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Association between Temperature–Humidity Index during the Recipient Estrous Cycle and Pregnancy Establishment Following Embryo Transfer in Dairy Heifers

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Abstract

This study evaluated 480 dairy heifers receiving ET, including 279 pregnant and 201 non-pregnant recipients. Daily temperature–humidity index (THI) values were obtained from standing estrus (Day 0) through Day 21 and summarized for estrus, embryo transfer (Day 7), Weeks 1–3, and the entire 21-day period. Associations between THI and pregnancy establishment were assessed using Welch's t-tests and multivariable logistic regression, with separate critical-window models adjusted for relevant recipient, embryo, semen, and seasonal factors. Pregnancy was established in 279/480 recipients (58.1%). Mean whole-cycle THI was 68.89 ± 7.47 in pregnant recipients and 68.01 ± 7.89 in non-pregnant recipients (P = 0.220), and no significant differences were detected for THI at estrus, Day 7, or Weeks 1–3. In the multivariable model, whole-cycle THI was not associated with pregnancy establishment (OR = 0.97 per 1-unit increase, 95% CI: 0.90–1.06, P = 0.542). Similarly, none of the critical-window models showed a significant association when THI was expressed per 5-unit increase (all P > 0.05). Sexed semen showed a borderline association with pregnancy establishment (OR = 0.57, 95% CI: 0.33–1.00, P = 0.050), which should be interpreted cautiously. Under the environmental conditions represented in this cohort, characterized predominantly by no-stress to mild-to-moderate THI exposure and no recipient-days exceeding a THI of 80, no statistically detectable association was observed between thermal load during the recipient estrous cycle and pregnancy establishment following ET.

Introduction

Heat stress is an important environmental constraint on dairy cattle production and reproductive efficiency. Elevated ambient temperature, particularly when accompanied by high relative humidity, can increase the physiological burden imposed on cattle and disrupt endocrine, metabolic, ovarian, uterine, and embryonic processes required for successful reproduction (Khan et al. 2023; Rhoads 2023; Mićić et al. 2025). Reproductive performance may be particularly sensitive to thermal conditions because fertility depends on a sequence of closely coordinated events extending from follicular development and ovulation through early embryonic development and establishment of pregnancy. Consequently, the reproductive effects of environmental heat exposure may depend not only on the magnitude of thermal load but also on its duration and timing relative to specific reproductive events (De Rensis et al. 2017; Hansen 2019).

The temperature–humidity index (THI) is one of the most widely used environmental indicators for characterizing thermal load in cattle because it integrates ambient temperature and relative humidity into a readily interpretable measure. However, THI should not be regarded as a universal biological threshold for reproductive impairment. Reported relationships between THI and fertility vary according to breed, production level, physiological state, climate, management, and the reproductive endpoint evaluated. Studies in lactating dairy cows have reported reductions in conception or pregnancy rates at THI values around 68–73, whereas other populations may tolerate higher environmental loads before measurable reproductive impairment becomes evident (Schüller et al. 2014; De Rensis et al. 2017; Vinet et al. 2024). Recent genetic studies further demonstrate substantial between-animal variation in

reproductive responses to thermal load, reflecting differences in genetic background and individual thermotolerance (Ojo et al. 2025; Bolormaa et al. 2026). Thus, a given THI value should be interpreted in the context of the animal population, physiological state, environmental conditions, and reproductive management system rather than treated as an absolute biological threshold.

The biological plausibility of an adverse effect of thermal load on reproduction is well established. Heat stress can alter hypothalamic–pituitary–ovarian signaling, follicular dynamics, steroidogenesis, corpus luteum function, metabolic status, and oxidative balance (Khan et al. 2023; Rhoads 2023). At the ovarian level, thermal stress can impair oocyte competence through oxidative stress, mitochondrial dysfunction, altered cellular responses, and apoptosis (Miętkiewska et al. 2022; Lang et al. 2024). Heat exposure can also compromise early embryonic development, although susceptibility changes as the embryo progresses through successive developmental stages (Sakatani 2017). Experimental work in cattle demonstrated that embryos are particularly susceptible to maternal heat stress during the earliest stages after fertilization, whereas resistance increases as development progresses (Ealy et al. 1993). These findings provide a biological basis for considering not only the magnitude of thermal exposure but also its timing within the reproductive cycle.

The timing of thermal exposure may therefore be particularly important because reproductive processes are not equally susceptible to environmental stress throughout the estrous cycle. Following ovulation, the developing corpus luteum and uterine environment undergo coordinated changes that support progesterone secretion, conceptus development, and establishment of the maternal–conceptus interaction. Early embryonic development is also characterized by stage-specific changes in cellular proliferation, gene expression, and sensitivity to environmental perturbation (Ealy et al. 1993; Sakatani 2017). Consequently, exposure to elevated thermal load during a specific interval may have different consequences from exposure of similar magnitude occurring at another stage of the reproductive cycle. This temporal dimension is particularly relevant following embryo transfer because the recipient's uterine and endocrine environment remains essential for embryo survival and pregnancy establishment even though fertilization and the earliest pre-transfer stages of embryonic development have already occurred (Putney et al. 1989; Hansen 2019).

Embryo transfer (ET) provides a useful model for investigating these temporal relationships. Unlike artificial insemination, ET bypasses several heat-sensitive events associated with sperm transport, fertilization, and the earliest stages of embryonic development. Earlier field research demonstrated that ET could partially reduce the adverse reproductive effects of summer heat stress compared with artificial insemination, supporting the concept that transfer of a more developmentally advanced embryo can circumvent some heat-sensitive stages of reproduction (Putney et al. 1989). Nevertheless, ET does not eliminate potential effects of environmental heat on pregnancy establishment because recipient pregnancy depends on corpus luteum function, progesterone secretion, uterine receptivity, conceptus development, and appropriate embryo–maternal synchronization (Hansen 2019; Batool et al. 2025). Environmental exposure during the post-transfer period may therefore remain relevant even when the transferred embryo has already undergone fertilization and early development.

Despite substantial evidence linking heat exposure with reduced fertility in dairy cattle, relatively fewer field studies have examined the recipient animal during ET using exposure windows spanning the recipient estrous cycle. Importantly, the available evidence is not entirely consistent. Abdel Aziz et al. (2022) specifically evaluated heat exposure during critical stages of the estrous cycle in embryo-recipient dairy heifers and found no statistically significant effect of exposure to THI ≥ 73 on pregnancy establishment, although pregnancy rates showed numerical reductions at higher mean THI levels. This finding suggests that recipient heifers undergoing ET may respond differently from lactating cows undergoing conventional insemination and highlights the importance of evaluating continuous environmental exposure across biologically relevant periods rather than relying exclusively on categorical heat-stress thresholds. Moreover, heifers generally have lower metabolic heat production than high-producing lactating cows, and their reproductive response to a given environmental load may therefore not be directly extrapolated from studies conducted in lactating animals (Hansen 2019; Gujar et al. 2023).

Against this background, the present study investigated the association between environmental THI during the recipient estrous cycle and pregnancy establishment following embryo transfer in dairy heifers. A cohort of 480 recipient heifers was evaluated, with daily THI characterized from standing estrus (Day 0) through Day 21. Exposure was examined at standing estrus, at embryo transfer on Day 7, during each of the three cycle weeks, and across the complete 21-day period. This design allowed discrete reproductive windows to be distinguished from cumulative thermal exposure. Multivariable analyses additionally accounted for relevant recipient, embryo, semen, and seasonal characteristics. By evaluating continuous THI exposure across predefined stages of the recipient cycle, this study aimed to determine whether environmental thermal load was associated with pregnancy establishment following ET under field conditions and whether particular peri-transfer or post-transfer periods showed stronger associations than cumulative exposure.

Material and methods

This retrospective study used records from an existing commercial embryo-transfer program. The use of animal records was reviewed and approved by the Institutional Animal Care and Use Committee of Beni-Suef University, Egypt (BSU-IACUC: 022–305).

Study design and recipient population

This retrospective cohort study evaluated the association between ambient thermal load and pregnancy establishment following embryo transfer (ET) in dairy heifers. Records from 480 ET recipient heifers enrolled in a commercial embryo-transfer program were retrospectively compiled. The recipient population comprised Holstein (n = 368), Brown Swiss (n = 90), and Simmental (n = 22) heifers. Recipients received embryos associated with Holstein, Brown Swiss, or Simmental sires. Embryos were produced using either conventional semen (n = 412) or sexed semen (n = 68).

The experiment was conducted on a well-managed dairy farm located in northwestern Egypt (30.66° N, 30.06° E). The heifers were kept in free-access yards that were partially shaded, providing approximately 25 m² of space and 70 cm of feed-bunk length for each animal. Fresh, clean drinking water was available to the heifers throughout the day. At the time of embryo transfer, the recipient heifers had an average body weight of 398.5 ± 10.4 kg and an average age of 14.5 ± 0.5 months.

Animals at this stage were carefully inspected for estrous behaviour either visually or using pedometers. Heifers fulfilling the criteria of recipient selection were enrolled in the study. On day six after recipient estrus, the quality of the corpus luteum was checked by ultrasonography (6 MHz linear probe, Easi-scan, BCF technology, USA) and those with adequate CL characters were selected. On day seven after estrus, heifers with satisfactory ultrasound findings received either frozen-thawed or fresh embryos. Embryos were transferred either fresh (n = 126) or following cryopreservation and thawing (n = 354).

Embryo transfers were distributed across autumn (n = 107), winter (n = 131), spring (n = 104), and summer (n = 138).

Embryo-transfer management

Standing estrus was designated as Day zero of the recipient cycle, and embryo transfer was performed on Day 7 according to the established ET program. The developmental stage and morphological grade of each embryo were recorded at the time of transfer. The study therefore included recipient-level information on breed, donor-sire breed, semen type, embryo developmental stage, embryo grade, embryo state, season of transfer, pregnancy outcome, and daily environmental conditions throughout the recipient cycle.

On day seven after recipient estrus, individual heifers were restrained in a special ET stanchion, given a low dose of lignocaine HCl (4 ml) via epidural route to induce posterior epidural anaesthesia. The the external genitalia and the perineal region were washed, cleaned and thoroughly disinfected and dried using a towel. The process of embryo transfer was carried out via trans-rectal route using a standard ET gun. For cryopreserved embryos, the process of thawing was carried out at 35 degrees for 30 seconds in a special thawing device, then frozen-thawed straws were dried and loaded into the standard ET gun. The embryo was deposited into the uterine horn ipsilateral to the ovary containing the CL.

Environmental measurements and calculation of the temperature–humidity index

Daily ambient temperature and relative humidity were used to characterize environmental thermal conditions during the recipient estrous cycle. Daily data regarding ambient temperature, relative humidity were retrieved from the data provided by the Egyptian meteorological Authority for temperature and relative humidity prevailing around the farm. For each study day, the 24-h mean ambient temperature (T,

°C) and 24-h mean relative humidity (RH, %) were used to calculate the temperature–humidity index (THI).

THI was calculated according to the equation described by the National Research Council (1971):

$$THI = (1.8T + 32) - [(0.55 - 0.0055RH)(1.8T - 26)]$$

where T represents the 24-h mean ambient temperature (°C) and RH represents the 24-h mean relative humidity (%).

Daily THI values were assigned to each recipient from the day of standing estrus (Day 0) through Day 21, encompassing the day of embryo transfer (Day 7) and the subsequent post-transfer period. Six exposure variables were derived from the daily THI series: (1) THI at standing estrus (Day 0); (2) THI at embryo transfer (Day 7); (3) mean THI during Week 1 (Days 0–7); (4) mean THI during Week 2 (Days 8–14); (5) mean THI during Week 3 (Days 15–21); and (6) mean THI across the complete observation period from Day 0 through Day 21.

THI was analyzed primarily as a continuous measure of environmental thermal exposure rather than as a categorical heat-stress classification. Across the study period, daily THI values ranged approximately from 50 to 80, and no recipient-day exceeded THI 80. Therefore, the study population predominantly represented no-stress to mild-to-moderate environmental thermal conditions and did not provide sufficient exposure to evaluate pregnancy responses under severe thermal stress.

Pregnancy outcome

Pregnancy diagnosis was carried out on day 35 after ET using ultrasound. The primary outcome was pregnancy establishment following embryo transfer, coded as pregnant or open according to the recorded pregnancy diagnosis. All 480 recipients contributed to the descriptive and univariable analyses for which the relevant variables were complete.

Two recipients had incomplete embryo-related information. One recipient had missing embryo developmental stage and embryo grade, whereas another recipient had a recorded developmental stage but missing embryo grade. Thus, the embryo developmental-stage variable contained 479 observations and the embryo-grade variable contained 478 observations. One recipient accounted for both missing stage and grade values.

Multivariable regression analyses were therefore conducted using complete cases for the variables included in each model, resulting in an analytical sample of 478 recipients.

Statistical analysis

All statistical analyses were performed using Python version 3.12 with the pandas, SciPy, and statsmodels libraries. Pregnancy establishment was treated as a binary outcome.

Categorical recipient-, embryo-, and management-related variables, including season of embryo transfer, recipient breed, donor-sire breed, semen type, embryo developmental stage, embryo grade, and embryo state, were initially described using frequencies and percentages. Associations between each categorical factor and pregnancy outcome were evaluated using Pearson's chi-square test.

Continuous THI exposure variables were summarized using means and standard deviations and compared between pregnant and open recipients using Welch's independent-samples t-test. Welch's test was used without assuming equal variances between the two outcome groups. A multivariable binary logistic regression model was fitted to evaluate the association between whole-cycle mean THI and pregnancy establishment while accounting for prespecified recipient-, embryo-, and seasonal factors. Pregnancy outcome was coded as 1 for pregnant and 0 for open recipients.

The primary model included whole-cycle mean THI as a continuous exposure and recipient breed, donor-sire breed, semen type, embryo developmental stage, embryo grade, and season of embryo transfer as covariates. These variables were selected a priori based on their biological relevance to embryo-transfer success and potential to confound the association between environmental thermal load and pregnancy establishment rather than solely on univariable statistical significance.

Embryo state (fresh versus frozen–thawed) was not included simultaneously with embryo grade because embryo state was completely collinear with embryo grade in the study dataset; all frozen–thawed embryos were Grade 1. Including both variables would therefore result in non-identifiable model coefficients. Embryo grade was retained because it provides a direct morphological assessment of embryo quality.

The association between whole-cycle THI and pregnancy establishment was expressed as an adjusted odds ratio (OR) with a 95% confidence interval (CI) for each 1-unit increase in mean THI. Model adequacy was assessed using the likelihood-ratio test and McFadden's pseudo-R^2.

To examine whether thermal exposure during specific periods of the recipient cycle was associated with pregnancy establishment, six separate logistic regression models were fitted. Each model included one THI exposure variable: THI at standing estrus, THI on the day of embryo transfer, mean THI during Week 1, mean THI during Week 2, mean THI during Week 3, or mean THI across the complete Day 0–21 period.

The same prespecified adjustment strategy was applied across the critical-window models. Each model included semen type, season of embryo transfer, and embryo grade as covariates in addition to the relevant THI exposure variable. The THI variables were modeled separately because the exposure windows were strongly correlated with one another, and simultaneous inclusion would introduce substantial multicollinearity.

For each critical window, the adjusted OR for pregnancy establishment was expressed per 5-unit increase in THI, together with its 95% CI and Wald P value. A 5-unit increment was selected to provide an interpretable estimate of the association across the observed environmental exposure range.

All statistical tests were two-sided. A P value < 0.05 was considered statistically significant. Exact P values and 95% CIs were reported where applicable. P values between 0.05 and 0.10 were not classified as statistical trends; instead, estimates in this range were described descriptively and interpreted in conjunction with their confidence intervals.

Results

Recipient characteristics and pregnancy establishment

Of the 480 embryo-transfer (ET) recipients included in the study, 279 (58.1%) established pregnancy and 201 (41.9%) remained open. The distribution of pregnancy outcomes according to recipient, embryo, semen, and seasonal characteristics is presented in Table 1. No statistically significant association with pregnancy establishment was observed for season of ET (P = 0.10), recipient breed (P = 0.55), donor-sire breed (P = 0.89), semen type (P = 0.18), embryo developmental stage (P = 0.21), embryo grade (P = 0.12), or embryo state (P = 0.16). Pregnancy rates varied numerically among categories, with the highest proportion observed in Simmental recipients (68.2%) and the lowest among Grade 3 embryos (40.7%); however, these differences were not statistically significant.

Table 1
Baseline characteristics of embryo-transfer recipients and univariable association with pregnancy establishment (N = 480).

Factor / category	n	Pregnant, n (%)	Open, n (%)	P value[1]
Season of embryo transfer				
Autumn	107	61 (57.0)	46 (43.0)	0.10
Spring	104	71 (68.3)	33 (31.7)	
Summer	138	78 (56.5)	60 (43.5)	
Winter	131	69 (52.7)	62 (47.3)	
Recipient breed				
Holstein	368	210 (57.1)	158 (42.9)	0.55
Brown Swiss	90	54 (60.0)	36 (40.0)	
Simmental	22	15 (68.2)	7 (31.8)	
Sire (embryo donor) breed				
Holstein	343	197 (57.4)	146 (42.6)	0.89
Brown Swiss	102	61 (59.8)	41 (40.2)	
Simmental	35	21 (60.0)	14 (40.0)	
Semen type				
Conventional	412	245 (59.5)	167 (40.5)	0.18
Sexed	68	34 (50.0)	34 (50.0)	
Embryo developmental stage				
Compact morula	318	175 (55.0)	143 (45.0)	0.21
Early blastocyst	58	40 (69.0)	18 (31.0)	
Blastocyst	97	59 (60.8)	38 (39.2)	
Expanded blastocyst	6	4 (66.7)	2 (33.3)	
Embryo grade				

[1] Pearson's chi-square test comparing the distribution of pregnancy outcome across categories of each factor. [2] Embryo state is reported separately because, herein, it is fully collinear with embryo grade (frozen–thawed = Grade 1 in all cases) and was therefore not entered simultaneously with grade in the multivariable model (Table 3). Note: The total sample comprised 480 recipients. Row totals for embryo developmental stage (n = 479) and embryo grade (n = 478) are lower because of missing values in one and two recipients, respectively; one recipient had missing information for both variables. All other variables were complete for all 480 recipients.

Factor / category	n	Pregnant, n (%)	Open, n (%)	P value[1]
1 (excellent/good)	354	213 (60.2)	141 (39.8)	0.12
2 (fair)	97	54 (55.7)	43 (44.3)	
3 (poor)	27	11 (40.7)	16 (59.3)	
Embryo state[2]				
Frozen–thawed	354	213 (60.2)	141 (39.8)	0.16
Fresh	126	66 (52.4)	60 (47.6)	

[1] Pearson's chi-square test comparing the distribution of pregnancy outcome across categories of each factor. [2] Embryo state is reported separately because, herein, it is fully collinear with embryo grade (frozen–thawed = Grade 1 in all cases) and was therefore not entered simultaneously with grade in the multivariable model (Table 3). Note: The total sample comprised 480 recipients. Row totals for embryo developmental stage (n = 479) and embryo grade (n = 478) are lower because of missing values in one and two recipients, respectively; one recipient had missing information for both variables. All other variables were complete for all 480 recipients.

Temperature–humidity index across the recipient estrous cycle

The mean THI values for pregnant and open recipients across the predefined exposure windows are presented in Table 2. No statistically significant between-group differences were detected for THI at standing estrus (67.76 ± 7.87 vs. 66.88 ± 8.31; P = 0.244), embryo transfer on Day 7 (68.04 ± 8.38 vs. 66.66 ± 8.71; P = 0.082), Week 1 (69.10 ± 7.15 vs. 68.21 ± 7.63; P = 0.195), Week 2 (68.92 ± 7.64 vs. 68.17 ± 8.01; P = 0.303), Week 3 (68.56 ± 8.07 vs. 67.56 ± 8.41; P = 0.189), or the complete Day 0–21 cycle (68.89 ± 7.47 vs. 68.01 ± 7.89; P = 0.220). Thus, although pregnant recipients showed numerically higher mean THI values across all exposure windows, none of these differences reached statistical significance. The daily THI profiles of the two outcome groups were broadly similar throughout the 21-day observation period (Fig. 1).

Table 2
Temperature–humidity index (THI, unitless) during defined windows of the embryo-transfer estrous cycle, by pregnancy outcome.

THI exposure window	Pregnant (n = 279) mean ± SD	Open (n = 201) mean ± SD	P value[1]
THI at standing estrus (Day 0)	67.76 ± 7.87	66.88 ± 8.31	0.244
THI at embryo transfer (Day 7)	68.04 ± 8.38	66.66 ± 8.71	0.082
Mean THI – Week 1 (Day 0–7)	69.10 ± 7.15	68.21 ± 7.63	0.195
Mean THI – Week 2 (Day 8–14)	68.92 ± 7.64	68.17 ± 8.01	0.303
Mean THI – Week 3 (Day 15–21)	68.56 ± 8.07	67.56 ± 8.41	0.189
Mean THI – whole cycle (Day 0–21)	68.89 ± 7.47	68.01 ± 7.89	0.220

[1] Welch's (unequal-variance) independent-samples t-test. Values are mean ± SD.

Multivariable association between whole-cycle THI and pregnancy establishment

The multivariable logistic regression analysis is presented in Table 3. Whole-cycle mean THI was not significantly associated with pregnancy establishment after adjustment for recipient breed, donor-sire breed, semen type, embryo developmental stage, embryo grade, and season of ET (adjusted OR = 0.97 per 1-unit increase in THI, 95% CI: 0.90–1.06, P = 0.542). Among the covariates, sexed semen was associated with lower odds of pregnancy establishment compared with conventional semen (adjusted OR = 0.57, 95% CI: 0.33–1.00, P = 0.050). However, this estimate was borderline and should be interpreted cautiously. None of the remaining covariates showed a statistically significant association with pregnancy establishment (all P > 0.09). The overall model was not statistically significant (likelihood-ratio P = 0.184), and the McFadden pseudo-R^2 was 0.029.

Table 3
Multivariable logistic regression model of factors associated with pregnancy establishment after embryo transfer (n = 478).

Variable	Adjusted OR	95% CI	P value
Mean THI, whole cycle (per 1-unit increase)			
Mean THI (Day 0–21)	0.97	0.90–1.06	0.542
Semen type (ref. Conventional)			
Sexed	0.57	0.33–1.00	0.050
Season of ET (ref. Autumn)			
Spring	2.08	0.87–4.98	0.098
Summer	1.23	0.45–3.36	0.680
Winter	0.81	0.36–1.80	0.608
Embryo grade (ref. Grade 1)			
Grade 2	0.90	0.54–1.49	0.672
Grade 3	0.48	0.20–1.19	0.113
Embryo developmental stage (ref. Blastocyst)			
Compact morula	0.89	0.54–1.47	0.637
Early blastocyst	1.55	0.76–3.16	0.225
Expanded blastocyst	3.21	0.33–30.97	0.313
Recipient breed (ref. Brown Swiss)			
Holstein	1.04	0.58–1.87	0.895
Simmental	1.64	0.53–5.09	0.392
Sire breed (ref. Brown Swiss)			
Holstein	0.92	0.52–1.61	0.767
Simmental	0.71	0.29–1.77	0.466

OR, odds ratio (from a single logistic regression model including all listed variables simultaneously); CI, confidence interval. Model likelihood-ratio P = 0.184; pseudo-R^2 (McFadden) = 0.029, indicating that, collectively, the factors examined explain only a small share of the variation in pregnancy outcome in this dataset. Note: Regression analyses were conducted using 478 complete cases because two recipients had missing embryo stage and/or embryo grade information.

Critical-window analysis of THI and pregnancy establishment

The associations between THI during individual exposure windows and pregnancy establishment are presented in Table 4 and Fig. 2. After adjustment for semen type, season of ET, and embryo grade, none of the evaluated exposure windows was significantly associated with pregnancy establishment. The adjusted ORs per 5-unit increase in THI were 0.95 (95% CI: 0.65–1.39; P = 0.773) at standing estrus, 1.15 (95% CI: 0.81–1.62; P = 0.433) on the day of embryo transfer, 0.93 (95% CI: 0.62–1.40; P = 0.722) during Week 1, 0.80 (95% CI: 0.56–1.13; P = 0.208) during Week 2, 0.92 (95% CI: 0.66–1.28; P = 0.613) during Week 3, and 0.85 (95% CI: 0.57–1.27; P = 0.433) across the complete Day 0–21 period. All confidence intervals included the null value of 1.0. Collectively, these analyses provided no statistically detectable evidence of an association between THI during any predefined stage of the recipient estrous cycle and pregnancy establishment under the environmental conditions represented in this cohort.

Table 4
Critical-window analysis: association between THI at each stage of the estrous cycle and pregnancy establishment, adjusted for semen type, season and embryo grade.

Exposure window	Adjusted OR[3]	95% CI	P value	n (models)
THI at standing estrus	0.95	0.65–1.39	0.773	478
THI at embryo transfer (Day 7)	1.15	0.81–1.62	0.433	478
Mean THI – Week 1 (Day 0–7)	0.93	0.62–1.40	0.722	478
Mean THI – Week 2 (Day 8–14)	0.80	0.56–1.13	0.208	478
Mean THI – Week 3 (Day 15–21)	0.92	0.66–1.28	0.613	478
Mean THI – whole cycle	0.85	0.57–1.27	0.433	478

[3] Adjusted odds ratio for pregnancy per 5-unit increase in the respective THI variable, from separate logistic regression models (one THI window per model), each additionally controlling for semen type, season and embryo grade. n: Two recipients with missing covariate data were excluded, resulting in 478 complete cases for each model.

Discussion

The present study evaluated whether ambient heat exposure during defined stages of the recipient estrous cycle was associated with pregnancy establishment following embryo transfer in dairy heifers. Contrary to the expected adverse influence of thermal stress on reproductive efficiency, none of the examined THI windows, including standing estrus, embryo transfer on Day 7, the post-transfer weeks, or the entire 21-day cycle, was significantly associated with pregnancy outcome. The overall pregnancy rate was 58.1%, and mean whole-cycle THI differed only slightly between pregnant and open recipients. These findings indicate that, under the environmental conditions represented in this cohort, ambient THI alone was not a significant predictor of pregnancy establishment.

Heat stress is widely recognized as an important challenge to dairy reproduction. Elevated ambient temperature can increase body temperature, alter endocrine regulation, reduce feed intake, and induce metabolic and oxidative disturbances. These responses may affect follicular development, oocyte competence, fertilization, embryonic development, and maternal recognition of pregnancy (Gujar et al. 2023; Rhoads 2023; Lang et al. 2024; Batool et al. 2025).

The reproductive consequences of heat stress are therefore biologically plausible, particularly during the periovulatory period and early embryonic development, acting through multiple pathways affecting cellular, gamete, and embryonic function (Rhoads 2023; Lang et al. 2024; Castro et al. 2026). The absence of a significant association in the present study does not contradict this biological evidence. Rather, it suggests that the magnitude and duration of environmental exposure, together with the physiological characteristics of the recipients, may determine whether these mechanisms translate into measurable differences in pregnancy establishment. The distinction between biological susceptibility and an observable population-level association is important when interpreting the present results.

Mean THI values in this study were approximately 68–69 across the recipient estrous cycle, although individual daily values approached 80. Importantly, no recipient-day exceeded a THI of 80. This restricted exposure range is a key consideration when interpreting the null findings. Thermal indices such as THI provide useful estimates of environmental heat load, but their relationship with physiological heat strain can vary with environmental conditions, management, and animal characteristics (Shephard and Maloney 2023). A study conducted predominantly under no-stress to mild-to-moderate thermal conditions may therefore have limited ability to detect an association that becomes more apparent under higher or prolonged heat loads (Nishisozu et al. 2023). Accordingly, the present results should not be extrapolated to conditions characterized by sustained severe thermal exposure

The use of THI is practical for field-based studies, but THI is an indirect environmental indicator rather than a direct measurement of the animal's internal thermal burden. It does not fully capture solar radiation, air movement, individual thermoregulation, or the effectiveness of cooling and shade. Consequently, two recipients exposed to the same THI may experience different physiological heat loads (Gujar et al. 2023; Stojnov et al. 2023). Future studies should therefore consider incorporating rectal temperature, respiration rate, activity, and other animal-based indicators alongside environmental measurements.

The current results are consistent with findings from other retrospective field evaluations of embryo transfer success in dairy cattle (Aziz et al. 2022; Lee et al. 2024). Their study reported no statistically significant effect of heat stress on pregnancy establishment following embryo transfer, although numerical differences were observed at higher mean THI levels. The agreement between these studies is relevant because both focus specifically on recipient heifers undergoing embryo transfer rather than relying exclusively on conventional artificial insemination outcomes. However, the absence of a significant association should not be generalized to all thermal environments. Previous research has demonstrated that heat stress can impair reproductive performance through direct effects on the ovary,

oocyte, embryo, and maternal reproductive tract, as well as through systemic metabolic changes (Rhoads 2023). The current findings may therefore reflect the exposure conditions and recipient population rather than an absence of biological sensitivity to heat.

An important feature of this study was the separate evaluation of THI at standing estrus, embryo transfer, and each subsequent cycle week. None of these windows was significantly associated with pregnancy establishment after adjustment for semen type, season, and embryo grade. The adjusted OR for THI at embryo transfer was 1.15 per 5-unit increase, whereas the OR for whole-cycle THI was 0.85. These estimates do not establish a consistent direction of effect, and their confidence intervals included the null value. The lack of a significant association across all windows suggests that no single exposure period was identified as a dominant predictor within the observed conditions. Nevertheless, the wide confidence intervals indicate that clinically relevant effects cannot be excluded. Larger datasets spanning a broader range of THI values would improve precision and help determine whether specific stages are more vulnerable.

The multivariable analysis showed that recipient breed, sire breed, embryo developmental stage, and embryo grade were not significantly associated with pregnancy establishment after adjustment. Embryo grade 3 showed numerically lower odds than Grade 1, but the confidence interval included unity, aligning with broader retrospective evidence emphasizing that morphological and developmental competence metrics play critical roles in predicting transfer success (Darfour-Oduro et al. 2024; Lee et al. 2026). These findings should be interpreted cautiously because the sample sizes for some categories, particularly expanded blastocysts and Grade 3 embryos, were relatively small.

Sexed semen was associated with lower odds of pregnancy than conventional semen, with an adjusted OR of 0.57 and P = 0.050. This borderline finding is consistent with the possibility that semen-related factors may influence reproductive outcomes, but it should not be interpreted as definitive evidence of an independent causal effect in this study. The model's likelihood-ratio P value was 0.184, and its McFadden pseudo-R^2 was 0.029, indicating that the examined variables collectively explained only a small proportion of pregnancy-outcome variation.

The principal strength of this study was the relatively large cohort of 480 recipient heifers and the assessment of THI across the complete 21-day embryo-transfer cycle. The use of multivariable and critical-window analyses also allowed the potential contribution of environmental exposure to be examined while accounting for several embryo-transfer characteristics.

However, limitations should be acknowledged. First, the observational design does not establish causality. Second, THI was used as the environmental exposure measure without direct physiological measurements of recipient thermal load, although environmental indices do not necessarily capture individual variation in physiological heat strain (Shephard 2023). Third, the restricted exposure range and absence of severe-stress days may have limited the ability to detect associations. Conceivably, ambient THI during the recipient estrous cycle was not significantly associated with pregnancy establishment following embryo transfer in this cohort of dairy heifers. The findings support the interpretation that,

under predominantly mild-to-moderate thermal conditions, THI alone may have limited predictive value for pregnancy establishment. Further research should evaluate larger populations exposed to higher and prolonged heat loads, incorporate animal-based thermal indicators, and investigate interactions between environmental stress, embryo characteristics, and recipient physiology. Such approaches would help clarify the conditions under which heat stress becomes a meaningful determinant of reproductive success.

Conclusions

In this retrospective cohort of 480 embryo-transfer recipient heifers, ambient THI measured from standing estrus through Day 21 was not significantly associated with pregnancy establishment at standing estrus, embryo transfer, individual post-transfer weeks, or across the complete recipient cycle. These findings indicate that, within the predominantly mild-to-moderate THI conditions represented in this cohort, environmental thermal load alone was not a statistically detectable predictor of pregnancy establishment. However, the restricted THI range and absence of severe heat exposure limit extrapolation to more extreme or prolonged thermal conditions. Future prospective studies should combine continuous environmental measurements with animal-based indicators of heat load and evaluate broader thermal gradients, nonlinear exposure-response relationships, and interactions with recipient and embryo characteristics.

Declarations

Ethics Approval:

This retrospective study used records from an existing commercial embryo-transfer program. The use of animal records was reviewed and approved by the Institutional Animal Care and Use Committee of Beni-Suef University, Egypt (BSU-IACUC: 022–305). The study was conducted in accordance with the applicable institutional guidelines for the care and use of animals in research.

Consent to Participate:

Not applicable. This retrospective study involved the analysis of existing animal records and did not involve human participants.

Consent to Publish:

Not applicable. This study does not contain any individual person's data, images, or other identifiable personal information.

Competing Interests:

The authors declare no competing interests.

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Author contributions:

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Data Availability:

All data will be available on request

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Figures

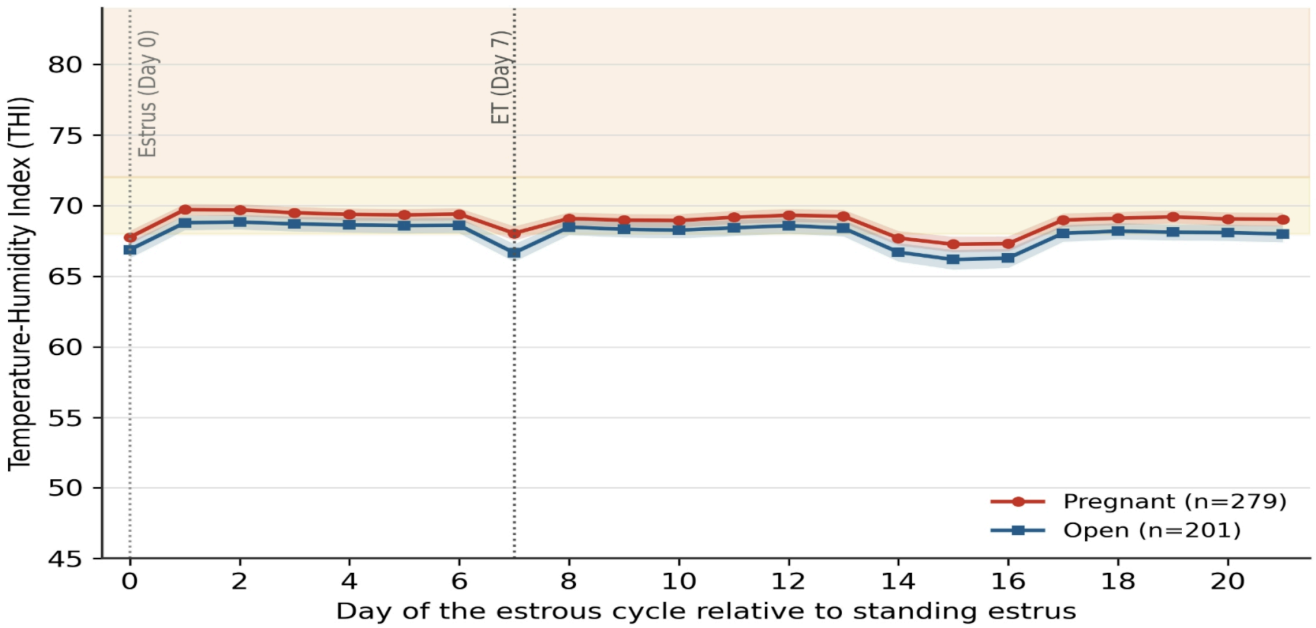


Figure 1

Daily temperature–humidity index (THI) trajectory across the 21-day embryo-transfer cycle in pregnant versus open recipients. Points show the daily group mean; shaded bands show ± SEM. Vertical dotted lines mark the day of standing estrus (Day 0) and embryo transfer (Day 7).

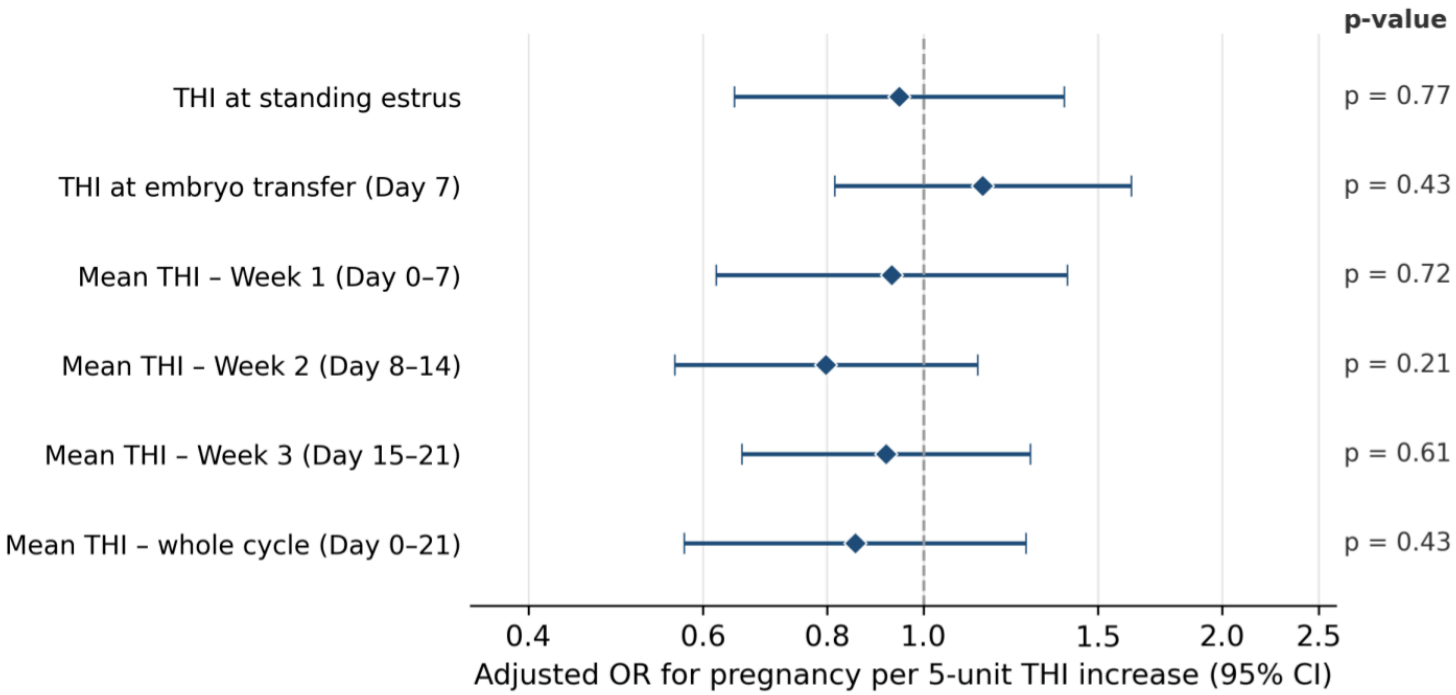


Figure 2

Forest plot of the adjusted odds ratio for pregnancy establishment per 5-unit increase in THI, estimated separately for each exposure window of the embryo-transfer cycle. Diamonds show the point estimate and horizontal lines the 95% confidence interval; the dashed reference line marks OR = 1 (no association).