Factors Affecting Seasonal Variation in 90-Day Nonreturn Rate To First Service in Lactating Holstein Cows in a Hot Climate

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ABSTRACT

The objective was to determine factors controlling seasonal variation in 90-d nonreturn rate to first service (90-d NRR) including effects of location, milk yield, and weather variables on specific days before and after breeding. Dairy Herd Improvement Association records on first services from 8124 Holstein cows in south Georgia (GA, n = 7 herds), north Florida (NF, n = 5), and south Florida (SF, n = 5) were used. The 90-d NRR was affected by location × month of breeding. The summer drop in 90-d NRR was of lower magnitude and duration in GA than in NF or SF and of lower magnitude and duration for NF than SF. When cows were grouped according to mature equivalent milk yield, there was a milk yield class × month of breeding interaction. As milk yield class increased, the summer depression in 90-d NRR was more pronounced. In a second series of analyses, effects of average air temperature at d –10, 0, and 10 relative to breeding were evaluated with sub-sets of cows in which average air temperature on the 10 d before the reference day were cool (<25°C). The 90-d NRR for cows having average temperatures >20°C on d –10 was less than 90 d NRR for cows with average temperatures ≤20°C on d –10 (60.1 vs. 36.5%). Similar results were found on d 0 (59.6 vs. 41.4%) and d 10 (56.9 vs. 41.1%). Thus, heat stress before and after breeding, and on the day of breeding, is associated with low 90-d NRR. (Key words: nonreturn rate, cattle, heat stress, fertility)

INTRODUCTION

Fertility in dairy cows is depressed during the summer months in warm areas of the world (11). This phenomenon is caused primarily by heat stress. Experimental application of heat stress to cows reduced pregnancy rate (22) and increased embryonic mortality (7, 13), whereas abatement of heat stress during the summer increased pregnancy rate (8, 16, 19, 20). The extent of the seasonal depression in fertility is influenced by environmental factors that define the magnitude of heat stress and internal factors of the cow that determine its ability to regulate body temperature. Environmental factors such as air temperature and humidity are determined in large part by location and by characteristics of animal housing. One of the features of the cow that determines thermoregulatory ability is milk yield, which affects metabolic rate and the hyperthermia experienced during heat stress (3). However, although fertility is greatly reduced during the summer for lactating cows compared with nonlactating heifers (1), it is not known if heat stress causes a more severe depression in fertility for cows producing higher milk yields.

The deleterious effect of heat stress on embryonic development depends on the day relative to ovulation at which cows are subjected to heat stress. Exposure of cows to high air temperature and humidity for 10 h beginning at the onset of estrus increased the incidence of retarded embryos recovered on d 7 of pregnancy (14). Thus, reproductive events in the preovulatory period are susceptible to disruption by heat stress in a way that leads to altered embryonic development. Thereafter, embryos appear to acquire some thermal resistance; exposure of superovulated lactating Holsteins to heat stress on d 1 after estrus decreased viability and development of embryos recovered at d 8, but heat stress had no effect if applied on d 3, 5, or 7 after insemination (7). Similar observations have been noted in embryos exposed to heat shock in culture (10). It is not known if heat stress before ovulation can compromise subsequent fertility. Such effects are possible because heat stress can alter follicular development (24), and the oocyte can be disrupted by exposure to elevated temperature (9). Research in sheep (6) indicates that heat stress 12 d before estrus reduces subsequent fertility.

The present study had two major series of objectives. The first objective was to utilize records compiled by DHIA to determine if the seasonal variation in 90-d
nonreturn rate to first service (90-d NRR) of lactating dairy cows in south Georgia and Florida is more pronounced in the southern part of the two-state region and to test whether this variation was affected by milk yield. A second objective was to use DHIA data to evaluate the association between heat stress at specific days relative to breeding with subsequent fertility.

MATERIALS AND METHODS

Data

The study utilized DHIA breeding records for 8124 Holstein cows in 17 herds from south Georgia [n = 7 herds located within 45 to 170 km of Tifton (31°28′ N 83°32′ W); 943 cows], north Florida [n = 5 herds located within 27 to 62 km of Gainesville (29°38′ N 82°20′ W); 4878 cows], and south Florida, [n = 5 herds located in Okeechobee County (27°16′ N 80°46′ W); 2303 cows] from January 1994 until May 1996. Records for each cow were for one lactation. Furthermore, the data were screened to include only those cows that had at least one service, were lactating, and had an interval to first service greater than 35 d and less than 150 d. The 90-d NRR was used to estimate pregnancy rate. Cows were considered pregnant to first service if they were not observed in estrus or rebred within 90 d from first breeding date.

Weather data were obtained for south Georgia (the meteorological station in Tifton) and north Florida (the agricultural weather station in Gainesville). Data included daily minimum and maximum temperature, minimum and maximum relative humidity, wind speed, and solar radiation.

Analysis of Location and Milk Yield Effects

The effects of location and mature equivalent (ME) milk yield on 90-d NRR were analyzed by least-squares analysis of variance by the general linear models procedure of SAS (17). Data were analyzed as one data set. In addition, subsets of data were analyzed to compare north Florida to south Florida and south Georgia to north Florida. For location effects on 90-d NRR, the model included main effects of location, herd(location), month of first service and interactions between main effects. Interval to first service was used as a covariate. Data were initially analyzed with all interactions in the model and then reanalyzed after removing nonsignificant interactions and higher-order (3 and 4 way) interactions that did not solve. In initial analyses, year was included in the model. Levels of significance for effects of interest were similar whether year was included or not. Data in one year (1996) were not distributed across all months and, as a result, least-squares means gave distorted results. Accordingly, year was not included in analyses reported in the paper.

In the second analysis, cows were grouped according to ME milk yield [1 < 4536 kg, n = 123 cows; 2 = 4536-9072 kg, n = 3833; 3 > 9072 kg, n = 4168], and data were analyzed by least squares analysis of variance as described above. Main effects in the model were location, herd(location), month of first service and ME milk yield, and interactions between main effects. Interval to first service was used as a covariate.

Regression analysis was carried out to determine if the relationship between ME milk yield and 90-d NRR varied with month of first service. Initial analysis indicated the R² for the model with only the linear effect of milk yield included as a continuous variable was similar to the R² for the model that also included quadratic and cubic effects of milk yield. Therefore, only the linear effect was included for subsequent analysis. Data were analyzed by least-squares analysis of variance with 90-d NRR as the dependent variable and with location, herd(location), month of first service, and location × month of first service as independent class effects and with ME milk yield as a continuous independent effect. Data were then reanalyzed with the same model, except with the term milk yield × month replacing milk yield. Heterogeneity of regression was determined by calculating whether there was a significant reduction in residual sums of squares caused by using milk yield × month of first service in the model instead of milk yield (23).

Analysis of the Relationship between Meteorological Data and 90-d NRR

Weather data were obtained for two locations (south Georgia and north Florida). Data included daily minimum and maximum temperature, minimum and maximum relative humidity, wind speed, and solar radiation. Average daily temperature (T<sub>av</sub>) was calculated as the average of the daily minimum and maximum dry bulb temperature. Canonical discriminant analysis was carried out by the discriminant analysis procedure of SAS (17) to derive linear combinations of the weather variables that had the highest possible multiple correlation with 90-d NRR. This procedure derives canonical variables, which are linear combinations of the quantitative variables (different combinations of weather variables in this data set) that summarize between class variation in much the same way that principal components summarize total variation. This procedure allowed determination of the combination of weather variables on the day of breeding that gave the best prediction of 90-d NRR.
The third analysis was performed to evaluate the relationship between weather variables at various times relative to first insemination on 90-d NRR to first service. The goal was to 1) confirm prior observations that heat stress on the day of insemination causes reduced fertility (14) and 2) to test whether heat stress during the period of follicular growth preceding ovulation and during the period of embryonic development is associated with reduced fertility. Canonical discriminant analysis indicated that Tav on the day of insemination was as good a predictor of subsequent 90-d NRR as more complex combinations of weather variables. Therefore, only Tav was related to 90-d NRR. Three days were chosen for analysis: d 0 (i.e., the day of insemination), d –10 (i.e., 10 d before insemination; to determine effects of heat stress during follicular growth preceding ovulation) and d 10 (i.e., 10 d after insemination; to determine effects of heat stress on the developing embryo). For each day examined, each cow was classified according to the Tav it was exposed to on that day and placed in one of six temperature categories (≤6°C, 7 to 10°C, 11 to 15°C, 16 to 20°C, 21 to 25°C, >25°C). Analysis of variance was then performed to determine effects of temperature category on 90-d NRR to first service. The model included main effects of the temperature class on the day of interest, location, and herd (location). Orthogonal contrasts were performed to separate temperature effects into individual degree-of-freedom comparisons.

One problem with interpreting such an analysis is that temperatures on a given day are highly correlated with temperatures on preceding and ensuing days. Thus, a relationship between high Tav on any day with fertility may not represent an effect of heat stress on that day but rather reflect effects of heat stress from other days. To reduce this problem, the data set was reduced before analysis as follows. For analysis of effects of Tav on d –10, only cows with Tav less than 25°C on d –9 to 0 were analyzed. Thus, effects of high daily air temperature on d –10 likely represent effects of heat stress on d –10 or before but not on days closer to ovulation because only cows exposed to cool temperatures on d –9 to 0 were used for the analysis. For analysis of effects at d 0, only cows with Tav less than 25°C on d –10 to –1 were analyzed. Effects of high daily air temperature on d 0 are thus not likely to represent effects of heat stress before estrus. For analysis of effects on d 10, the subset of cows analyzed were those cows exposed to Tav <25°C on d 0 to 9.

RESULTS

Effect of Location on Seasonal Variation in 90-d NRR

There was a location × month of first service interaction affecting 90-d NRR (Figure 1) (P<0.001). Interaction of location × month of first service occurred when comparing north Florida to south Florida only (P<0.05) and south Georgia to north Florida only (P<0.05). These interactions resulted because, although the 90-d NRR declined during warm months in all locations, the decline was of greater magnitude and occurred for a longer period in south Florida than in north Florida and for north Florida than in south Georgia. For example, the least-squares means for 90-d NRR in July was 18.9 ± 7.2, 7.0 ± 2.8, and 4.7 ± 3.7% for south Georgia, north Florida, and south Florida, respectively. The number of months where least squares means for 90-d NRR were ≤20% was two for south Georgia (June, July), five for north Florida (May to September), and seven for south Florida (April to October).

Effect of ME Milk Yield on Seasonal Variation in 90-d NRR

There was a milk yield × month of first service interaction (P=0.06) when cows were grouped according to ME milk yield (1 < 4536 kg, n = 123 cows; 2 = 4536 to 9072 kg, n = 3833; and 3 > 9072 kg, n = 4168; Figure 2). As milk yield class increased, the summer depression in 90-d NRR was of greater magnitude and lasted for more months. For example, the 90-d NRR in July was 44.9, 13.5, and 5.3% for classes 1, 2, and 3, respectively. The number of months where 90-d NRR was ≤20% was 0, 3, and 6 mo for classes 1, 2, and 3, respectively.

Figure 1. Seasonal variation in 90-d nonreturn rate to first service in south Georgia (●), north Florida (○), and south Florida (▲). Data are least-squares means ± SEM adjusted for interval to first service.
Variation of 90-d NRR Due to ME Milk Yield and Interval to First Service

Regression coefficients for the relationship between 90-d NRR and ME milk yield by month of first insemination are shown in Figure 3. An increase in milk yield was associated with a depression in 90-d NRR. The magnitude of the depression was affected by month (heterogeneity of regression; \( P < 0.005 \)). This effect was not significant if data for the month of April were eliminated from the analysis, although the magnitude of the change in 90-d NRR per change in ME milk yield tended to be greater in warm months.

Effect of Environmental Variables at Specific Days Relative to Insemination on 90-d NRR

To determine effects of heat stress at d 10 before insemination (d –10), we analyzed a subset of cows that experienced \( T_{av} < 25^\circ C \) from d –9 before insemination until insemination (d 0). As shown in Table 1, the 90-d NRR decreased as \( T_{av} \) on d –10 increased. Orthogonal contrasts indicated that 90-d NRR for cows with \( T > 20^\circ C \) on d –10 (i.e., temperature classes 5 and 6) was less (\( P < 0.001 \)) than 90-d NRR for cows with \( T \leq 20^\circ C \) (i.e., temperature classes 1 to 4) on d –10 (36.5 vs. 60.1%). For the analysis of temperature effects on d 10 after insemination (d +10), we analyzed a subset of cows that experienced \( T_{av} < 25^\circ C \) from the day of insemination (d 0) until d 9 after insemination. The 90-d NRR decreased as temperature on d +10 increased (Table 1). Orthogonal contrasts indicated that 90-d NRR for cows with \( T_{av} > 20^\circ C \) on d 10 after insemination was less (\( P < 0.001 \)) than for cows with \( T_{av} \leq 20^\circ C \) on d +10 (41.1 vs. 56.9%). To determine the effect of \( T_{av} \) on the day of insemination on subsequent fertility, a subset of cows which experienced \( T_{av} < 25^\circ C \) from d 10 until d 1 before insemination was analyzed. As shown in Table 1, 90-d NRR decreased as \( T_{av} \) on d 0 increased. Orthogonal contrasts indicated that 90-d NRR for cows having \( T_{av} > 20^\circ C \) on the day of insemination was less (\( P < 0.001 \)) than that for cows having \( T_{av} \leq 20^\circ C \) on d 0 (41.4 vs. 59.6%).

DISCUSSION

Results obtained from analysis of data of cows inseminated in south Georgia and Florida indicated that the magnitude of the summer decline in 90-d NRR depends on location and ME milk yield. In particular, summer infertility is exacerbated as cows are located further south and as milk yield increased. It is to be expected that location would affect the magnitude of summer infertility, but it is notable that such effects were seen over relatively short distances. In particular, cows in north Florida were more affected by season than cows in south Georgia although they were only \( \sim 200 \text{ km} \) south. Analysis of the data also suggest that heat stress may act at several physiological time points to disrupt establishment of pregnancy, including before ovulation, on the day of insemination, and after embryonic development has proceeded.

The measure of fertility used in this study, 90-d NRR, overestimates pregnancy rate because any cow with
no breeding record 90 d from the first insemination is classified as pregnant. In this way, some nonpregnant cows with silent or undetected estrus will be classified as pregnant. In fact, the depression in pregnancy rate in the summer is likely to be even stronger than indicated by 90-d NRR because of the increased frequency of unobserved estrus in summer (21). Possible differences in culling rate between seasons could also lead to seasonal bias in the accuracy of 90-d NRR.

The increase in milk yield experienced by dairy cows in the last 25 yr has been coincident with a reduction in pregnancy rate (18). The many reasons for this decline include alterations in follicular function (5) and endocrine differences between Holstein cows selected for milk yield and control cows (12). The present results indicate that high milk yield exacerbates the effects of heat stress on fertility. In fact, the summer decline in 90-d NRR was greater for cows with high milk yield than cows with low milk yield. Although not significant, the relationship between milk yield and 90-d NRR tended to be larger during the summer months of the year. The major reason high milk yield exacerbates effects of heat stress on reproduction is likely related to the increased metabolic rate and decreased thermoregulatory ability for cows with high milk yield (3).

Discriminant analysis was used to predict which combination of the weather variables available (average daily temperature, minimum and maximum relative humidity, wind speed, and solar radiation) on the day of insemination gave the highest prediction of 90-d NRR. That average daily minimum and maximum temperature on the day of breeding was as good a predictor of fertility as various combinations of weather variables does not mean that factors such as humidity or solar radiation are unimportant to the thermal biology of cattle, but rather that limitations in the nature of data (e.g., data were collected at a central weather station rather than on site; housing differed between herds) limited the predictive power of weather variables. Nonetheless, analysis of the relationship between average temperature on various days and subsequent 90-d NRR revealed some potential biological effects of heat stress on embryo loss. In particular, 90-d NRR was reduced when heat stress (i.e., $T_{av} >20^\circ C$) occurred on d –10 before breeding or at d 10 after breeding. One problem with interpreting relationships between temperature at specific times with fertility is that there is high correlation between temperature on a certain day with subsequent and previous days. To reduce this problem, the data set was reduced to subsets of cows that were exposed to cooler temperatures 10 d before or after the day of interest. For example, to evaluate effects of air temperature on d 10 before breeding, only those cows with average daily temperatures less than 25°C on d –9 to 0 relative to insemination were analyzed. Thus, the relationship between air temperature on day –10 and fertility is less likely to reflect heat stress on day –9 to 0.

Earlier, Dutt (6) reported that heat stress 12 d before breeding reduced fertility in ewes, suggesting heat stress can compromise oocyte function. One way such an effect could be mediated is through alterations in

<table>
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<th>Days relative to insemination where $T &lt; 25^\circ C$</th>
<th>Temperature class on day of interest (°C)</th>
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fOLLICULAR function as reported elsewhere (2, 24). Ealy et al. (7) and Edwards (10) reported that embryos become more resistant to heat stress as development proceeds and so the relationship between heat stress at d 10 after breeding and 90-d NRR was somewhat unexpected. However, Biggers et al. (4) reported that heat stress from d 8 to 16 of pregnancy reduced embryo after breeding and 90-d NRR was somewhat unex-
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