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# *Short communication*: Effect of heat stress on nonreturn rate of Italian Holstein cows

**S. Biffani,* U. Bernabucci,† A. Vitali,† N. Lacetera,† and A. Nardone†[1]**
*Istituto di Biologia e Biotecnologia Agraria (IBBA), Consiglio Nazionale delle Ricerche (CNR), Via Einstein - Località Cascina Codazza, 26900 Lodi, Italia
†Dipartimento di Scienze Agrarie e Forestali (DAFNE), Università degli Studi della Tuscia-Viterbo, 01100, Viterbo, Italia

## ABSTRACT

The data set consisted of 1,016,856 inseminations of 191,012 first, second, and third parity Holstein cows from 484 farms. Data were collected from year 2001 through 2007 and included meteorological data from 35 weather stations. Nonreturn rate at 56 d after first insemination (NR56) was considered. A logit model was used to estimate the effect of temperature-humidity index (THI) on reproduction across parities. Then, least squares means were used to detect the THI breakpoints using a 2-phase linear regression procedure. Finally, a multiple-trait threshold model was used to estimate variance components for NR56 in first and second parity cows. A dummy regression variable ($t$) was used to estimate NR56 decline due to heat stress. The NR56, both for first and second parity cows, was significantly (unfavorable) affected by THI from 4 d before 5 d after the insemination date. Additive genetic variances for NR56 increased from first to second parity both for general and heat stress effect. Genetic correlations between general and heat stress effects were −0.31 for first parity and −0.45 for second parity cows.
**Key words:** dairy cow, temperature-humidity index breaking point, heat stress, reproduction trait, heritability

## Short Communication

Declining fertility of dairy cows represents a major concern among producers due to the effect on the profitability of the herd, leading to increased culling rates, larger veterinary expenses, and reduced genetic potential. Indeed, heat stress due to warm environment is one of the major factors that can negatively affect production, reproduction, and health of dairy cows (Bernabucci et al., 2010). The temperature-humidity index (**THI**), which represents the combined effects of air temperature and humidity (Armstrong, 1994), is generally used as a bioclimatic index (Hahn et al., 2003). In a comprehensive review by Hansen (2007), the deleterious effects of heat stress on oocyte development and maturation, on early embryonic death, and on fetal or placental development were elucidated. This author highlighted 2 specific aspects: the time at which the heat stress occurs (e.g., before or after the insemination event) and the role of genetics.

Jordan (2003) reported that negative effects of heat stress could be detected as early as 42 d before insemination until 40 d after. Ravagnolo and Misztal (2002) reported that THI on the day of insemination seems to be the most informative weather parameter to be used for reproduction studies. However, they stressed that geographical variations do exist and the right parameter should be estimated from actual data.

Apart from the onset of heat stress effect and its further implications on reproductive traits, some studies (Nardone and Valentini, 2000; Ravagnolo and Misztal, 2002a; Bernabucci et al., 2014) have demonstrated the existence of both a genetic component of heat tolerance and its negative genetic relationship with production and reproduction. Moreover, the onset of heat stress varies between and within animals (Aguilar et al., 2009), as well as between traits and parities, depending on their physiological status (Bernabucci et al., 2014).

Recently, Bernabucci et al. (2014) proposed a 2-phase linear regression procedure to detect THI thresholds in production traits to estimate variance components for heat tolerance and to infer its genetic relationship with milk production traits in Italian Holstein cows.

The objective of the present study was to investigate the effect of heat stress on a reproductive trait, namely, nonreturn rate at 56 d after first insemination (**NR56**), to detect the threshold point and time period when NR56 of primiparous and multiparous Italian Holstein cows are affected by heat stress and to estimate its genetic component. The most controversial issue regarding fertility is which is the best trait to be used. This is due to the fact that female fertility is quite a complex

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[1]Corresponding author: nardone@unitus.it

trait and several indicator traits have been proposed for its evaluation. Among them, nonreturn rate within 56 d after first insemination (NR56) has been internationally recommended and widely used to assess the ability of conception and maintaining pregnancy over the period of early gestation (Miglior, 1999; Jorjani, 2006; Biffani and Canavesi, 2007; Tiezzi et al., 2015). Moreover, as Andersen-Ranberg et al. (2005) pointed out, this NR56 data are less biased due to selection and have fewer missing records than other fertility traits. On the other side, the main disadvantage is that a cow that does not return within 56 d might not be pregnant or get a subsequent calving (Sun and Su, 2010). Currently, the NR56 is the trait officially used in the national genetic evaluation for fertility in the Italian Holstein breed, and in other 13 countries worldwide (Interbull, 2016).

Data were provided by the Italian Holstein Breeder Association and comprised 1,016,856 inseminations of 191,012 Holstein cows (first, second, and third parity) from 484 farms, collected from 2001 through 2007. Nonreturn rate at 56-d after first insemination was calculated for all cows. First, second, and third parity cows were retained for successive analysis. A value of 1 was assigned to cows that did not return to insemination (assumed pregnant) and 0 for cows that were inseminated a second time within 56 d. A detailed description of the rules used to validate fertility traits that relied on insemination information is given in Biffani et al. (2003). The weather data were obtained from 35 meteorological stations located within a maximum of 5 km from each herd. Data consisted of daily weather information over a 7-yr period (2001–2007). Briefly, THI index was calculated using the following formula:

$$\text{THI} = (1.8 \times \text{AT} + 32) - (0.55 - 0.55 \times \text{RH}) \times [(1.8 \times \text{AT} + 32) - 58],$$

where AT is the ambient temperature (°C), and RH is the relative humidity as a fraction of the unit. With respect to the original equation, this formula includes terms (1.8 × AT + 32) that account for conversion of temperature data from degrees Celsius (°C) to degrees Fahrenheit (°F). Maximum AT and minimum RH were used for calculating THI index. More details can be found in Bernabucci et al. (2014).

Reproduction data were merged with weather information, assigning each insemination record to the daily weather records at the nearest weather station. Weather station data provide an accurate source of ambient air temperature and relative humidity outside of dairy barns; however, the values within a barn can often be different as reported by Schüller et al. (2013). In the same study, high positive relationships were found between meteorological data and on-farm measurements confirming the validity of using data from meteorological stations in studies in which the sample is very large as in the present study. Age intervals were established for each parity according to the following thresholds: 20 to 36, 31 to 50, and 42 to 65 mo of age for first, second, and third parity cows, respectively. Additionally, cows were required to have no insemination before 14 or beyond 150 d from calving. Days in milk classes were defined as one class for every 30 d, resulting in 11 classes. The weather data set was divided into 36 THI classes, with the first class beginning at THI = 50, and the subsequent classes were set at each 1 point THI thereafter until the last class, which was THI = 85. After editing, the final data set consisted of 119,195 NR56 records for 76,943 cows. Distribution of data across parities was 57.5, 30.1, and 12.4% for first, second, and third parity cows, respectively. Descriptive statistics of THI per month of calving are in Table 1 and Figure 1.

**Table 1**. Descriptive statistics for temperature-humidity index by month of calving

| Month of calving | Number of animals | Number of records |
|---|---|---|
| January | 3,015 | 5,056 |
| February | 4,784 | 7,547 |
| March | 6,867 | 9,924 |
| April | 8,809 | 13,077 |
| May | 7,946 | 11,460 |
| June | 6,111 | 8,742 |
| July | 5,931 | 8,426 |
| August | 7,278 | 11,003 |
| September | 8,328 | 13,523 |
| October | 7,641 | 13,081 |
| November | 6,676 | 11,071 |
| December | 3,557 | 6,285 |

The effect of THI (heat stress) was analyzed by fitting the following logit model (model 1) using the GLIMMIX procedure in SAS version 9.2 (SAS, 2008):

$$\text{logit}(p_{ijklmno}) = \eta + e = \mu + \text{herd}(yi)_i + \text{mc}(yi)_j + \text{cdim}_k + \text{thir}_l + \text{age}_m + \text{ord}_n + \text{thir}_l \times \text{ord}_n + \text{cdim}_k \times \text{ord}_n + b \times \text{ecm} + e_{ijklmno},$$

where logit() is the link function, log(odds), between the probability of either returning to insemination or not for cow $o$ in herd $i$, month of calving, and year of insemination $j$, days in milk $k$, THI $l$, age $m$, parity $n$, and the linear predictor $\eta$, where $\mu$ is the overall mean; $\text{herd}(yi)_i$ is the random effect of herd nested within year of insemination $i$; $\text{mc}(yi)_j$ is the fixed effect of month of calving nested within year of insemination $j$; $\text{cdim}_k$ is the fixed effect of DIM class $k$; $\text{thir}_l$ is the fixed effect of