Effect of short- and long-term heat stress on the conception risk of dairy cows under natural service and artificial insemination breeding programs

L.-K. Schüller, O. Burfeind, and W. Heuwieser¹

Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Koenigsweg 65, 14163 Berlin, Germany

ABSTRACT

The objectives of this retrospective study were to examine the effect of heat stress on natural service and artificial insemination (AI) breeding methods. We investigated the influence of short- and long-term heat stress on the conception risk (CR) of dairy cows bred by natural service or by AI with frozen-thawed or fresh semen. In addition, the relationship between breeding method and parity was determined. Cows bred by AI with frozen-thawed semen exposed to long-term heat stress (mean temperature-humidity index ≥ 73 in the period 21 d before breeding) were 63% less likely to get pregnant compared with cows not exposed to heat stress. Cows bred by AI with fresh semen were 80% less likely to get pregnant during periods of short-term heat stress than during periods without heat stress. Furthermore, multiparous cows bred by AI with frozen-thawed or fresh semen were 22 and 67% less likely to get pregnant, respectively, than primiparous cows. No influence of heat stress or parity was noted on the CR of cows bred by natural service. The present study indicates that the likelihood of dairy cows becoming pregnant is reduced by short- and long-term heat stress depending on the type of semen employed. In particular, CR of cows inseminated with fresh semen is negatively affected by short-term heat stress and CR of cows inseminated with frozen-thawed semen is negatively affected by long-term heat stress.

Key words: heat stress, conception risk, artificial insemination, natural service, dairy cow

INTRODUCTION

Heat stress can have major effects on fertility and embryonic survival in lactating dairy cows (Hansen and Arechiga, 1999). These include disturbances in hormonal balance (Wilson et al., 1998; Roth et al., 2000),

decreased follicle and oocyte quality (Wolfenson et al., 1995; Roth et al., 2000), and decreased embryo development and embryo survival (Wolfenson et al., 2000; De Rensis and Scaramuzzi, 2003). These processes lead to a decrease in conception rate during periods of heat stress ranging between 30 and 60% compared with periods without heat stress (De Rensis et al., 2002; Schüller et al., 2014). Even in temperate climates, conception rate of dairy cows decreases from 31% during cold climate conditions to less than 13% during periods of heat stress (Schüller et al., 2014).

Furthermore, reproductive performance of bulls is negatively affected by heat stress, resulting in reduced libido and sperm motility and an increased number of abnormal sperm (Nichi et al., 2006; Menegassi et al., 2015). Generally, ambient temperatures in the range of 5 to 15°C were found to be optimal for semen production (Parkinson, 1987; Fuerst-Waltl et al., 2006). In a study conducted in a temperate climate, a negative relationship between ambient temperature in periods of 11 and 65 d before sperm collection and percentage of viable spermatozoa and sperm motility was detected (Fuerst-Waltl et al., 2006).

Reduced reproductive performance of dairy cows in combination with reduced libido and semen quality of bulls during summer months can cause important economic losses to the dairy industry (Collier et al., 2006). Therefore, the breeding method used in periods of heat stress should be considered to maximize reproductive performance. As a result of impaired reproductive performance, AI has become popular, and AI upon natural estrus is still the most prevalent method to breed cows for first and subsequent services (Overton and Sischo, 2005; National Animal Health Monitoring System, 2009). In contrast to natural service, it is assumed that a decrease in semen quality due to heat stress can be avoided in AI breeding programs by the use of frozen semen (Hansen and Arechiga, 1999). Natural service bulls, however, have been considered as a profitable alternative for managing dairy herds when estrus-detection rates are abysmal during summer months (Jobst et al., 2000); therefore, natural service continues to be a popular component of the breeding system in

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¹Corresponding author: w.heuwieser@fu-berlin.de

many dairy herds (de Vries et al., 2005). Furthermore, in European dairy farms, it is common practice to breed repeat breeder cows and cows with reproductive issues by natural service. Few studies have compared reproductive performance between AI and natural service breeding systems during periods of heat stress, and none have done so in a temperate climate. Niles et al. (2002) found pregnancy rates of dairy cows bred by AI or natural service did not differ significantly during the hot season, although the level of heat stress was not assessed. In contrast, de Vries et al. (2005) reported lower pregnancy rates for AI-breeding herds than for natural service and combined breeding herds during periods of heat stress. In contrast, a dearth of information exists on the effect of different periods of heat stress on AI and natural service breeding programs. Finally, we found no documentation of the effect of heat stress on AI compared between frozen-thawed or fresh semen. Thus, the objective of our study was to determine the influence of short- and long-term exposure to heat stress on the conception risk (**CR**) of lactating dairy cows in different breeding programs. Furthermore, the relationship between breeding method and parity was determined.

MATERIALS AND METHODS

Design of the Barn

The retrospective study was conducted on a commercial dairy farm in Sachsen-Anhalt, Germany, from May 2010 to October 2012. The herd consisted of 1,150 Holstein dairy cows with an average milk production of 10,345 kg (4.0% fat, 3.3% protein). The barn was positioned in a northeast-southwest orientation (51°77'N, 12°91'E) with open ventilation and a mechanical fan system. Sixty fans were installed above the stalls and controlled manually by the farm manager. All cows were housed in a freestall facility with slatted floors and beds equipped with rubber mats. Group composition was dynamic, with cows entering and leaving the study barn depending on their calving dates. Cows were fed a TMR. The rations were formulated to meet or exceed the requirements of the NRC (2001). Lactating cows were milked 3 times a day at starting at 0600, 1400, and 2200 h.

Reproductive Management

The voluntary waiting period was set at 55 d postpartum. Between 35 and 49 d, cows received an initial injection of 25 mg of PGF_{2α} (Dinoprost, Dinolytic, Zoetis Deutschland GmbH, Berlin, Germany) and a second injection of 25 mg of PGF_{2α} 2 wk later to regress the

corpora lutea. Cows that showed estrus after the second injection of PGF_{2α} were AI or received natural service by a bull. Natural service was provided for all cows after caesarean section, with adhesions of the uterus and repeat breeder cows in third or higher lactation. Pedometers (Milkline, Gariga di Podenzano, Italy) and visual observation were used to detect estrus. Cows that did not show estrus after the second injection of PGF_{2α} within 3 d were excluded from the analysis. Inseminations were performed by 7 technicians within 12 h after detection of estrus with frozen-thawed or fresh semen from bulls (n = 219) used randomly across inseminations depending on the availability of semen in fresh or frozen state. Pregnancy diagnoses were performed 35 to 42 d after the day of breeding with transrectal ultrasonography by the herd veterinarian. Cows with unclear pregnancy diagnosis were reexamined with transrectal ultrasonography 1 wk later. Cows that were diagnosed not pregnant were reassigned to breeding with natural service or AI with frozen-thawed or fresh extended semen depending on the results of the transrectal ultrasonography and the number of previous inseminations. Cows that were observed in estrus >10 d after AI were assumed nonpregnant and reinseminated.

Frozen-thawed and fresh semen used for AI in this study was processed by the local AI center and sent daily to the dairy farm. Frozen semen was stored in 0.25-mL straws within liquid nitrogen at −196°C. During the course of thawing, semen samples were taken out of the liquid nitrogen and thawed in a water bath at 37°C for 30s. Fresh semen was stored in thermoinsulated canisters at 10 to 15°C until insemination.

Data Collection

Ambient temperature and relative humidity within the experimental barn were recorded using a Tinytag Plus II logger (Gemini Loggers Ltd., Chichester, UK) secured at a beam 3 m from the ground within the barn. This logger measured ambient temperature from −25 to 85°C, with an accuracy of ±0.3°C and a resolution of 0.01°C, and relative humidity from 0 to 100%, with an accuracy of ±3% and a resolution of 0.3%. These data were recorded hourly. Ambient temperature (AT) and relative humidity (RH) data were used to calculate the temperature-humidity index (**THI**) according to the equation reported by the NRC (1971): $THI = (1.8 \times AT + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times AT - 26)]$. Cow identification, parity, service dates, breeding method, number of services, and results of pregnancy diagnosis were obtained from computerized herd records managed using Herde (Version 5.5, Software Projektierungs- und Handels GmbH, Aschare, Germany).

Statistical Analyses

Data from the onsite climate logger and from Herde were exported into Excel spreadsheets (Office 2013, Microsoft Deutschland GmbH, Munich, Germany) and statistical analysis was performed using SPSS for Windows (Version 19.0, SPSS Inc., IBM, Ehningen, Germany). Daily and monthly mean ambient temperature, relative humidity, and resulting THI were calculated from hourly measures. Short-term heat stress was defined as a mean THI ≥ 73 at the day of breeding because heat stress around the day of breeding was consistently associated with reduced CR (García-Ispuerto et al., 2007; Morton et al., 2007; Schüller et al., 2014). Long-term heat stress was defined as a mean THI ≥ 73 in the period from d 21 to 1 before the day of breeding because heat stress beginning on d 1 of the previous estrous cycle leads to a reduced number of follicles, a reduced concentration of estradiol in follicles, and to an earlier emergence of the dominant follicle (Wolfenson et al., 1995, 1997). In a study that compared the effect of heat stress on CR between 7 different periods around the day of breeding, heat stress at 3 wk before breeding had the greatest negative influence on CR (Schüller et al., 2014). In the current study, a THI ≥ 73 on the day of breeding (short-term heat stress) or during the 21-d period before breeding (long-term heat stress) did not preclude presence of heat stress after breeding, in particular when heat stress is seasonal in contrast to sporadic heat stress episodes. Descriptive statistics of continuous variables were presented by median and range. For categorical data, frequencies of occurrence were presented. Conception risk was defined as the number of pregnant cows divided by the number of total services multiplied by 100.

The association between heat stress (no heat stress, short-term heat stress, long-term heat stress), parity (primiparous vs. multiparous), number of services (1, 2, 3, ≥ 4 services), and CR was determined in a multivariable logistic regression model for each breeding method (AI with frozen-thawed semen, AI with fresh semen, natural service). Furthermore, the interactions between heat stress and each variable were determined. The model was constructed in a backward stepwise manner with variables being continuously removed from the model by the Wald statistic criterion if the significance was greater than 0.20. Odds ratios, including 95% confidence interval, were reported for all associations and significance was set at $P < 0.05$. For all variables, one class of each variable was considered as the reference and an odds ratio significantly greater (or lower) than 1 for any other class of this variable was indicative of an increased (or reduced) risk for conception when compared with the class used as reference.

RESULTS

The data set contained 5,192 breeding records from 1,537 lactating dairy cows on a single dairy farm. The overall CR obtained was 33.0% and average days open were 129.3. Inseminations with frozen-thawed and fresh semen were conducted in 80.6 and 5.6% of the cows, respectively, and 13.8% of the cows were bred by natural service. The population of cows enrolled in the study consisted of 33.0% primiparous cows and 67.0% cows in the second or higher lactation. Frequency distribution of number of inseminations needed to establish a pregnancy was 32.8 (1 insemination), 28.0 (2 inseminations), 18.8 (3 inseminations), and 20.4% (≥ 4 inseminations).

Minimum, maximum, and mean ambient temperature during the study period were 2.3, 29.8, and $16.0 \pm 5.5^\circ\text{C}$, respectively. Minimum, maximum, and mean relative humidity during the study period were 48.9, 95.8, and $76.0 \pm 8.2\%$, respectively. Minimum, maximum, and mean THI during the study period were 34.6, 83.7, and $60.4 \pm 8.5\%$, respectively. Descriptive statistics for the mean THI per month are depicted in Figure 1.

In the multivariable logistic regression model investigating cows inseminated with frozen-thawed semen, the variables heat stress, parity and number of services remained significant (Table 1). No significant interactions were noted between heat stress and the variables parity and number of services. Multiparous cows bred by AI with frozen-thawed semen were 22% less likely to get pregnant than primiparous cows (Table 1). Cows bred by AI with frozen-thawed semen exposed to long-term

Figure 1. Mean (—), minimum (---), and maximum (···) temperature-humidity index in the barn from May 2010 to October 2012 ($n = 913$ experimental days).

Table 1. Results of multivariable logistic regression model and interactions of factors associated with conception risk in dairy cows inseminated with frozen-thawed semen

Variable	Odds ratio	95% CI	n	P-value
Parity			4,184	<0.05
Primiparous	Referent		1,408	
Multiparous	0.78	0.68–0.90	2,776	<0.05
Heat stress			4,184	<0.05
No heat stress	Referent		3,928	
Short-term heat stress	0.75	0.53–1.06	174	0.10
Long-term heat stress	0.37	0.20–0.67	82	<0.05
Number of services			4,184	<0.05
1	Referent		1,373	
2	0.96	0.82–1.14	1,173	0.67
3	1.04	0.86–1.25	785	0.71
≥4	0.75	0.62–0.91	853	<0.05

heat stress were 63% less likely to get pregnant than cows not exposed to heat stress (Table 1). Cows bred by AI with frozen-thawed semen receiving ≥4 services were 15% less likely to get pregnant than cows receiving 1 service (Table 1).

In the multivariable logistic regression model investigating cows inseminated with fresh semen, the variables heat stress and parity remained significant (Table 2). Number of services had no significant association with CR ($P = 0.28$) and therefore was not included in the multivariable model. No significant interactions between heat stress and parity were observed. Multiparous cows bred by AI with fresh semen were 67% less likely to get pregnant than primiparous cows (Table 2). Cows bred by AI with fresh semen exposed to short-term heat stress were 80% less likely to get pregnant than cows not exposed to heat stress (Table 2). In the multivariable logistic regression model investigating cows bred by natural service, the variables heat stress ($P = 0.21$), parity ($P = 0.98$), and number of services ($P = 0.24$) had no significant association to CR.

DISCUSSION

The present study demonstrates that both short- and long-term heat stress significantly reduce the likelihood of cows becoming pregnant bred by AI. Previous studies demonstrated that the CR of cows is negatively

affected by extended heat stress, as well as on single days around the day of breeding (Morton et al., 2007; Schüller et al., 2014). In our study, however, the effect of short- and long-term heat stress differed depending on the breeding method. Long-term heat stress significantly reduced the likelihood of cows becoming pregnant when inseminated with frozen-thawed semen, whereas short-term heat stress significantly reduced the likelihood of cows becoming pregnant when inseminated with fresh semen. Furthermore, the effect of long-term heat stress on the likelihood of cows becoming pregnant when inseminated with fresh semen was barely statistically significant ($P = 0.05$, Table 2). These results are in agreement with previous studies demonstrating that long-term heat stress in the period before breeding significantly reduces CR of dairy cows (Wolfenson et al., 2000; Morton et al., 2007; Schüller et al., 2014).

Conception risk of cows inseminated with frozen-thawed semen during periods of long-term heat stress decreased about 32.4 percentage points compared with periods without heat stress (Figure 1). Long-term heat stress before the day of breeding leads to a reduced number of follicles, a reduced concentration of estradiol in follicles and to an earlier emergence of the dominant follicle (Wolfenson et al., 1995, 1997). The advantage arising from the storage of semen in a frozen state is the possibility to maintain spermatozoa for a very long period. Hansen and Arechiga (1999) assumed that AI

Table 2. Results of multivariable logistic regression model and interactions of factors associated with conception risk in dairy cows inseminated with fresh semen

Variable	Odds ratio	95% CI	n	P-value
Parity			317	<0.05
Primiparous	Referent		42	
Multiparous	0.33	0.16–0.67	275	<0.05
Heat stress			317	<0.05
No heat stress	Referent		261	
Short-term heat stress	0.20	0.07–0.56	30	<0.05
Long-term heat stress	0.41	0.17–1.01	26	0.05

with frozen-thawed semen represented the best method for bypassing effects of heat stress on male fertility and the use of natural breeding squanders this benefit of AI. However, inadequate maintenance of storage temperatures in liquid nitrogen may occur during periods of high ambient temperature and can lead to far shorter time periods of survival of frozen semen (Vishwanath and Shannon, 2000). Furthermore, the effect of heat stress on the fertility and semen quality of AI bulls in the period before breeding may appear delayed due to the preservation of frozen storage of semen until the day of insemination.

Conception risk of cows inseminated with fresh semen during periods of short-term heat stress decreased by 25.4 percentage points (Figure 2) when compared with periods without heat stress. Fresh semen used for inseminations in our study was sent daily from the local AI station and stored in thermoinsulated canisters at 10 to 15°C until insemination. The optimum storage temperature of fresh semen ranges between 16 to 20°C, and storage temperatures above this led to reduced semen fertility (Shannon and Curson, 1984; Vishwanath and Shannon, 2000). Long storage periods in combination with high ambient temperature can lead to an increase of the storage temperature and might explain poor CR during periods of short-term heat stress in our study. These findings support our hypothesis that climate conditions should be considered to optimize insemination programs and resulting CR on dairy farms. Particularly during periods of short-term heat stress, the on-site handling of fresh semen needs to be improved; however, temperatures for storage, freezing, and thawing processes were not recorded in our study. Further studies should investigate the effect of heat stress on fertility of bovine frozen and fresh semen under field conditions considering storage conditions and thawing procedures.

In contrast to cows bred by AI, cows bred by natural service were not affected by short- or long-term heat stress in the current study. Heat stress in bulls can lead to a reduced libido, sperm output, and motility and an increase in sperm abnormalities (Nichi et al., 2006; Menegassi et al., 2015). Although the natural service bulls in our study were housed in a freestall facility within the dairy farm, CR after natural service did not differ significantly between periods of heat stress and periods without heat stress. In a recent study, scrotal temperatures measured by infrared thermography were highly correlated with the daily THI (Menegassi et al., 2015), and increased testicular temperatures led to impaired spermatogenic cycle and impaired sperm quality (Nichi et al., 2006). In the current study heat stress just reached the maximum THI of 83 (Figure 1), and in the study of Menegassi et al. (2015) the aver-

age THI of 83 did not cause significant morphological changes in sperm quality. However, a scarcity of studies exists investigating THI thresholds for the effect of heat stress on the libido and sperm quality of bulls in a temperate climate. Further research is necessary to investigate the effect of heat stress on the reproductive performance of bulls in the temperate climate. Some evidence exists that the use of natural service increases herd reproductive performance because bulls are supposed to be better at detecting estrus than humans because errors in estrus detection might be avoided (Vishwanath, 2003; Lima et al., 2009). During periods

Figure 2. Conception risk of dairy cows bred by AI with frozen-thawed ($n = 4,184$) or fresh semen ($n = 317$) or by natural service ($n = 716$) during periods of short-term heat stress (striped bar) and long-term heat stress (black bar) compared with periods without heat stress (white bar). Bars with different letters (a,b) differ significantly ($P < 0.05$).

of heat stress, estrus-detection rates can be reduced because mounting occurs more frequent during night and intensity of estrus is reduced (Orihuela, 2000). In the current study, a pedometer system in combination with visual observation was used to detect estrus in AI cows. Using an automated activity monitoring system for estrus detection in combination with visual observation led to estrus-detection rates of 56.3% in the first 21 d after the voluntary waiting period (Michaelis et al., 2014). Although manufacturers of automated activity monitoring systems claim high estrus-detection rates even under conditions that influence the cow movements because of individual threshold monitoring (Milkline, 2014), science-based information is not available. Further research is necessary to evaluate such estrus-detection systems under heat stress conditions, because the use of natural service bulls in dairy herds is a critical issue (Vishwanath, 2003; Lima et al., 2010). We emphasize that the benefit of natural service should be weighed against considerable risks associated with natural service, such as occupational safety, omission of gain in genetic merit, decreased predictability of time of birth, birth weight, and infertility in the dominant bull (Vishwanath, 2003; Lima et al., 2010).

In our study, the number of services is intrinsically related to CR of cows inseminated with frozen-thawed semen. These results corroborate earlier publications that report an inverse relationship for number of inseminations to CR (González-Recio et al., 2005; Weller et al., 2006). The number of inseminations to conception reflects the actual variation of female fertility and therefore is a direct measurement for the fertility in dairy cows (González-Recio et al., 2004). This negative relationship, however, was only observed for cows receiving ≥ 4 inseminations with frozen-thawed semen and not observed in cows inseminated with fresh semen. These observations can be explained by lower case numbers for cows inseminated with fresh semen combined with a relatively small effect of number of inseminations on CR. Therefore, in the current study, this relationship was only apparent in cows receiving ≥ 4 inseminations with frozen-thawed semen.

We observed that multiparous cows inseminated with frozen-thawed and fresh semen were 22 and 67% less likely to get pregnant, respectively, than primiparous cows (Tables 1 and 2). This observation agrees with previous studies that reported lower reproductive performance for multiparous cows (Chebel et al., 2004; Tenhagen et al., 2004; Astiz and Fargas, 2013). A possible reason for better fertility in primiparous cows is the lower milk production and the reduced risk of metabolic disorders in early lactation impairing reproductive efficiency (Tenhagen et al., 2004). In our study, no interaction was detected between heat stress and

parity. Multiparous cows have higher milk production than primiparous cows (Hansen et al., 2006) and milk yield is closely related to increased DMI and increased metabolic heat production (Kadzere et al., 2002; Kononoff et al., 2006). This metabolic stress can lead to a decrease in CR during the hot season by 20 to 30% compared with the winter season (De Rensis et al., 2002). Therefore, multiparous cows can be affected by heat stress because an increase in milk yield is related to a decrease in heat tolerance (Berman et al., 1985; West, 2003). In our study, however, heat stress significantly decreased the likelihood of pregnancy in multiparous and primiparous cows. Our results are in line with a most recent study conducted in Spain including 7,805 cows demonstrated that CR was reduced during the hot season to a similar extent for primiparous and multiparous cows (Astiz and Fargas, 2013).

CONCLUSIONS

The present study indicates that the likelihood for dairy cows to become pregnant is reduced by short- and long-term heat stress depending on the form of semen used. Conception risk of cows inseminated with fresh semen was negatively affected by short-term heat stress, whereas CR of cows inseminated with frozen-thawed semen was negatively affected by long-term heat stress. Conception risk of naturally serviced cows was not affected by either duration of heat stress. Therefore, climate conditions on dairy farms should be considered when selecting breeding strategies to optimize AI and resulting CR, particularly during periods of short-term heat stress when fresh semen is used.

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