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A transgenerational study on the effect of great-granddam birth month on granddaughter EBV for production traits in Italian Simmental cattle

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ABSTRACT

Heat tolerance is a key feature of resilient animals. Offspring of animals that suffer environmental stress during pregnancy could show physiological, morphological, and metabolic modifications. This is due to a dynamic reprogramming of the epigenetics of the mammalian genome that occurs in the early life cycle. Thus, the aim of this study was to investigate the extent of the transgenerational effect of heat stress during the pregnancy of Italian Simmental cows. The effects of dam and granddam birth months (as indicator of pregnancy period) on their daughter and granddaughter estimated breeding values (EBV) for some dairy traits as well as of the temperature-humidity index (THI) during the pregnancy were tested. A total of 128,437 EBV (milk, fat, and protein yields, and somatic cell score) were provided by the Italian Association of Simmental Breeders. The best birth months (of both dam and granddam) for milk yield and protein yield were May and June, whereas the worst were January and March. Great-granddam pregnancies developed during the winter and spring seasons positively affected the EBV for milk and protein yields of their great-granddaughters; in contrast, pregnancies during summer and autumn had negative effects. These findings were confirmed by the effects of maximum and minimum THI in different parts of the great-granddam pregnancy on the performances of their great-granddaughters. Thus, a negative effect of high temperatures during the pregnancy of female ancestors was observed. Results of the present study suggest a transgenerational epigenetic inheritance in Italian Simmental cattle due to environmental stressors.

Key words: epigenetics, heat stress, milk production, temperature-humidity index

INTRODUCTION

The ability of animals to face climate changes represents a key topic for the livestock industry. Future climate scenarios will be characterized by increase of earth temperature, reduction of rainfall, and frequent occurrence of extreme events such as heat waves and storms (Bernabucci, 2019). Among the various aspects that define animal adaptability to climate constraints, heat tolerance in dairy cattle is one of the widely most studied. The condition of heat stress (**HS**)—that is, when the animal is not able to dissipate the excess of heat to maintain body thermal balance (Bernabucci et al., 2014)—exerts a direct negative effect on productive and reproductive performances (Lees et al., 2019) and on the welfare of dairy cattle (Vitali et al., 2020; Kipp et al., 2021a). Economic losses due to the direct effects of heat stress for the US dairy industry have been estimated to be more than 1.5 billion dollars per year (Laporta et al., 2020).

Genetic aspects of HS tolerance have been investigated using both traditional quantitative and genomic approaches (Ravagnolo and Misztal, 2000; Bernabucci et al., 2014; Dikmen et al., 2015; Nguyen et al., 2016). In general, HS tolerance, measured either directly using physiological variables or indirectly using production performances, seems to have a low to moderate genetic component and unfavorable correlations with productive performances in dairy animals. Some genes involved in the mechanism of heat tolerance have been discovered (Dikmen et al., 2015; Macciotta et al., 2017). However, inheritance aspects of HS tolerance are further complicated by epigenetic mechanisms (Ghaffari, 2022).

Studies carried out in humans and livestock species have reported that exposures to environmental stress in the different phases of pregnancy result in lifelong physiological, morphological, and metabolic modifications of the offspring (Laplante et al., 2004; González-Recio et al., 2012). As observed for other stress events, the occurrence of HS during pregnancy affects not only the physiological status of the cow itself, by compromis-

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ing embryonic and fetal development (Ouellet et al., 2021), but also the offspring and future generations. The reason is a dynamic reprogramming of the epigenetics of the mammalian genome that occurs in the early life cycle. It has been widely assessed that in the first phases of fetal development in mammalian species, relevant changes of the level of methylation may take place (González-Recio et al., 2012), and that these epigenetic marks can be transmitted to subsequent generations. In particular, epigenetic reprogramming occurs in 2 waves: in the primordial germ cells and in the early embryo (Singh and Li, 2012; Wu et al., 2020; Bellver-Sanchis et al., 2021). Pinedo and De Vries (2017) found that cows conceived during winter months had better production and survival performances than those conceived in summer. Negative effects of HS experienced in late lactation on production performances of granddaughters have been observed in U.S. Holsteins (Laporta et al., 2020). The occurrence of HS in late pregnancy of dams has also been reported to affect genetic parameters estimated using their offspring records (Kipp et al., 2021b). Transgenerational effects on milk production and reproductive traits have recently been reported in a study carried out on 4 generations of Israeli Holsteins by Weller et al. (2021), who found an effect of the calving month and pregnancy season of great-granddams on the production and reproduction performances of great-granddaughters.

The underlying hypothesis of the transgenerational approach is that the effect of environmental stress is direct on the pregnant animal ($\mathbf{F_0}$), on the first filial generation as fetuses in utero ($\mathbf{F_1}$), and on its primordial germ cells that will generate the second filial generation ($\mathbf{F_2}$). Thus, effects detected in the third filial generation ($\mathbf{F_3}$), not directly exposed to parental stress, are the results of transgenerational epigenetic inheritance through the female lineage (Bellver-Sanchis et al., 2021; Weller et al., 2021).

Heat stress represents a major concern not only for tropical and subtropical farming areas but also for temperate regions, due to both global warming and higher metabolic heat production by high-yielding animals (Kadzere et al., 2002; Segnalini et al., 2011; Pryce et al., 2022). Direct effects of temperature-humidity index (**THI**) on milk production traits have been reported in dairy cattle farmed in several European countries (Hammami et al., 2013; Bernabucci et al., 2014; Carabaño et al., 2016; Maggiolino et al., 2020). Because most transgenerational studies of heat stress on cow performances have been carried out in hot and humid areas, it could be of interest to investigate whether similar effects can also be detected in temperate regions.

In this work, a study on 4 female generations was carried out to investigate the effects of granddam and dam month of birth on EBV for milk production traits and SCS of their granddaughters and daughters, respectively. This effect will be considered as representative of the period of pregnancy occurrence. Cows were Italian Simmental cattle, a dual-purpose breed that is farmed mostly in the mountainous areas of Northeastern Italy (Cesarani et al., 2020).

MATERIALS AND METHODS

Animal Care and Use Committee approval was not needed, as data were obtained from preexisting databases.

Production Data

Data were 128,437 Italian Simmental cows (F_3) calving from 1984 to 2014. Cows were farmed in commercial herds located mostly (about 88% of records) in Northeastern Italy, the traditional farming area of this breed. For each cow, data of the dam (F_2), maternal granddam (F_1), and maternal great-granddam (F_0) were also available. Only F_3 cows with complete records for the 3 previous generations were considered. For all 4 generations, EBV for the following traits were provided by the Italian Association of Simmental Breeders: milk (**MY**), fat (**FY**), and protein yields (**PY**), and SCS. The EBV were estimated during routine genetic evaluations using a repeatability test day model that included the fixed effects of herd-test-day, season-year-region of calving, lactation stage within age at calving within parity, pregnancy stage, and DIM as covariate. The EBV for the 4 generations considered in the analysis were estimated in the same run. Average EBV reliabilities were 60.8 ± 4.3, 57.2 ± 4.9, 58.2 ± 4.5, and 53.4 ± 4.9 (mean $\pm$ SD) for MY, FY, PY, and SCS, respectively. Calving dates were also available for all 4 generations. The EBV for MY, FY, and PY were expressed in kilograms per 305 d, whereas those for SCS were rescaled to mean 100 and standard deviation 12. Moreover, EBV for SCS were reported to have higher values, as desirable (i.e., the higher the EBV, the smaller the SCS). To avoid negative values, EBV were rescaled by adding a constant value.

Climate Data

Daily temperature (C°) and relative humidity for the years of the great-granddam pregnancy occurrence (1980–2014) were retrieved for all areas where herds are located using the *riem* R package (Salmon, 2021). Maximum and minimum temperature and humidity recorded during the first 5 and the last 4 mo of pregnancy were considered respectively. In particular, 285 d (the

Table 1. Basic statistics of temperature-humidity index (THI) of the 2 parts of great-granddam (F_0) pregnancy during the period from 1984 to 2014

Variable[1]	No. records	Average	SD	Minimum	Maximum
COMPLETE data set					
THI_max_1st	22,178	69.29	3.58	47.50	81.01
THI_min_1st	22,178	38.40	6.59	13.46	67.77
THI_max_2nd	22,178	71.22	3.40	59.79	82.69
THI_min_2nd	22,178	42.47	6.68	24.59	60.55
FIRST data set					
THI_max_1st	45,731	71.68	3.90	47.50	82.46
THI_min_1st	45,731	42.83	7.09	13.46	71.76
LAST data set					
THI_max_2nd	47,693	72.47	4.09	47.50	82.70
THI_min_2nd	47,693	44.32	1.09	18.82	71.75

[1]COMPLETE = data set with meteorological data for all the 9 mo of pregnancy. FIRST = data set with meteorological data only for the first 5 mo of pregnancy. LAST = dataset with meteorological data only for the last 4 mo of pregnancy. THI_MAX_1st = maximum THI registered in the first 5 mo of pregnancy. THI_MIN_1st = minimum THI registered in the first 5 mo of pregnancy. THI_MAX_2nd = maximum THI registered in the last 4 mo of pregnancy. THI_MIN_2nd = minimum THI registered in the last 4 mo of pregnancy.

average gestation length of the Italian Simmental) was subtracted from the birth date to estimate the conception date and the pregnancy months. Due to lack of data for some meteorological stations in the considered periods, 3 data set were created: (1) **FIRST**, with all the temperatures for the first 5 mo of pregnancy (47,531 records); (2) **LAST**, with data of the last 4 mo of pregnancy (47,693 records); (3) **COMPLETE**, with data for both parts (22,178 records).

The THI index was then calculated as follows (Kelly and Bond, 1971):

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

where AT is the daily temperature expressed in degrees Celsius, and RH is the relative humidity expressed as percentage. In particular, maximum THI (**THI_max**) was calculated using maximum AT and minimum RH, whereas minimum THI (**THI_min**) was calculated using minimum AT and maximum RH, respectively (Vitali et al., 2009). Basic statistics of the THI_max and THI_min in the 3 data sets data across the period from 1984 to 2014 are reported in Table 1. Monthly average values of THI_max and THI_min are reported in Figure 1.

Statistical Analysis

Production Data. The effect of the birth month of the dam, representing the period of the year during which the granddam pregnancy occurred, on the production performances of the cows was investigated by analyzing data with the following linear model:

$$y = \mathrm{BMF}_3 + \mathrm{BMF}_2 + \mathrm{bBDF}_2 + \mathrm{bEBVF}_2, \quad [1a]$$

where y is the EBV for the considered trait of F_3 cows; BMF_3 is the fixed effect of the birth month of the cow; BMF_2 is the fixed effect of the birth month of the dam; $bBDF_2$ is the fixed covariable of the exact birth date (dd/mm/yyyy) of the dam; and $bEBVF_2$ is the fixed covariable of the EBV of the dam. The $bBDF_2$ covariable was included in the model to account for the genetic trend, whereas $bEBVF_2$ was included to avoid a confounding between birth month and genetic value of the dam (Weller et al., 2021).

The effect of the birth month of the maternal granddam, which represents the period of the year during

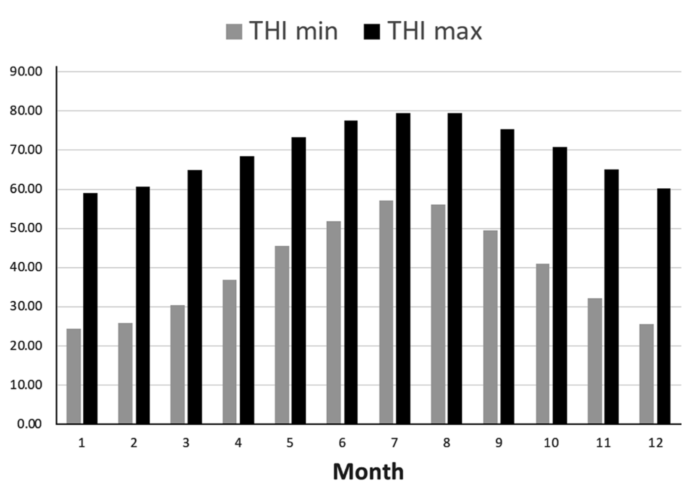

Figure 1. Monthly average maximum (max) and minimum (min) temperature-humidity index (THI) values in the considered period, 1984–2014.

which the great-granddam pregnancy occurred, was evaluated using the following model:

$$y = BMF_3 + BMF_1 + bBDF_1 + bEBVF_1, \quad [1b]$$

where BMF_1 is the fixed effect of the birth month of the granddam; $bBDF_1$ is the fixed covariable of the exact birth date (dd/mm/yyyy) of the granddam; and $bEBVF_1$ is the fixed covariable of the EBV of the granddam.

The 2 models were solved using SAS 9.4 software (SAS Institute Inc.).

According to the general hypothesis of the transgenerational model and considering that response variables were EBV (i.e., phenotypes corrected for pedigree relationships), the inclusion of a random effect of the interaction granddam × calving month (model [1a]) or, especially, of great-granddam × calving month (model [1b]) would allow estimation of a variance component that could represent a quantification of the epigenetic effect. Thus, models [1a] and [1b] were run a second time, also including the random effect of the interaction granddam × calving month or of the great-granddam × calving month effects, respectively. The 2 effects were assumed to be distributed as $N\left(0, \mathbf{I}\sigma^2_{GD}\right)$ and $N\left(0, \mathbf{I}\sigma^2_{GGD}\right)$, where $\mathbf{I}$ is an identity matrix whereas σ^2_{GD} and σ^2_{GGD} are the variance components associated with the granddam and the great-granddam, respectively. The contribution of the granddam $\left(r^2_{GD}\right)$ and great-granddam $\left(r^2_{GGD}\right)$ effects to the variance of the trait were calculated as follows:

$$r^2_{GD} = \frac{\sigma^2_{GD}}{\sigma^2_{GD} + \sigma^2_e}$$

and

$$r^2_{GGD} = \frac{\sigma^2_{GGD}}{\sigma^2_{GGD} + \sigma^2_e},$$

where σ^2_e is the component associated with the random residual variance. This contribution represents the correlation between repeated measurements (different months) within granddam or great-granddam, respectively (Macciotta et al., 2021). Models were solved using the HPMIXED procedure of SAS.

THI Effects. The 3 data sets with climate data were analyzed to test the effect of THI (maximum and minimum) of the first and second parts of pregnancy of F_0 great-granddams on F_3 cows' EBV. According to main 4 percentiles of THI distribution, 4 classes of THI_max (<69, 69–73, 73–75, >75) and THI_min (<38, 38–44, 44–49, >49) were created, respectively.

The FIRST and LAST data sets were analyzed to test effect of THI with the following linear model:

$$y = BMF_3 + BMF_2 + THI + bBMF_1 + YRBF_1, \quad [2]$$

where THI is the fixed effect of the THI class of the first or second part of F_0 pregnancy; $YRBF_1$ is the fixed effect of the year of birth of the granddam; and other symbols have the same meaning as in model [1]. Model [2] was run 2 times, using either THI_max or THI_min.

When model [2] was used to analyze the COMPLETE data set, THI_max or THI_min values of both pregnancy periods were considered together. Because these 2 variables are highly correlated ($r > 0.75$), a principal component analysis was carried out to derive uncorrelated covariables to be included in the model. In particular, principal component analysis was run on the 4 variables (THI_max and THI_min of both the first 5 and the last 4 mo of pregnancy). Eigenvectors were analyzed to infer the relationships between original variables and extracted principal components (**PC**). Finally, PC scores were calculated and included in the linear model instead of using THI data, to avoid collinearity problems.

RESULTS

Production Data

The birth month of the dam significantly affected the EBV for MY, PY, and SCS of F_3 cows (Table 2). In the case of MY, least squares means of dam birth months were greater in the spring–summer period compared with fall and winter (Table 3). In particular, the largest value was observed in May and the lowest in January (Tukey-adjusted P-value of the difference <0.0001), respectively. Therefore, pregnancies of F_1 granddams carried out mostly during summer and fall negatively affected F_3 performances compared with those developed in winter and spring, respectively. The effect of dam birth month showed a similar pattern also for PY

Table 2. Bonferroni-corrected P-values for the dam and maternal granddam effects included in models [1a] and [1b], respectively; correction was computed considering the 4 analyzed traits

Trait	Dam	Maternal granddam
Milk yield	<0.0001	<0.0001
Fat yield	NS	<0.0001
Protein yield	<0.0001	<0.0001
SCS	<0.05	<0.0001

Table 3. Least squares means (±SE) of the different dam birth months EBV of milk, fat, and protein yields, and of SCS, of F_3 cows estimated with model [1a][1]

Dam birth month	n	305-d yield (kg) Milk	Fat	Protein	SCS
January	12,060	1994.35 ± 2.94	58.99 ± 0.11	59.93 ± 0.09	144.51 ± 0.07
February	10,610	2004.92 ± 3.14	59.17 ± 0.12	60.22 ± 0.09	144.64 ± 0.07
March	10,338	2000.07 ± 3.14	59.32 ± 0.12	60.19 ± 0.09	144.71 ± 0.08
April	7,974	2013.68 ± 3.62	59.21 ± 0.13	60.36 ± 0.11	144.54 ± 0.09
May	7,482	2019.53 ± 3.74	59.43 ± 0.14	60.67 ± 0.11	144.50 ± 0.07
June	8,851	2015.65 ± 3.44	59.56 ± 0.13	60.55 ± 0.10	144.61 ± 0.08
July	10,804	2012.54 ± 3.12	59.44 ± 0.12	60.47 ± 0.09	144.84 ± 0.07
August	10,680	2013.22 ± 3.16	59.49 ± 0.12	60.56 ± 0.09	144.59 ± 0.07
September	11,748	2011.30 ± 2.99	59.40 ± 0.11	60.41 ± 0.09	144.69 ± 0.07
October	12,888	2001.04 ± 2.85	59.30 ± 0.11	60.25 ± 0.09	144.66 ± 0.07
November	12,545	2006.95 ± 2.89	59.34 ± 0.11	60.35 ± 0.09	144.80 ± 0.07
December	12,457	2003.37 ± 2.89	59.33 ± 0.11	60.31 ± 0.09	144.83 ± 0.07

[1]n = number of records. Due to the high number of levels (months) for each effect, mean comparisons results were not reported.

(Table 3), whereas SCS showed a less-defined trend, with greatest values for cows whose mother were born in July and lowest for those with mothers born in January and May.

Similar results were obtained for the granddam birth month. This factor significantly affected ($P < 0.001$; Table 2) the EBV for MY, FY, PY, and SCS. The largest EBV for MY were obtained for cows whose granddams were born in May and June, and the lowest were for F_1 born in January (difference $P < 0.0001$), respectively. A different pattern was observed for FY, where December showed the largest values. Protein yield exhibited the same behavior as MY, whereas SCS mimicked FY (Table 4).

The contributions of the random granddam × calving month effect to the F_3 EBV variance was below 10% for all the considered traits (Table 5). The largest values were observed for PY and the lowest for SCS. The same trend across traits was observed for the great-granddam × calving month contribution, but values were markedly larger, with values for MY and PY of about 23 to 24% (Table 5).

THI Effects

The class of THI_max during the first 5 mo of pregnancy affected all 4 considered dairy traits (Table 6). For MY, the third THI class (between 72 and 75) showed the lowest least squares mean, which was statistically different from the second and first classes. The lowest value for FY was obtained in the fourth class (THI_max > 75), whereas for PY and SCS it was found in both of the 2 highest classes. The THI_max class during the second part of pregnancy significantly affected milk and protein, with the lowest value for the third class. No effect was observed for FY and SCS.

Table 4. Least squares means (±SE) of the different granddam birth months for EBV of milk, fat, and protein yields, and of SCS, of F_3 cows estimated with model [1b][1]

Granddam birth month	n	305-d yield (kg) Milk	Fat	Protein	SCS
January	12,202	1988.48 ± 3.64	58.67 ± 0.13	59.67 ± 0.11	145.60 ± 0.09
February	10,486	1995.98 ± 3.93	59.00 ± 0.14	59.96 ± 0.12	145.95 ± 0.10
March	10,247	1990.49 ± 3.97	58.80 ± 0.15	59.88 ± 0.12	145.99 ± 0.09
April	8,106	2008.15 ± 4.47	59.29 ± 0.16	60.30 ± 0.11	145.69 ± 0.12
May	7,565	2020.42 ± 4.62	59.40 ± 0.17	60.71 ± 0.14	145.77 ± 0.09
June	8,737	2020.52 ± 4.30	59.52 ± 0.16	60.77 ± 0.13	146.21 ± 0.11
July	10,692	2015.11 ± 3.89	59.50 ± 0.14	60.56 ± 0.12	146.14 ± 0.09
August	10,227	2013.00 ± 3.97	59.34 ± 0.15	60.55 ± 0.12	145.80 ± 0.10
September	11,513	2007.33 ± 3.75	59.40 ± 0.14	60.38 ± 0.11	145.95 ± 0.10
October	13,319	2008.77 ± 3.49	59.58 ± 0.13	60.39 ± 0.10	146.17 ± 0.09
November	12,885	2009.69 ± 3.54	59.49 ± 0.13	60.42 ± 0.11	146.22 ± 0.09
December	12,458	2011.09 ± 3.61	59.61 ± 0.13	60.51 ± 0.11	146.23 ± 0.09

[1]n = number of records. Due to the high number of levels (months) for each effect, mean comparisons results were not reported.

Table 5. Variance components estimated on production data

Parameter[1]	Milk yield	Fat yield	Protein yield	SCS
Model [1a]				
σ^2_{GD}	8,313.32	7.63	7.72	1.01
σ^2_e	95,716.00	135.24	85.24	48.52
r^2_{GD} (%)	7.99	5.34	8.31	2.04
Model [1b]				
σ^2_{GGD}	37,720	41.21	34.93	12.24
σ^2_e	124,548	176.57	111.87	63.36
r^2_{GGD} (%)	23.24	18.92	23.79	16.19

[1]σ^2_{GD} = variance associated with the grandam; σ^2_e = variance associated with the random residual term; r^2_{GD} = contribution of the granddam to the total variance of the analyzed trait; σ^2_{GGD} = variance associated with the great-grandam; r^2_{GGD} = contribution of the great-granddam to the total variance of the analyzed trait.

The class of THI_min in the first 5 mo of pregnancy significantly affected EBV of F_3 cows for all the dairy traits (Table 7). The best values for MY and PY were observed for the fourth class (THI_min > 49), whereas fat yield and SCS showed an opposite pattern. In the second part of pregnancy, the THI_min class affected milk and protein yields and SCS. Also, in this case, milk and protein yields exhibited the best values in the fourth class, whereas SCS showed an opposite pattern.

Results obtained for the THI class (minimum and maximum) in the FIRST and LAST data sets were confirmed by fitting model [2] to the COMPLETE data set (results not reported, for brevity). The eigenvectors of the correlation matrix among THI_min and THI_max values of the 2 different parts of F_0 pregnancy obtained by performing principal component analysis on the COMPLETE data set are reported in Table 8. Explaining the largest amount of the total variance, PC1 was found to be related to all original variables, whereas PC2 and PC3 were associated mainly with minimum and maximum THI, respectively (Table 8). Model [2] was then run for the COMPLETE data set using the individual scores of the 4 principal components instead of the THI class. Scores for PC2 and PC3 were statistically ($P < 0.0001$) associated with all traits. Their regression coefficients, as well as those of PC1 and PC4, are reported in Table 9. Coefficients of PC2 (i.e., the one related to THI_min) exhibited positive values for MY and PY, whereas values were negative for FY and SCS. Coefficients for PC3 showed exactly the opposite pattern.

DISCUSSION

The results of the present study provide interesting insights on the transgenerational effects of climate stress during pregnancy on offspring performances in the Italian Simental cattle breed. Birth months of female parental generations F_2 and F_1 were found to affect F_3 cow EBV for production dairy traits. The best birth months for MY and PY, the 2 traits that showed the most defined effect in all the analyses, were May and June, and the worst were January and March, respectively. Because birth months are an indicator of the period during which the gestation occurs, F_0 and F_1 pregnancies developed in winter and spring had a positive effect on F_3 performances, whereas those developed in summer and autumn had a negative effect, respectively. Thus, a negative effect of high temperatures during pregnancy on the offspring performances has been detected. These findings were supported by the analysis carried out using the THI class (either minimum or maximum) during F_0 pregnancy as an explana-

Table 6. Least squares means (±SE) of the effects of the maximum temperature-humidity index estimated with model [2] in the first 5 and in the second 4 mo of great-granddam pregnancy, respectively

Class	n[1]	Milk yield	Fat yield	Protein yield	SCS
First 5 mo of pregnancy					
		$F = 10.21$ $P < 0.0001$	$F = 7.27$ $P < 0.0001$	$F = 11.71$ $P < 0.0001$	$F = 5.12$ $P = 0.0015$
<69	12,924	$2{,}066.60^{A} \pm 5.17$	$61.50^{A} \pm 0.19$	$62.55^{A} \pm 0.16$	$149.45^{A} \pm 0.11$
69–73	12,634	$2{,}072.84^{A} \pm 5.21$	$61.60^{A} \pm 0.19$	$62.63^{A} \pm 0.16$	$149.33^{AB} \pm 0.11$
73–75	11,446	$2{,}044.79^{B} \pm 5.38$	$60.98^{AB} \pm 0.20$	$61.86^{B} \pm 0.16$	$149.09^{AB} \pm 0.11$
>75	10,527	$2{,}051.92^{AB} \pm 5.45$	$60.76^{B} \pm 0.20$	$61.86^{B} \pm 0.17$	$149.02^{B} \pm 0.11$
Last 4 mo of pregnancy					
		$F = 5.55$ $P = 0.0008$	$F = 1.20$ $P = 0.308$	$F = 5.59$ $P = 0.0008$	$F = 1.46$ $P = 0.22$
<69	10,926	$2{,}059.97^{A} \pm 12.20$	60.80 ± 0.39	$62.36^{A} \pm 0.35$	149.49 ± 0.32
69–73	11,845	$2{,}059.80^{A} \pm 12.22$	61.00 ± 0.39	$62.37^{A} \pm 0.35$	149.61 ± 0.32
73–75	9,095	$2{,}037.85^{B} \pm 12.34$	60.57 ± 0.41	$61.70^{B} \pm 0.35$	149.66 ± 0.32
>75	15,827	$2{,}057.20^{A} \pm 11.73$	60.86 ± 0.38	$62.31^{A} \pm 0.35$	149.43 ± 0.31

[A,B]Means along the columns with different superscripts differ ($P < 0.001$).

[1]n = number of records.

Table 7. Least squares means of the effects of the minimum temperature-humidity index estimated with model [2] in the first 5 and in the second 4 mo of great-granddam pregnancy, respectively

Class	n[1]	Milk yield	Fat yield	Protein yield	SCS
First 5 mo of pregnancy					
		$F = 21.28$	$F = 7.36$	$F = 14.24$	$F = 36.99$
		$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.00001$
<38	12,316	2,055.95^{A} ± 5.27	61.78^{A} ± 0.19	62.27^{A} ± 0.16	149.58^{A} ± 0.11
38–44	10,895	2,058.86^{A} ± 5.44	61.25^{AB} ± 0.20	62.32^{A} ± 0.16	149.61^{A} ± 0.12
44–40	14,186	2,042.09^{A} ± 5.02	61.03^{B} ± 0.18	61.71^{B} ± 0.15	149.30^{A} ± 0.11
>49	10,134	2,088.36^{B} ± 5.56	60.84^{B} ± 0.20	62.86^{B} ± 0.17	148.38^{B} ± 0.12
Last 4 mo of pregnancy					
		$F = 10.14$	$F = 0.97$	$F = 8.57$	$F = 18.79$
		$P < 0.0001$	$P = 0.4063$	$P < 0.0001$	$P < 0.0001$
<38	10,389	2,052.84^{A} ± 12.03	60.99 ± 0.39	62.18^{A} ± 0.33	149.69^{A} ± 0.32
38–44	10,463	2,047.45^{A} ± 12.16	60.86 ± 0.39	62.10^{A} ± 0.32	149.73^{A} ± 0.32
44–40	12,436	2,042.13^{A} ± 12.06	60.88 ± 0.39	61.81^{A} ± 0.31	149.85^{A} ± 0.31
>49	14,405	2,070.42^{B} ± 12.15	60.64 ± 0.36	62.64^{B} ± 0.31	149.05^{B} ± 0.31

[A,B]Means along the columns with different superscripts differ (Tukey adjusted $P < 0.01$).

[1]n = number of records.

tory variable of F_3 EBV. The highest maximum THI classes showed the lowest least squares means for many of the considered traits. Moreover, a positive effect of the highest class of the minimum THI was observed. Both FY and SCS were affected by climate variables occurring in the first 5 mo of lactation only. Although the effect appeared not to be completely linear for MY and PY, the behavior of FY and SCS could be related to their intrinsic variability. Looking at F- and P-values (Tables 6 and 7), the first 5 mo of pregnancy appear to be more affected by the maximum and minimum THI class than the last 4 mo. All of this evidence suggests the hypothesis of a transgenerational inheritance, probably through epigenetic marks, of HS effects through the female lineage.

Previous reports of transgenerational studies on the effect of HS in dairy cattle substantially agree with the findings of the present work. Weller et al. (2021) found higher PTA for fat and protein yields for F_3 cows whose dams and granddams were born in the period from March to June, and lower for births between August and December. Those authors ascribed their results to the higher exposure to heat stress conditions for F_0 and F_1 cows that had their last 6 mo of pregnancy in summer to autumn. A meta-analysis of 10 years of studies carried out on U.S. Holsteins in Florida estimated a reduction of milk yield (−1.3 kg/d) in the first lactation of granddaughters of heat-stressed cows during the last 46 d of pregnancy compared with the granddaughters of non-heat-stressed cows (Laporta et al., 2020). The concordance of results between these studies and those of the present work is of interest also considering the different climates of the areas where the studies were carried out. The climate conditions of Israel and Florida are markedly different (hotter) than those of Northeastern Italy, where nearly 90% of the considered sample of cows were farmed. Because the trait considered is expressed as PTA, EBV, or measured phenotype, a comparison among studies on the magnitude of the effect is quite difficult. Moreover, the use of EBV as a response variable is undoubtedly challenging due to the adjustments for environmental factors that are made for their estimation. However, the use of direct phenotypes would lead to more complex models. In any case, possible confounding effects have been considered in models [1a] and [1b] by including the birth month of the F_3 cow and the date of birth of F_1 or F_2, respectively. Weller et al. (2021) reported

Table 8. Eigenvectors and eigenvalues of the correlation matrix (PC = principal component) among THI_min and THI_max values of the 2 different parts of great-granddam pregnancy in the complete data set

Item[1]	PC1	PC2	PC3	PC4
THI_max_1st	−0.513799	0.176500	0.760750	0.355130
THI_min_1st	−0.474466	0.740191	−0.333203	−0.340551
THI_max_2nd	0.513257	0.306885	0.548332	−0.584569
THI_min_2nd	0.497453	0.571652	−0.097810	0.645125
Eigenvalues %	86	9	3	2

[1]THI_max_1st = maximum temperature humidity index (THI) registered in the first 5 mo of pregnancy; THI_min_1st = minimum THI registered in the first 5 mo of pregnancy; THI_max_2nd = maximum THI registered in the last 4 mo of pregnancy; THI_min_2nd = minimum THI registered in the last 4 mo of pregnancy.

Table 9. Solutions of the covariables of the scores of the principal components (PC) of THI_min and THI_max values of the 2 different parts of great-granddam pregnancy in the complete data set

Item	Milk yield	Fat yield	Protein yield	SCS
PC1	−2.02 ± 1.61	−0.05 ± 0.06	−0.07 ± 0.05	−0.02 ± 0.03
PC2	48.20* ± 5.51	−0.98* ± 0.20	1.20* ± 0.17	−1.07 ± 0.12*
PC3	−50.76* ± 11.24	0.64 ± 0.43	−1.18* ± 0.35	1.71 ± 0.25*
PC4	7.54 ± 16.42	1.02 ± 0.61	0.67 ± 0.50	−0.64 ± 0.36

[1]THI_min = minimum THI registered during the pregnancy; THI_max = maximum THI registered during the pregnancy.

*$P < 0.0001$.

Bonferroni-corrected P-values of the effects of dam and maternal granddam birth months. Type I error probabilities for the dam and the granddam birth month effects estimated in the present study were of the same order as, or even smaller than, those reported by Weller et al. (2021). Kipp et al. (2021a) reported an effect of THI during the last 2 mo of pregnancy on milk test day, reproduction, and survival performance in offspring generations of cattle farmed in the climatic conditions of middle Europe. Results of the present study also highlight that transgenerational effects of temperature and humidity on production and reproduction traits could be detected in temperate climates.

Most of the studies on the effect of HS during pregnancy on next-generation performance have focused on the last period of gestation. However, both conception and gestation are crucial stages for the development of the offspring genotype at adulthood (Ouellet et al., 2021). A role of stressor factors during conception in dairy cattle was reported by González-Recio et al. (2012), who found that females conceived by lactating cows had lower milk yield and had a shorter lifespan than those conceived by nonlactating dams. Pinedo and De Vries (2017) investigated the effects of month of conception on future production and fertility of progeny of cows farmed in Florida. Their study reported larger milk yields, greater odds of survival to the second calving (about 20%), and better reproduction performances for cows that were conceived in winter compared with those conceived in summer. Transgenerational effects of the first part of the maternal great-granddam pregnancy were also found in the present study. Looking at the type I error probabilities of both maximum and minimum THI class, the effect of the climatic indicator appears to be more significant in the first part of F_0 pregnancy (Tables 6 and 7).

In the current scenario of climate change, with particular reference to global warming, a proper accounting of effects of thermal stress in genetic models applied to livestock species should be evaluated. Bernabucci et al. (2014) reported that the inclusion of THI in the genetic evaluation of Italian Holstein bulls resulted in relevant changes of their EBV rank for milk yield. A genetic evaluation procedure for predicting a genetic merit for heat stress tolerance has been implemented in Australia (Pryce et al., 2022). As far as transgenerational effects of HS are concerned, some studies have been carried out to identify genotype by environment interactions due to THI during pregnancy in the genetic parameters estimated on offspring data. Kipp et al. (2021b) used random regression models to estimate the pattern of heritability for milk production traits across different values of THI in late dam gestation. They found a sensible decrease of h^2 for milk yield for increasing THI values, due to a reduction of additive genetic variance, for THI >58. Moreover, they also found an effect on low-heritability traits with a reranking of sire EBV according to prenatal intrauterine environment. Also, those authors explained the lagged HS effects observed on variance components and EBV by hypothesizing an epigenetic fetal reprogramming. A similar result was reported by Lee et al. (2019), who analyzed heat tolerance in South Korea Holstein cattle. Those authors found that heritability decreased slightly as THI increased, and it started to increase at THI = 79; they also stated that bull EBV rank changed according to the THI. The latter was also observed by Negri and Cobuci (2021), who analyzed the inclusion of heat stress level in the fixed regression modeling of fat and protein yields in Holstein cattle. When heat stress indicators were included in the model, these authors found an important reranking of sires and increased reliabilities of EBV. The higher reliabilities could be due to a better fit of a model with heat stress levels to the analyzed data. Genotype by environment effects have also been reported on weaning and yearling weights in American Angus by Bradford et al. (2016), who concluded that the maternal component of growth can be selected with similar selection response despite environmental heat stress.

Epigenetics inheritance is currently not accounted for in models for evaluating the genetic merit of individuals. A main issue to be addressed is the quantification of the amount of phenotypic variation that is explained by epigenetic mechanisms (Varona et al., 2015). During each generation, epigenetic modifications occur, but they are also restructured, affecting covariance between relatives. Tal et al. (2010) proposed a method for quantifying epigenetic transmissibility, also considering the stability of epigenetic inheritance. This approach has been implemented into a Bayesian framework by Varona et al. (2015), who estimated a transgenerational epigenetic heritability of 0.04 for birth weight of Pirenaica cattle using a Bayesian model. Paiva et al. (2018) estimated an epigenetic heritability of 0.10 for body weight in meat quails. In the present study, the inclusion of the random effect of the interaction between great-granddam and month of calving allowed estimation of a variance component that accounted for 0.16 to 0.24 of the total variances in the 4 considered traits. Because EBV are already corrected for systematic and parentage effects, this component could be proposed as a rough estimator of the contribution of epigenetic inheritance to the variance of the milk production trait here considered. Further efforts are also needed, to achieve a reliable estimation of transgenerational epigenetic contribution to the phenotypic variance as well as to determine its possible consideration in the prediction of genetic merit of selection candidates.

CONCLUSIONS

Results of this study suggest a transgenerational epigenetic inheritance in Italian Simmental cattle due to environmental stressors. Great-granddam pregnancies developed in winter and spring positively affect EBV for milk and protein yields of their great-granddaughters, whereas summer and autumn pregnancies have a negative effect. These figures were also confirmed by the detected effects of THI (minimum and maximum) in the different stages of F_0 pregnancy on F_3 performances. Heat stress during pregnancy also affects further generations' performances for milk production traits in temperate climates. Results of the present study were obtained using EBV as response variables and should be validated also on raw phenotypes.

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