Effect of heat stress on non-return rate of Italian Holstein cows

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Key Words:	dairy cow, THI breaking point, heat stress, reproduction trait

Interpretative summary

***Short communication*: Effect of heat stress on non-return rate of Italian Holstein cows.** By *Biffani et al., page 000-000*. Heat stress has a negative impact of reproduction efficiency. Fertility of high yielding dairy cows is strongly compromised. Heat tolerance is considered one of the most important adaptive aspects in dairy cattle also in temperate zones. The definitions of temperature-humidity index thresholds for reproductive trait and the estimation of genetic values will be useful in helping to select heat-tolerant sires.

RUNNING HEAD: EFFECT OF HEAT STRESS ON REPRODUCTION

Short communication: Effect of heat stress on non-return rate of Italian Holstein cows

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ABSTRACT

Dataset comprised 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. Non-return rate at 56 d after first insemination (NR56) was considered. A linear model was used to estimate the effect of temperature-humidity index (THI) on reproduction across parities. Then, least square means were used to detect the THI breakpoints using a two-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. NR56, both for first and second parity cows, was significantly (unfavorable) affected by THI from 4 d prior to 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, THI breaking point, heat stress, reproduction trait, heritability

Declining fertility of dairy cows represents a major concern among producers due to the impact 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 bioclimatic index (Hahn et al., 2003). In a comprehensive review by Hansen (2007), the deleterious effect of heat stress on oocyte development and maturation, on early embryonic death and on fetal or placental development were elucidated. This author highlighted two 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) have 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 non-return rate at 56 d after first insemination (**NR56**), in order 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 NR56 has been used in the present study because it is the trait officially used in the national genetic evaluation for fertility in the Italian Holstein breed (Biffani and Canavesi, 2007), and is the most widely used trait by Interbull for genetic evaluation of female fertility (Jorjani, 2006).

Data

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. Non-return 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 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:

THI = (1.8 x AT + 32) – (0.55 – 0.55 x RH) x [(1.8 x AT + 32) – 58], where AT is the ambient temperature (˚C), and RH the relative humidity as a fraction of the unit. With respect to the original equation, this formula includes terms (1.8 x AT + 32) that account for conversion of temperature data from Celsius degree (˚C) to Fahrenheit degrees (˚F). Maximum AT and minimum RH were utilized 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 be often 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: 20–36, 31–50 and 42–65 months 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. Weather dataset 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 dataset consisted of 119195 NR56 records for 76943 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.

Statistical Model and Variance Component Estimation

The effect of THI (heat stress) was analyzed by fitting the following linear model (MODEL 1) using the Mixed procedure in SAS version 9.1 (SAS, 2002):

$$Y_{ijklmno} = herd(yi)_i + mc(yi)_j + cdim_k + thir_l + age_m + ord_n + thir_l \text{ x } ord_n + cdim_k \text{ x } ord_n + b \text{ x } ecm + e_{ijklmno}$$

where $y_{ijklmno}$ is NR56 calculated for each cow; $herd(yi)_i$ is the fixed effect of herd nested within year of insemination *i*; $mc(yi)_j$ is the fixed effect of month of calving nested within year of insemination *j*; $cdim_k$ is the fixed effect of DIM class *k*; $thir_l$ is the fixed effect of THI class *l*; age_m is the fixed effect age class *m*; ord_n is the fixed effect of parity *n;* $thir_l$ x ord_n is the fixed effect of the THI class *l* by parity class *n*; $cdim_k$ x ord_n is the fixed effect of the DIM class *k* by parity class *n* class; b is a fixed regression on daily energy-corrected milk (ecm), computed as suggested by Clay and McDaniel (2001); $e_{ijklmno}$ is the random residual effect. A minimum of 24 records per herd-year of insemination was required. The two-phase linear regression procedure was applied to least squares means from MODEL 1 to detect the exact THI at which NR56 was affected. Package "strucchange" (Zeileis et al., 2002), implemented in R software (http://CRAN.R-project.org), was used for data analysis. The effect of THI on NR56 in first, second and third parity cows, was also investigated 16 d before and 16 d after the insemination date, respectively. The idea of using a 2-phase linear regression, as proposed by Bernabucci et al. (2014), is based on the observation that the relationship between an environmental parameter (e.g., THI) and the response to that parameter (e.g., non-return rate) might not be correctly detected by a simple graphical inspection or by a standard linear regression. If, for example, THI and non-return rate are linearly related, a simple linear regression can be used to model their relationship. However, if we observe that above a particular THI value, the non-return rate increases faster than before (i.e. the slope before and after the THI value is not the same), a simple linear regression may not provide an adequate and accurate pattern description. Moreover it will not be able to identify the THI value at which the slope of the linear function changes (i.e., the breakpoint). In this kind of situation, test the following model can be used: $y_i = x_i^T \beta + u_i \ (i = 1, \ldots, n)$, where at time i, y_i is the observation of the dependent variable (NR56), x_i is a *k x 1* vector of n regressors (THI values) and β_i is the *k x 1* vector of regression coefficients, which may vary over time and u_i are $iid(0,\sigma^2)$. The hypothesis being tested is that the regression coefficients remain constant (*reduced model)*: $H0: \beta i = \beta 0 \ (i = 1, \ldots, n)$, compared to the alternative hypothesis that at least one coefficient varies over time (*complete model)*. If the increase in sum of square errors, going from the complete to the reduced model is significantly large, then the null hypothesis can be rejected concluding that the complete model works well and a significant "breakpoint" does exist, i.e. a significant change in NR56 due to THI has been detected.

Effect of THI on Non-return rate at 56 d

Non-return rate at 56 d after first insemination of first and second parity cows was significantly affected by THI, by 4 d before and 5 d after the insemination (Table 2). No breakpoint was detected for third parity cows. Several authors have reported a general decrease in fertility performance with increase of parity number, hence suggesting that 3rd parity cows have a lower non-return rate than 1st or 2nd parity cows (Ravagnolo and Misztal, 2002b). The absence of THI breakpoint in 3rd parity cows is not easy to explain. Possible hypotheses are that the sensitivity to heat stress across parities is not the same and that THI thresholds may also be affected by several environmental factors (Bernabucci et al., 2014).

THI-breaking points ranged from 64 to 78, and 72 to 78 for first and second parity cows, respectively. On average THI-breaking point was smaller in first than second parity cows (73 *vs*. 75). Both first and second parity cows showed a drop in NR56 (Figure 2), namely the slope ('b' value in Table 2) of the two-phase linear regression related above. The most critical period seems to be within 4 d before the insemination. However the unfavorable THI effect is still detected after the day of insemination. Even if first parity cows are affected at a lower THI than second parity cows, the latter suffer from a greater NR56 drop, especially during the 3 days prior to insemination (-0.087, -0.080, -0.081 *vs.* -0.047, -0.051, -0.042 for second and first parity cows, respectively). The observed and unfavorable effect of THI on reproduction confirms previous findings (Chebel et al., 2004; Al-Katanani et al., 1999; Ravagnolo and Misztal, 2002). Those authors related that from 20 to 27 % drop in conception rates or a decrease in 90-d non-return rate to the first service in lactating dairy cows occur during summer season, *i.e.* when THI reaches its maximum value. Moreover, heat stress has also been associated with altered hormones secretion during the estrus cycle (Ronchi et al., 2001), impaired embryo development and increased embryo mortality (Wolfenson et al., 2000; Hansen, 2007) in cattle. More recently, Schüller et al (2014), showed how the conception rate of lactating dairy cows was negatively affected by heat stress both before and after the day of breeding. THI thresholds of the present study, were slightly different (higher) than those reported by Ravagnolo and Misztal (2002a,b) for non-return rate trait. Such discrepancy might be due to several reasons. These include different experimental conditions, different methodological approach to detect the actual THI-breaking point and different number of days (56 *vs.* 90) to declare the successful insemination. With regard to this last point, using 56 days may lead to rely on some false positive records.

Genetic Aspects

After the detection of the THI-breaking points a multiple trait threshold model was fitted to estimate and quantify the genetic relationship between heat stress and NR56 (MODEL 2). A dummy regression variable (**t**) was defined and included in the model to estimate the variation of NR56 due to heat stress. If the observed THI was below a particular THI threshold, a value of 0 was assigned to the variable t (*i.e.*, no heat stress), while if the observed THI was above such threshold, variable t was the difference between the observed THI and that particular threshold. No THI breakpoint was observed in third parity cows, hence only first and second parities were included in the analyses using as THI breaking point 73 and 75, respectively. Those thresholds were obtained by averaging the THI values within 4 d before and 5 d after the day of insemination (Bernabucci et al., 2014). MODEL 2 was as follows:

$$Y_{ijklmno} = htd_{ij} + dim_k + age_{jm} + mi_l(yi)_{nm} + f(ecm)b_{jo} + a_{jp} + v_{jp}\,[f(THI)_i] + e_{ijklmnop}$$

where $y_{ijklmno}$ is NR56 after first insemination for cow *p* in age class m within parity *j* (1 or 2) and dim class *k* for herd test-day *i* within parity *j* and month of insemination *l* nested within year of insemination *m* within parity *j*; htd is the fixed effect of herd test-day within parity; mi(yi) is the fixed effect of month of insemination nested within year of insemination ; dim is the fixed effect of DIM class; age is the fixed effect of age class; a is the effect of the additive genetic merit of cow within parity; v is additive effect of heat tolerance of cow within parity; e_{ijklmn} is the residual Let $a' = [\, a'_{jo} \quad v_{jo}]$ be the vector of random additive genetic effects for parities j = 1 to 2. The (co)variance structure was :$Var\begin{vmatrix} a \\ e \end{vmatrix} = \begin{bmatrix} A \otimes G_0 & 0 \\ 0 & I \otimes R_0 \end{bmatrix}$ where **A** is the numerator relationship matrix; $\mathbf{G_0}$ is 4 x 4 matrices of (co)variances for additive effects; and $\mathbf{R_0}$ is a diagonal matrix of residual variances corresponding to each trait. Residual variances equal to 1 (Gianola and Foulley, 1983) was assumed. A minimum of 10 records per herd-year of insemination and 20 daughters per sire were required to estimate variance components. Variance component estimation was performed using the THRGIBBS1F90 software of Tsuruta and

Misztal (2006) that implements a Gibbs sampling algorithm and allows for different combinations of continuous and threshold traits.

Parameter values for NR56 are given in table 3. Additive genetic variances for NR56 increased from first to second parity both for general and heat stress effect. Heritability estimates for NR56 trait were 0.024 and 0.095 for first and second parity, respectively. Those values agree with previous findings (Jamrozik et al., 2005). Heritability for second parity cows seems to be a little bit overestimated. Even if Jamrozik et al. (2005) stressed the fact that heritability obtained using threshold models is usually higher than those obtained with linear models, there could be additional reasons. Non-return rate at 56 d considers successful those inseminations that are not followed by a subsequent breeding within 56 d, without validation through subsequent calving. In case of a second parity cows, this value might be “favorably” overestimated because it does not take into account the possible successive culling. Indeed, this hypothesis is supported by the average NR56 value for second parity cows, which was lower than first parity cows (Table 3).

Genetic correlation between NR56 after first insemination for first and second parity cows, as expected, was very strong ($r_g = 0.90$). Moreover, genetic correlations ($r_{g\text{-}THI}$) between general and heat stress effects with NR56 were -0.31 and -0.45, for first and second parity cows, respectively. These results confirm the negative (genetic) effect of heat stress on reproductive efficiency.

Ravagnolo and Misztal (2002b) used data from multiparous cows but they fitted a repeatability model. Indeed, they analyzed jointly the non-return rate of primiparous and multiparous cows including in the model the “parity” fixed effect. Their results showed that primiparous cows were more sensitive to THI increase. In the present study, the reverse was true. The effect of heat stress was stronger on multiparous than primiparous (higher negative genetic correlation). Those results are quite interesting and support the hypothesis by Aguilar et al. (2010) who related that if records of later parities are going to be used they should be treated as a separate trait. This will model the different behavior observed across parities, leading to more reliable results.

Recently, in two different and independent studies, Dikmen et al. (2015) and Biffani et al. (2015) identified several specific genetic markers responsible for genetic variations in thermoregulation during heat stress. Those findings, obtained using DNA data (i.e. SNPs) do confirm results of the present study coming from a "quantitative genetics" approach (field data and classical pedigree data), suggesting a genetic component of heat stress.

In conclusion, heat stress represents a significant component of low fertility in dairy cattle. Indeed, both first and second parity cows are unfavorably affected by hot environment before and after insemination day even if their sensitivity is not the same. Second parity cows start to be affected by heat stress at a threshold higher than first parity cows, but the effect on their NR56 is greater (i.e., the proportion of second parity cows who return to heat is larger). From a practical point of view, it is important for the farmer to know that a heat wave occurring after insemination may be as dangerous as a very hot period before insemination. Farmers usually try to avoid inseminating their cows during very hot periods, but knowing for how long a hot period might jeopardize the success of an insemination could help them in setting up the right management. So far, no genetic evaluation for heat stress resistance in dairy cattle are available worldwide; however, due to the increasing importance of animal welfare heat-tolerance trait should be included in the selection objective of dairy cattle populations.

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Table 1. Descriptive statistics for THI by month of calving

Month of Calving	Number of animals	Number of records
January	3015	5056
February	4784	7547
March	6867	9924
April	8809	13077
May	7946	11460
June	6111	8742
July	5931	8426
August	7278	11003
September	8328	13523
October	7641	13081
November	6676	11071
December	3557	6285

Table 2. Estimated weighted average THI from the breaking point analysis considering 16 d before and after the insemination for non-return rate at 56 d (NR56). No break points were detected for third parity dairy cows.

			Days relative to first insemination-day										
Trait	Parity		-4	-3	-2	-1	0	1	2	3	4	5	Weighted THI breaking points
NR56 %	1	THI[§]	75	67	69	64	77	77	77	72	78	78	73
		b[¥]	-0.062[b]	-0.047[c]	-0.051[c]	-0.042[c]	-0.08[a]	-0.080[a]	-0.074[a]	-0.049[b]	-0.067[b]	-0.057[b]	
		R^2	0.65	0.53	0.54	0.33	0.69	0.70	0.62	0.45	0.50	0.44	
	2	THI	72	78	77	75	74	72	74	74	76	78	75
		b	-0.066[b]	-0.087[c]	-0-080[a]	-0.081[a]	-0.080[a]	-0.072[b]	-0.070[a]	-0.066[b]	-0.068[b]	-0.069[c]	
		R^2	0.62	0.57	0.50	0.65	0.60	0.57	0.73	0.68	0.64	0.50	

[a]$P < 0.00001$; [b]$P < 0.0001$; [c]$P < 0.001$

[§]THI break-point

[¥]slope

Table 3. Estimates of variance components for 56-d non-return rate (NR56)

Parameters[1]	NR56, (% x 100)[2]		
	1st parity		2nd parity
σ^2_a	0.024		0.095
σ^2_{thia}	0.015		0.013
$^2_{a-thia}$	-0.006		-0.026
σ^2_e	1		1
r_{g-thi}	-0.35		-0.45
h^2_a	0.024		0.095
h^2_{thi}	0.0251		0.076
r_g		0.90	
n. cows		19153	
Animals in the pedigree		56271	
Mean , %	54		48
Standard deviation	49		49

[1] σ^2_a = additive genetic variance; σ^2_{thia} = additive genetic variance for temperature-humidity index (THI); σ^2_{a-thia} = additive genetic covariance between the general effect and the effect of THI; σ^2_e = residual variance; r_{gt-thi} = genetic correlation between the general effect and the effect of THI; h^2_a = general effect heritability; h^2_{thi} = heat stress heritability.; r_g = genetic correlation between NR56 in fisrt and second parity cows

Figure 1. Changes of monthly average values of maximum temperature-humidity index (THI) in north, center and south Italy.

Figure 2. Variation of 56-d non-return rate (NR56) estimated by using the "weighted temperature-humidity index breaking point" for first and second parity cows, considering four days before and five days after the insemination-day**.**

355 **Figure 1**

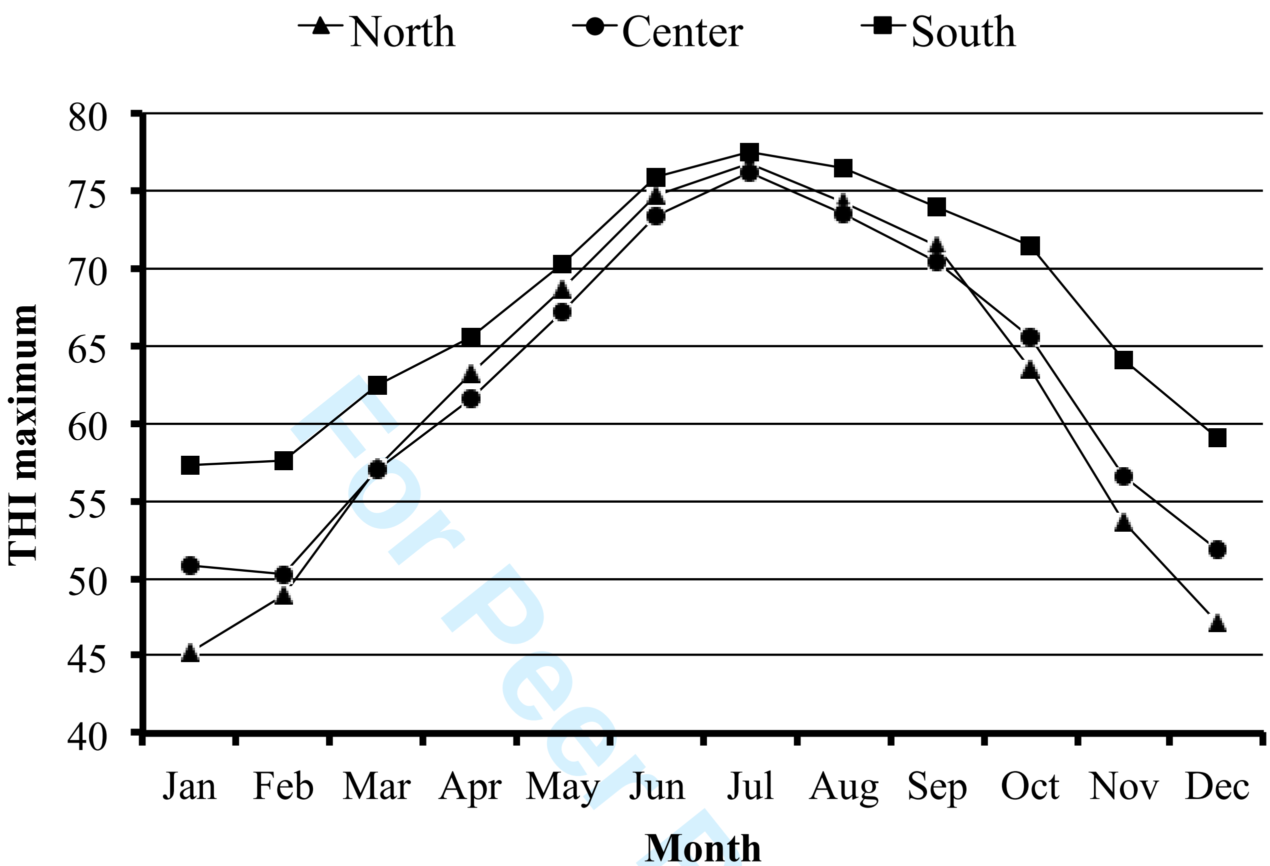

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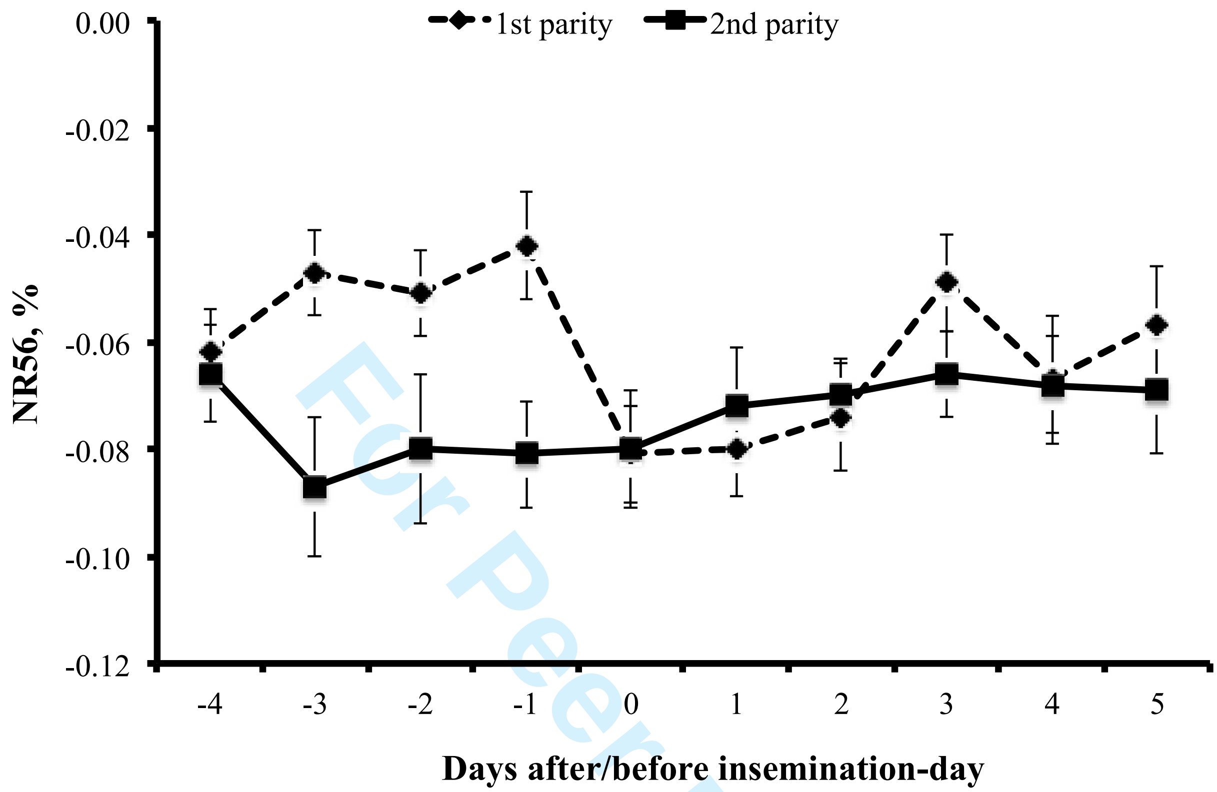
Figure 2.


1st parity
2nd parity
NR56, %
0.00
-0.02
-0.04
-0.06
-0.08
-0.10
-0.12
-4
-3
-2
-1
0
1
2
3
4
5
Days after/before insemination-day