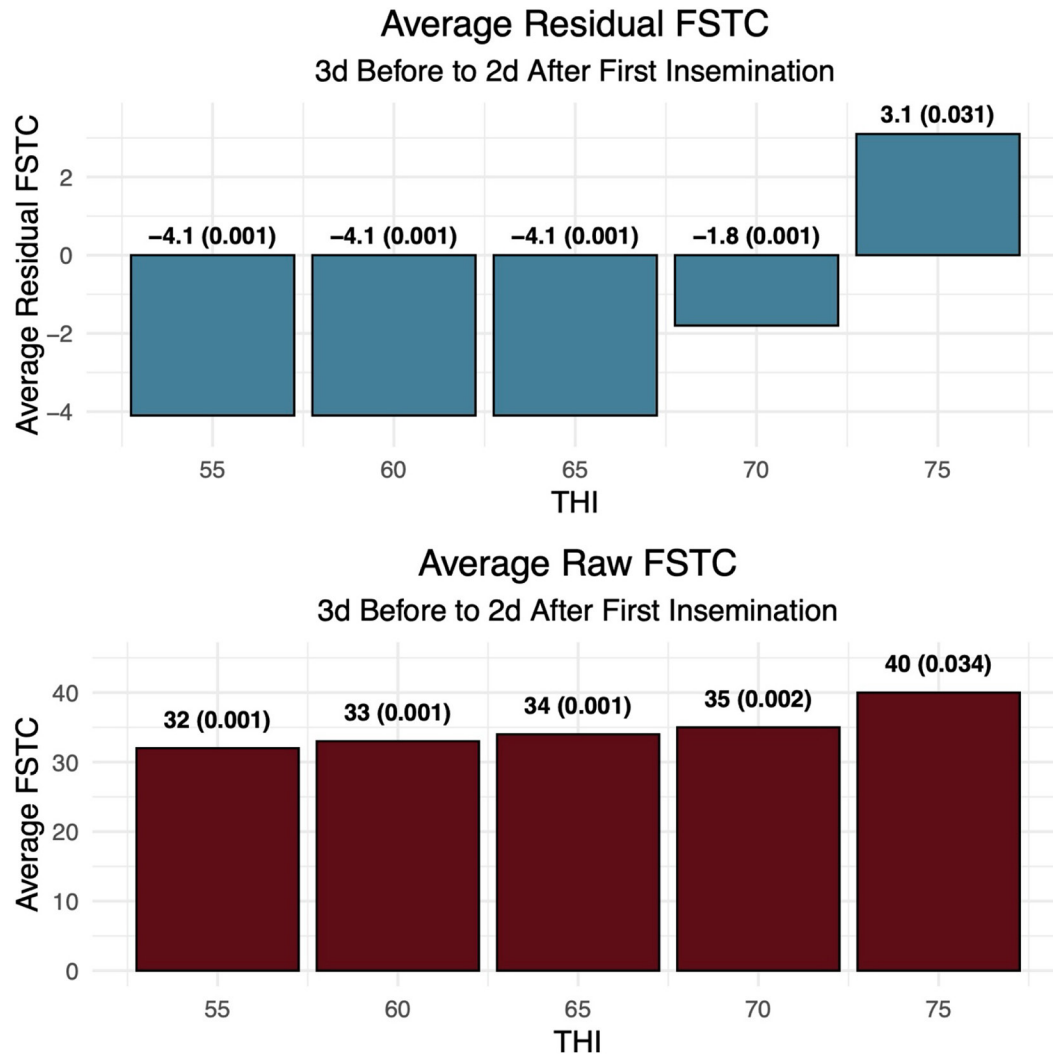
AI cows did not display estrus at the time of insemination, and in cases where estrus behavior was not present, pregnancy per AI was significantly lower than in cows that showing estrus behavior. In contrast, two-thirds of inseminations based on heat detection were accompanied by high intensity of estrus behavior. Lack of observed estrus behavior may also be the result of other stressors such as health challenges or metabolic stress during the transition period, which have been shown to decrease fertility (Ribeiro et al., 2016; Bruinjé et al., 2023).

Although use of timed AI in Canada has increased over the past 20 years (Van Doormaal, 2018), in most herds it is not uniformly applied across all cows. Timed AI protocols are often used to inseminate cows that did not display estrus, due to either anovularity or undetectable estrus behavior that may be attributed to other underlying issues. Given that 92% of the records used in this analysis came from mixed-use herds, the observed differences in performance between timed AI and heat detection protocols may be influenced by sampling bias. Specifically, the lower THI threshold FSTC may not be due solely to the insemination method, but rather to the pre-existing stress loads of the cows selected for that

protocol. Additionally, this observed pattern may suggest that cows facing some form of stress are more sensitive or responsive to additional stressors, such as HS. Herds that exclusively use timed AI or heat detection should be compared to truly understand the influence of estrus synchronization on HS response.

### Weather Patterns

Average summer THI was mapped across Canada to regionally identify areas of concern and any variation within province (Figure 6). Ontario, particularly southern Ontario, had the highest summer heat load, reaching THI of nearly 70 on average. Quebec, Alberta, and Saskatchewan had THI averages ranging from 60 to over 65. This observed pattern is particularly concerning for Ontario and Quebec, which house 80% of Canadian dairy farms and 70% of Canadian dairy cows as of 2024 (CDIC, 2024). Although Alberta and Saskatchewan together account for only 7% of Canadian dairy farms and 12% of dairy cows, these areas are concerning, as temperatures are projected to rise across the country. Climatic models predict an increase in surface temperature of 1°C to 6°C

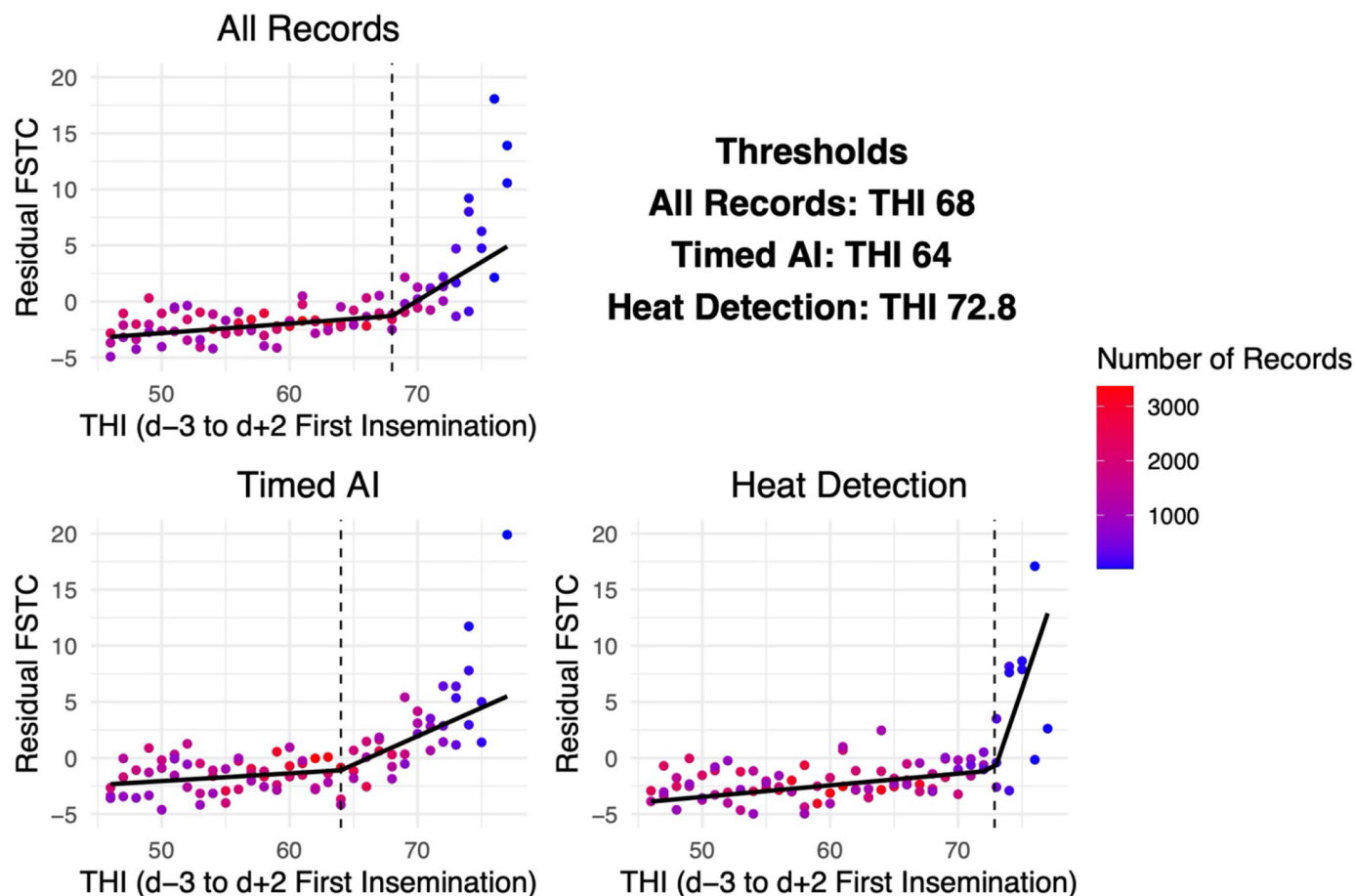


**Figure 4.** Bar plots of the average days from first service to conception (Raw FSTC) and average FSTC adjusted by known sources of variation (residual FSTC) at averaged temperature-humidity index (THI) across the window of 3 d before to 2 d after first insemination (W1) for THI 55, 60, 65, 70, and 75. Mean value with SEM listed above each bar.

by 2081, which corresponds to an increase of 1 to 11 units in THI (Collins et al., 2013). Although Alberta and Saskatchewan currently have average summer THI at or below the threshold of 66, these projected increases suggest that incidence of HS may rise in the future.

Assuming the identified THI threshold of 66, weather data were examined to assess degree of exposure to HS across provinces. The average percentage of the 24-h day exceeding this threshold varied considerably across provinces (Figure 7). British Columbia and Alberta tended to experience 25% of the day during the summer above THI 66, whereas Quebec and Saskatchewan were closer to 30% of the day and above. Ontario was an area of concern, with over 50% of daily hours above THI 66 between 2009 and 2021. Across all provinces, the highest average percentage of hours in a day that were above THI

66 occurred in the past 4 years of the dataset across all 5 provinces. A high percentage of the day with THI above the identified threshold is concerning, as cows may live in a constant state of HS. Overnight cooling is crucial for recovery, as it allows for dissipation of accumulated heat during the day (Igono et al., 1992; Becker et al., 2020; Gaughan et al., 2023). Without extended periods of cooler temperatures, HS effects may be exacerbated. Similar patterns were found when examining the percentage of days in the summer months with an average THI above 66 (Figure 8). Ontario again had the highest sustained exposure to HS, with many years exceeding 50% of days in the summer above the identified threshold, whereas British Columbia and Alberta generally had less than 25% of the summer above THI 66. All provinces, except for British Columbia, experienced their hottest summer



**Figure 5.** Segmented polynomial representing the relationship between the days from first service to conception adjusted by known sources of variation (residual FSTC) and average temperature-humidity index (THI) across the window of 3 d before to 2 d after first insemination (W1) for subset of data containing information on whether timed artificial insemination (timed AI) or heat detection was used for insemination protocol. Dotted line indicates identified THI threshold and solid line indicates the identified polynomial.

within the last 2 years of the dataset. These results indicate that, in addition to longer duration of HS during the day, there is also a broader seasonal pattern of prolonged exposure to HS.

The degree of sustained exposure in this data suggests a pressing need to address these challenges through the use of management adaptations or genetic selection.

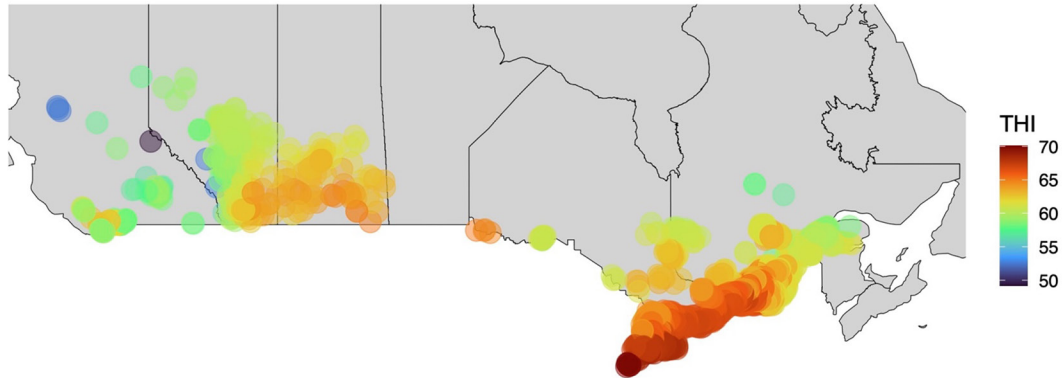
Many mitigation techniques have been evaluated for the ability to reduce HS effects in dairy cows, including ventilation barns, sprinklers, and fans (Titto et al., 2013; Dikmen et al., 2020; Roth, 2022). These strategies offer management solutions that can be implemented to limit the experienced heat by cows during times of high environmental stress. Investment in cooling infrastructure,

**Table 4.** Coefficient estimates (estimate  $\pm$  SE) from nested segmented linear regression models relating residual FSTC to average THI during W1 (3 d before to 2 d after insemination), using group-specific thresholds (timed AI = 64; heat detection = 72.8)<sup>1</sup>

Item	Timed AI records	Heat detection records	<i>t</i> -test <sup>2</sup>
Intercept	-5.50 $\pm$ 5.17	-8.65 $\pm$ 2.54	NA
Pre-threshold slope	0.07 $\pm$ 0.09	0.10 $\pm$ 2.62	<i>P</i> = 0.7132
Post-threshold slope	0.44 $\pm$ 0.14	3.27 $\pm$ 0.47	<i>P</i> < 0.0001

<sup>1</sup>Estimates are shown for all records and by insemination protocol, followed by SE. The final column presents pairwise contrasts between timed AI and heat detection for pre- and post-threshold slopes; no contrast was applied for intercepts (NA).

<sup>2</sup>Results reflect pairwise contrasts between timed AI and heat detection records from the nested segmented regression model.

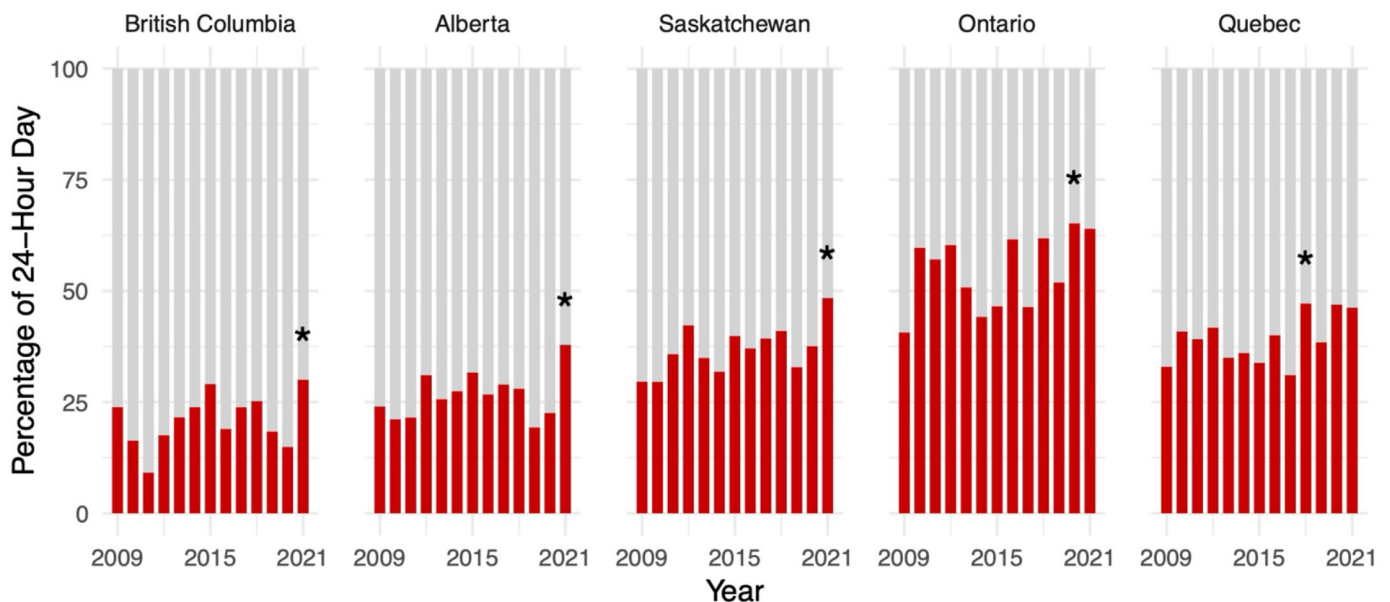


**Figure 6.** Average temperature-humidity index (THI) in summer months (June–August) across Canada between 2009 and 2021.

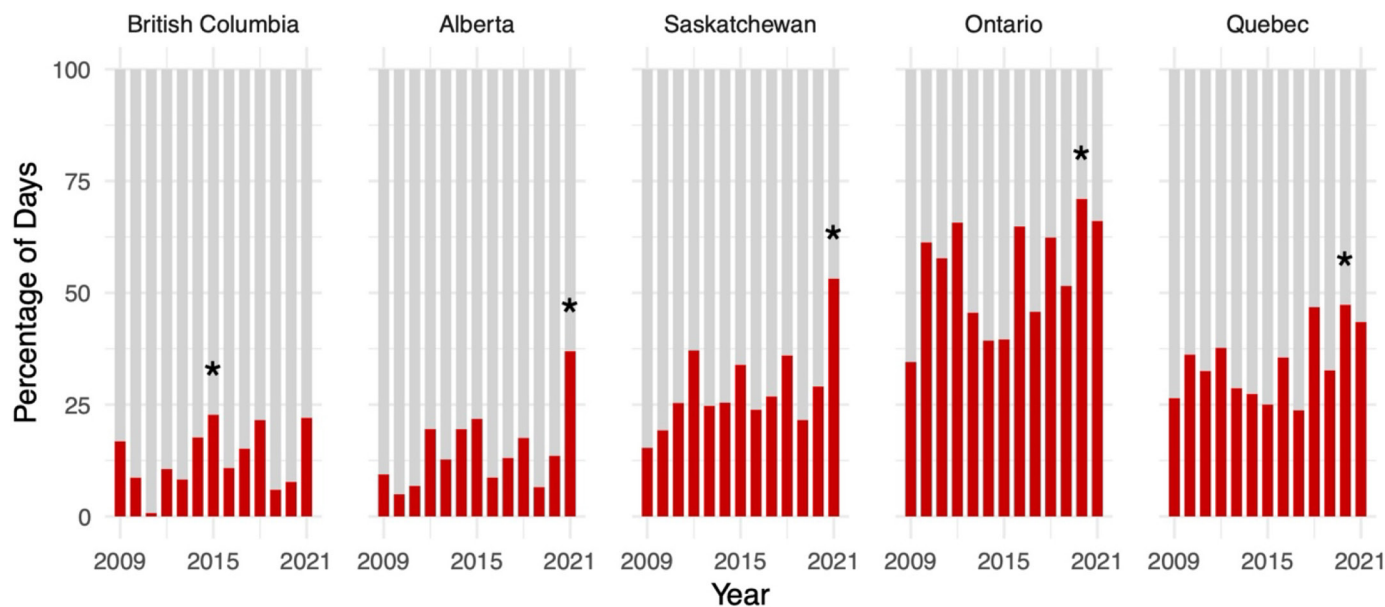
such as fans, sprinklers, or shaded resting areas, may become increasingly critical. These strategies should be regionally tailored, especially in Ontario and Quebec, where chronic HS exposure may have long-term effects on reproductive performance and herd longevity. In addition to management and housing changes, genetic selection offers a permanent and cumulative approach to help mitigate the effects of HS. Studies have demonstrated a genotype by environment interaction resulting in the re-ranking of sires in the thermoneutral zone and under HS for production traits (Campos et al., 2022a; Rockett et al., 2023) and fertility traits (Biffani et al., 2016; Vinet et al., 2024; Ojo et al., 2025). These studies also indicate that it is possible to select for heat tolerance in dairy cattle, which may help alleviate the impacts of HS in the future.

## CONCLUSIONS

This study estimated the temperature-humidity index (THI) threshold at which heat stress (HS) affects the first-service to conception (FSTC) interval, evaluated interactions with estrus synchronization methods, and examined regional weather patterns across Canada. Minimal variation in THI thresholds was observed across parities, with moderate variation across HS time windows. The shortest window (W1: 3 d before to 2 d after first insemination) provided the most consistent estimates, identifying a threshold near THI 66. Cows bred by timed artificial insemination (AI) appeared more susceptible to HS than those bred by heat detection, with an observed lower THI threshold but a shallower response slope.



**Figure 7.** Average percentage of 24-h day in which temperature-humidity index (THI) was above 66 during summer months (June–August) across Canadian provinces between 2009 and 2021. Year within dataset with the highest percentage for each province marked by asterisk.



**Figure 8.** Percentage of days in which average daily temperature-humidity index (THI) was above 66 during summer months (June–August) across Canadian provinces between 2009 and 2021. Year within dataset with the highest percentage for each province marked by asterisk.

Ontario demonstrated highest heat exposure, frequently exceeding THI 66 during summer. Results demonstrate a measurable HS effect on FSTC at THI 66 and suggest management practices may influence susceptibility to HS during early breeding.

## NOTES

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No approval by an Institutional Animal Care and Use Review Board was required because data were obtained from an existing database. The authors have not stated any conflicts of interest.

**Nonstandard abbreviations used:** AI = artificial insemination; ApMf = age at previous calving with month of first service; FSTC = interval from first service to conception; HS = heat stress; HY = herd and year; NASA = National Aeronautics and Space Administration; POWER = Prediction of Worldwide Energy Resources; RYM = region, year, and month of birth; THI = temperature-humidity index; W1 = THI time window from 3 d before to 2 d after first insemination; W2 = THI time window from 7 d before to 2 d after first insemination; W3 = THI time window from 10 d before to 2 d after first insemination.

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