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Effect of Heat Stress on Subsequent Estrous Cycles Induced by PGF2*α* in Cross-Bred Holstein Dairy Cows Raised in Ratchaburi Province, Thailand

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Simple Summary: Heat stress influenced reproductive performance and physiological responses in cross-bred Holstein dairy cows under tropical conditions. First service conception rates of cows under moderate heat stress tended to be lower than those under low heat stress. The average interval between calving and first service in cows under moderate heat stress was longer than those under moderate heat stress. Cows in moderate heat stress responded less to PGF2*α* than cows in mild heat stress. The cows' average rectal temperature and respiratory rate were most likely affected by moderate HS rather than mild HS. The study indicated that heat stress influenced the reproductive performance of cows raised in Thailand throughout the year, especially when heat stress was relatively intense.

Abstract: The study aimed to determine the effect of heat stress (HS) on reproductive parameters [calving to first service (CTFS) and first service conception rate (FSCR)] and general physiological responses [rectal temperature (RT) and respiratory rate (RR)] in tropical Holstein Friesian dairy cows raised in Ratchaburi province, Thailand. Cows were divided into a moderate HS (n = 22) and a mild HS (n = 22) group. Cycling cows were detected and injected with PGF2*α* at the beginning of the experimental period. Reproductive and physiological parameters were recorded. The average temperature humidity indices experienced by the cows were 80.67 ± 0.79 and 77.81 ± 1.09 for the moderate and mild HS groups, respectively. Cows showed significantly lower RT and RR in the mild (38.47 ± 0.21°C and 41.04 ± 4.55 bpm, respectively) than in the moderate HS group (38.87 ± 0.15°C and 51.17 ± 10.52 bpm). Under mild HS, cows showed a better response to injection of PGF2*α* than those under moderate HS. The percentage of cows that ovulated after being induced by PGF2*α* and showed estrus signs was higher in the mild than the moderate HS groups (54.55% versus 18.18%). Furthermore, the FSCR of cows under mild HS tended to be higher than in the moderate HS group (54.55 % and 42.11%, respectively), while the average CTFS interval was significantly shorter under mild than moderate HS (69.47 ± 18.18 and 84.60 ± 27.68, respectively). These results indicate that HS impairs reproductive performance in cross-bred Holstein cows, especially in moderate HS conditions.

Keywords: heat stress; dairy cow; rectal temperature; respiratory rate; reproduction

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1. Introduction

Global warming has been increasing worldwide, including in Thailand, a tropical country with high ambient temperatures and relative humidity for most of the year. Between 2006 and 2011, the highest average temperature in the western part of Thailand was 32.6°C, which is higher than a cow's thermoneutral zone and could cause heat stress (HS) due to undissipated body heat [1].

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HS can be detected by the Temperature Humidity Index (THI), a single value representing the combined effects of environmental temperature and relative humidity. The comfort zone of many animals is a THI lower than 71. Values between 72 and 79 imply the potential for mild HS, between 80 and 89 are considered moderate HS, and above 90 are considered severe HS for animals [2]. On average, in Thailand, a high THI is present for eight months of the year (March to October), with durations of 9 h/day (from 10 am to 7 pm) [3]. Ratchaburi is a province located in western Thailand with high dairy farm density [4]; cows raised in this area are likely to face HS.

HS has a severe impact on health performance and the reproductive system. Cows can adapt to HS by quickly dissipating heat by increasing their respiratory rate (RR) [5]. HS also affects food intake capacity [6–8] and reduces rumen activity [9]. Consequently, it reduces body condition scores and could aggravate negative energy balance during postpartum [10]. HS has various adverse effects on milk production, including yield and composition [11,12], and it can affect the body's immune system [13,14].

Furthermore, HS is a significant factor in poor reproductive performance [15,16]. Several studies have reported that HS could alter various aspects of the hypothalamic-pituitary-ovarian axis, especially in follicular growth [6,17,18]. HS induced apoptosis of granulosa cells and resulted in poor follicle quality [19,20]. Granulosa cells are the primary cells that produce estrogen and inhibin concentrations. Thus, HS reduced steroidogenesis [6,17–19] and decreased estrous behavior and silent heat [21]. Less estrogen was also affected by positive and negative feedback related to the reduced pre-ovulatory follicle dominance process, delayed ovulation, and formed poor quality luteal cells, which altered progesterone concentration [22]. Hence, these adverse effects (especially in summer) led to low reproductive performance in Thailand, including delayed postpartum ovulation, prolonged days open, and low first-service conception rates (FSCRs) [23–25].

Evaluating general physiological responses associated with the effect of HS can improve understanding and confirm the severity of HS in cows raised in Thailand. The result is necessary to develop and improve the farm management of heat-stressed cows. In addition, the information might be used to reference the reproductive index of cows under HS conditions and improve hormone administration protocols to reduce reproduction problems due to HS. Furthermore, the effect of HS on reproductive parameters and general physiological responses was very limited in the field study in Thailand.

Therefore, this research focuses on the effect of HS on reproductive parameters and general physiological responses during subsequent estrous cycles induced by PGF2α in tropical Holstein Friesian dairy cows in Ratchaburi province, the western part of Thailand.

2. Materials and Methods

This study was approved by the Committee for Animal Ethics in Scientific Procedures of Kasetsart University, Bangkok, Thailand (animal ethics permission number ACKU62-VET-047)

2.1. Daily Temperature and Humidity Data

Air temperature and relative humidity in the barn were recorded every three hours using three data loggers (Ibutton®, model: DS1923, Maxim Integrated USA) throughout the experiments. These data were transferred monthly through the 1-Wire® program and exported to a .CSV file (Microsoft® Excel). The temperature humidity index (THI) was calculated according to the following formula: $THI = (0.8\ T) + ((RH/100) \times (T–14.4)) + 46.4$, where T was the ambient temperature of the air (°C), and RH was the relative humidity (%) [3]. The average monthly THI ranged between 81.89 and 76.64 between April 2018 and January 2019.

2.2. Animals and Management

The study was conducted from April 2018 to January 2019 on a medium-sized dairy farm with approximately 90 lactating dairy cows in Ratchaburi province, Thailand. According to the THI and the report from the Thai Meteorological Department, all healthy pregnant dry cows (crossbred

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Holstein Friesian) had an expected calving date during one of two periods. The summer and rainy group consisted of cows that calved from April 2018 to August 2018 (n = 29), including 11 primiparous and 18 multiparous cows (2nd–8th lactation); the average number of lactations in this group was 2.82 ± 2.18. The winter group consisted of cows that calved from November 2018 to January 2019 (n = 37). This group consisted of 15 primiparous and 24 multiparous cows, averaging 2.94 ± 2.36 lactations.

The cows were raised in open barns with a loose housing system. They were fed commercial concentrates twice daily but had free access to roughage (grasses, corn stalks, corn leaves, and rice straw) and water. The cows were milked twice daily (at 5.30 am and 4.30 pm) with the pipeline system. Electric fans under the roof were used during the afternoon until milking in the evening.

2.3. Reproductive Management and Blood Sampling

The voluntary waiting period was established at 30 days or four weeks after delivery. Cows were examined by transrectal palpation and ultrasound (Honda HS 1600 with a 7.5 MHz rectal probe, Honda, Tokyo, Japan) to measure ovarian structure and uterine involution. Cows with a corpus luteum (CL) and uterine involution structure were included. Cyclic cows were synchronized by the PGF2α protocol: they received an injection of 2ml PGF2α (Cloprostenol, Estrumate®, Intervet International B.V., Bangkok, Thailand) to regress the CL on day 0. The owner observed them twice a day (morning and evening) for estrus detection, and blood collection began 1 day after injection of PGF2α. If a CL was not found in at least one ovary in the fourth week after delivery, the veterinarian rechecked these cows using transrectal palpation and ultrasound in the sixth and eighth weeks. Cows without a CL at the second and third examinations were considered anestrous and excluded from the study. Subsequently, after estrous cycles, blood collection was also performed, starting 22 days after injection of PGF2α.

Blood samples (5ml) to monitor progesterone concentration were taken daily from the jugular vein on day 1 after PGF2α injection and continuously until day 8 (i.e., blood samples were collected eight times), including periods of early heat, standing heat, and after standing heat. Blood samples were collected again eight times during the subsequent estrous cycle (days 22–29). After collection, all blood samples were left at room temperature for 30 minutes and then centrifuged (Eppendorf Centrifuge 5804 R) with 1500 xg for 10 minutes. The serum was stored at –20°C until progesterone was assayed.

2.4. Rectal Temperature, Respiratory Rate, and Reproductive Performance Record

The rectal temperature (RT, in °C) was measured with a thermometer. RR (breaths per minute; bpm) was measured using a stethoscope with the ability to perform a physical examination. Both measurements were made prior to blood collection. The reproductive performance was explored and recorded by a veterinarian. Cows with clinical illness were recorded and treated by a veterinarian.

2.5. Evaluation of Ultrasound and Progesterone Concentration for Detection of Ovulation

The cows' owners visualized estrous behavior during the blood sampling period. These conclusions were confirmed by a veterinarian using transrectal palpation and ultrasound on day 8 and day 29 following PGF2α injection. The presence of a suspected new CL was confirmed by progesterone concentration (P4). If the hormone decreased to less than 1 ng/ml during the blood sampling period, it was defined as the day of regression of a mature CL in a cow [17,26] and was recorded as a new CL. Furthermore, the cows were also inseminated or showed estrous signs.

Progesterone concentration was measured by secondary antibody enzyme immunoassay using the procedure of Brown et al. [27] in cooperation with the Endocrine Laboratory at the Khaokheow Open Zoo. The intra-assay and inter-assay coefficients of variation in progesterone concentration were 2.53% and 4.95%, respectively.

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2.6. Statistical Analysis

Statistical analysis was performed using the R program. THI data for each month and season were determined using one-way ANOVA. Differences in mean RR, RT, and calving to first service (CTFS) interval in each group were compared using Mann-Whitney tests or *t*-tests. The proportional data of estrus behavior with CL detection, the incidence of retained fetal membrane, and FSCR were analyzed using Chi-square tests. The distribution of the data was checked for normality using the Shapiro-Wilk test. Data were presented as means and SEM. Analyses were considered statistically significant when $p < 0.05$.

3. Results

3.1. Temperature-Humidity Index and General Data

The mean THI for the hot and rainy season was 80.67 ± 0.79 (ranging from 79.80 to 81.89) and was defined as moderate HS. The mean monthly THI for the cool season was 77.81 ± 1.09 (range: 76.64–78.80) and was defined as mild HS.

The number of cows selected under both conditions is presented in Table 1. At the beginning of the experiment, there were 29 cows in the moderate HS group, and 22 returned to the estrous cycle. However, 13 of 22 cows returned within 30 days after delivery and were included in the experiment. For the mild HS group, there were 37 at the start of the experiment, and 23 of 28 returned to the estrous cycle within 30 days after delivery. The occurrence of retained fetal membranes (RFM) in the moderate HS group was 3.03 times higher than in the mild HS group. Furthermore, data on the incidence of cystic ovary (within 30 days postpartum) and the cause of culling (where necessary) are also shown in Table 1.

Table 1. Distribution of the cows with general data.

Parameter	Moderate HS group	Mild HS group
Total sample size	29	37
Cyclic cows received an injection of $PGF_{2\alpha}$[1]	22	22 (28*)
Cyclic cows within 30 days	13	23
Cyclic cows during 30–60 days	9	5
Retained fetal membranes	12	7
Cystic ovary 30 days[2]	4	2
Culling	3	1
Anaplasmosis	1	0
Cystic ovary > 30 days	1	1
Hip luxation	1	0

[1] $PGF2\alpha$ = Estrumate® (cloprostenol sodium). [2] Cystic ovaries within 30 days postpartum (spontaneous recovery).
* Number of cows returned to estrous cycle in mild HS group.

3.2. Effect of Heat Stress on Rectal Temperature and Respiratory Rate

The average RT and RR in moderate and mild HS are shown in Table 2. The average RR was significantly lower in the mild HS group than in the moderate HS group ($p < 0.05$). Furthermore, the average RT was also significantly lower for the mild HS group than for the moderate HS group ($p < 0.05$). These results indicate that moderate HS had more effects on general responses than mild HS.

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Table 2. Mean ± SEM of maximum, minimum, and mean respiratory rate (RR) and rectal temperature (RT) in moderate (n = 22) and mild HS (n = 22) groups.

Parameter	HS group	Maximum	Minimum	Mean
RT (°C)	Mild	38.96 ± 0.22[a]	37.78 ± 0.78[a]	38.47 ± 0.21[a]
	Moderate	39.45 ± 0.52[b]	38.38 ± 0.20[b]	38.87 ± 0.15[b]
RR (bpm)	Mild	48.55 ± 7.54[a]	33.45 ± 3.16[a]	41.04 ± 4.55[a]
	Moderate	62.82 ± 13.48[b]	39.82 ± 9.76[b]	51.17 ± 10.52[b]

[a–b] Different letters in the same column indicate significant differences ($p < 0.05$) between groups. bpm = breaths per minute.

3.3. Effect of Heat Stress on Estrous Behavior and the Response of PGF$_{2\alpha}$ in Cycling Cows

The 22 cows in each group received an injection of 2 ml PGF2α. Estrous behavior and CL detection as responses to PGF2α confirmation and progesterone concentration (less than 1 ng/ml) were then investigated to classify the cow group. The group of PGF2α-induced cows were classified in that cycle and the subsequent estrous cycle, as shown in Table 3. After injection of PGF2α, the percentage of cows in estrus + new CL in mild HS was significantly higher than in moderate HS. Interestingly, the percentage of silent heat cows (without estrus + new CL) was higher under moderate than mild HS. Therefore, these findings indicated that the percentage of cows with a good response to PGF2α and estrus behavior (estrus + new CL) was mainly in cows in mild HS conditions. The incidence of new CL was also detected in moderate HS cows without estrous behavior. As a consequence, the percentage of cows in estrus and/or artificial insemination (AI) did not differ between the two groups in the next estrous cycle.

Table 3. Distribution of the cows with estrous behavior and new corpus luteum (CL) in the PGF2α-induced cycle and the subsequent estrous cycle.

Parameter	Moderate HS group (n = 22)	Mild HS group (n = 22)
Estrous cycles induced by PGF2α		
Estrus + new CL[1]	18.18% (4/22) [a]	54.55% (12/22) [b]
Estrus + No new CL[2]	13.64% (3/22)	13.64% (3/22)
No Estrus + new CL[3]	40.91% (9/22) [a]	13.64% (3/22) [b]
No Estrus + No new CL[4] (non-response)	27.27% (6/22)	18.18% (4/22)
Subsequent estrous cycle	(n = 22)	(n = 22)
Estrus and/or AI[5]	40.91% (9/22)	36.36% (8/22)
No Estrus + new CL[6]	13.64% (3/22)	13.64% (3/22)
No Estrus + No new CL[7]	36.36% (8/22)	40.91% (9/22)
Culling	9.09% (2/22)	0% (0/22)
Other events	–	9.09% (2/22) [8]

[1] PGF2α-induced cows showed estrous behavior, and a new CL was found. [2] PGF2α-induced cows showed estrous behavior, and a new CL was not found. [3] PGF2α-induced cows did not show estrous behavior, but the level of P4 was ≤ 1 ng/ml and/or a new CL was found. [4] Cows did not respond to PGF2α and were excluded from the study. [5] Cows showed estrous behavior and/or were artificially inseminated. [6] Cows did not show estrous behavior, but the level of P4 ≤ 1 ng/ml and/or a new CL was found. [7] Cows did not show estrous behavior or a new CL. [8] Cows were inseminated in the previous estrous cycle. [a–b] Different letters in the same row indicate significant differences ($p < 0.05$) between groups.

In addition, the FSCR and CTFS data were recorded and presented in Table 4. The FSCR of cows in mild HS tended to be higher than cows in moderate HS conditions (42.11% vs. 15%) ($p = 0.06$), while the average CTFS of cows in mild HS was significantly shorter than cows in moderate HS (69.47

± 18.18 vs. 84.60 ± 27.68) (Table 4). These findings indicated that HS suppresses reproductive performance in cows, especially in moderate HS conditions.

Table 4. Distribution of the mean first service conception rate (FSCR) and calving to first service (CTFS) in moderate and mild HS groups.

Parameter	Moderate HS group (n = 20)	Mild HS group (n = 19)
FSCR (%)	15 (3/20) [a]	42.11 (8/19) [a]
CTFS (days)	84.60 ± 27.68 [a]	69.47 ± 18.18 [b]

[a–b] Different letters in the same row indicate significant differences ($p < 0.05$) between groups.

To clarify whether the incidence of RFM is caused by environmental factors such as HS, the association between HS and the incidence of RFM was measured in Table 5. The result showed an association between the HS group and the incidence of RFM, such that the incidence of RFM was higher in moderate HS than under mild HS conditions. However, there was no difference between CTFS from RFM and non-RFM cows in the moderate HS group (Table 6). Therefore, RFM did not affect reproductive performance in this study.

Table 5. Association between moderate or mild HS and incidence of retaining the fetal membrane (RFM) in cows.

Parameter	Moderate HS group	Mild HS group	Chi-square value	*p*
RFM cow[1]	9	3	4.125	0.042*
Non-RFM cow[2]	13	19		

[*] Significant association ($p < 0.05$). [1] Cows failed to expel fetal membranes within 24 hours after calving. [2] Cows did not fail to expel fetal membranes within 24 hours after calving.

Table 6. Comparison of the average calving to first service (CTFS) between cows that retained the fetal membrane (RFM) and those that did not in moderate HS.

Parameter	RFM cow (n = 8)	Non-RFM cow (n = 12)
CTFS (days)	94.25 ± 36.60 [a]	78.17 ± 18.92 [a]

[a–b] Different letters in the same row indicate significant differences ($p < 0.05$) between groups.

4. Discussion

The value of THI was relatively high throughout the experimental period. The temperature gradually increased in April to reach the maximum level in May. It then slightly decreased until September and October, fell to its lowest at the end of 2018, and remained low until January 2019. In contrast, average humidity was stable during the summer period (May–August), which saw high temperatures and low humidity. The humidity gradually increased in September to reach its highest level in October, which was the rainy season. Humidity then decreased from November to January (the winter season). The THI was similar to previous reports in Thailand [24,28]. Therefore, chronic HS occurred in cows raised in Thailand throughout the year, and the cows in this study were affected by moderate and mild HS during the experiment.

The RR and RT results were higher in moderate than in mild HS. According to the field study of Shehab-El-Deen et al. [10], cows had significantly higher RR and RT (95.5 ± 1.1 and 39.88 ± 0.06, respectively) in summer (when the THI was 82.10) than in winter (when THI was 66.40) (RR: 43.89 ± 0.61; RT: 38.94 ± 0.07). Furthermore, when cows were exposed to acute HS in heat chambers, it was found that RR increased immediately after heat exposure (76.02 ± 1.70 bpm) compared to the control (39.70 ± 0.71 bpm) [34]. Moreover, in this study, daily THI was moderately correlated with both RT ($r = 0.42$) and RR ($r = 0.41$), consistent with previous studies [35–37]. The increase in body temperature represented by RT would increase as a physiological mechanism in homeothermic animals, signifying a response in the cows from the induced heat load in their environment. The cows still showed a significant difference in RR and RT between moderate and mild levels of HS even though

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they were chronically exposed to HS. In this study, cows in moderate HS showed more adapted behavior to heat dissipation for an immediate return to the thermoneutral zone than those in mild HS. These results confirm that the cows in the two groups were affected by different levels of HS and had different general responses.

The percentage of cows with a good response to PGF2α (estrus + new CL) was significantly higher in mild than in moderate HS conditions (p < 0.05). This difference could suggest that cows under mild HS were more responsive to PGF2α and showed estrous signs better than cows under moderate HS. However, the percentage of cows that had a new ovulation (estrus + new CL and no estrus + new CL groups) did not differ between moderate and mild HS (p = 0.75). Typically, the CL is regressed by PGF2α, after which the dominant follicle will ovulate. HS alters follicular development and steroid hormone synthesis [6]. The heat condition affected the poor quality of the dominant follicle with low granulosa cells [19,38]. In this study, the development of the follicle during moderate HS might be qualitatively less than that during mild HS. Moreover, failure of heat detection could have resulted from either human error or silent heat, which is a typical problem in cows with HS. One of the possible causes for the reduction in estrous behavior in summer could be a reduction in estradiol level [22]. Low estradiol levels would not be sufficient to induce the LH (luteinizing hormone) surge, resulting in a prolonged estrous cycle in the summer period. Furthermore, the percentage of non-estrus + new CL cows in moderate HS (9/22, 40.91%) was significantly higher than in mild HS (3/22, 13.64%). Therefore, these findings were assumed to show that cows with mild HS responded to PGF2α administration and showed better follicular development and estrous signs than cows with moderate HS. Using hormone injections such as PGF2α (one or double injection protocol) could increase the estrous detection rate during HS conditions.

The number of estrous and/or those artificially inseminated cows was observed in the following estrous cycle and did not differ between the two groups. Possible reasons for this result were that the number of new ovulations in the previous cycle did not differ because the hormone injections were sufficient to trigger ovulation equally well even under moderate HS or that temperature variation throughout the day could affect follicular development. The result of this study is similar to that of Pongpiachan et al. [39], who reported that the incidence of spontaneous estrous behavior after calving did not differ significantly in each season. However, the percentage of estrus observed between November and January (the cold to dry season; 74/338, 21.9%) was higher than between March and May (the hot to dry season; 29/195,14.9%) and June to September (41/265, 15.5%). The latter two seasons may be lower than the percentages in this study because the cows were induced with hormones after calving (36.36% and 40.91%, for moderate and mild HS, respectively).

In this study, the reproductive index was concerned with FSCR and CTFS. Both indices could be affected by the level of HS as a result of its effects on follicular development and the estrous cycle. These measures did not consider repeat breeding cows and prolonged anestrus with many unknown factors. However, neither estrous indicators, the presence of the CL, nor the incidence of artificial insemination differed in this study. The FSCR under mild HS tended to be higher than in moderate HS (p = 0.06), and the average CTFS of cows under mild HS was significantly shorter than cows under moderate HS. During HS, altered hormonal levels have affected follicular development, delayed ovulation [6,19], and resulted in an inappropriate timing of fertilization between the oocyte and the sperm. Furthermore, HS reduces the potential for oocyte development [40]. Therefore, HS led to a lower FSCR [41,42] and a prolonged average CTFS [23]. Therefore, it might also directly affect the extension of open days (calving-to-conception interval) [24].

In this study, RFM was recorded in cows during moderate and mild HS (Tables 1 and 5). The incidence of RFM was higher in moderate than in mild HS conditions, which aligns with previous studies [29–31]. HS increased reactive oxygen species and the immune response [7,32], causing atony of the uterus during calving [33]. This mechanism might increase the percentage of RFM during the summer and explain the results reported here.

5. Conclusions

HS influenced reproductive performance and general physiological responses in cross-bred Holstein Friesian dairy cows under tropical conditions in Ratchaburi province, Thailand. The FSCR of cows in mild HS tended to be higher than that of cows in moderate HS. The average CTFS of cows under mild HS was shorter than that of cows under moderate HS. Cows with mild HS had a relatively better response to PGF2α when compared to cows with moderate HS. The average RT and RR of cows were more affected in moderate than mild HS. This research indicates that HS potentially impairs the reproductive health of cows raised in tropical climates like Thailand across the year and has a more significant impact on cows during moderate than mild HS conditions.

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Institutional Review Board Statement: The present study was approved by the Institutional Animal Care and Use Committee and performed in accordance with the guidelines of animal care and use under the Ethics Board of the Office of the National Research Council of Thailand for the performance of scientific research. The approval number is ACKU62-VET-047, dated on 25 September 2019.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

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