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RESEARCH ARTICLE

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Effects of *in utero* heat stress on subsequent reproduction performance of first-calf Holstein heifers

María I. Chavez (Chavez, MI)[1], José E. García (García, JE)[2], Francisco G. Véliz (Véliz, FG)[1], Leticia R. Gaytán (Gaytán, LR)[1], Ángeles de Santiago (de Santiago, A)[1] and Miguel Mellado (Mellado, M)[2]

[1] *Autonomous Agrarian University Antonio Narro, Dept. Veterinary Science, Torreon, Coahuila, 27054 Mexico.*
[2] *Autonomous Agrarian University Antonio Narro, Dept. Animal Nutrition, Saltillo, Coahuila, 25315 Mexico.*

Abstract

Aim of study: To determine the reproductive performance of heifers gestated under maternal conditions of heat stress in late gestation.

Area of study: Northern Mexico (25° 32' N, 103° 23' W).

Material and methods: The study included reproductive records of 4976 first-calf Holstein heifers in a hot environment.

Main results: Heifers born to cows experiencing no heat stress three months before parturition but with a THI >83 at calving were older ($p<0.05$) at first calving (743 ± 67 *vs.* 729 ± 55 days) than heifers gestated under maternal conditions of heat stress. A two-fold increase ($p<0.01$) in pregnancy rate occurred in heifers gestated under maternal conditions of no heat stress during two or three months before pregnancy and no heat stress at parturition, compared with heifers gestated under maternal conditions of no heat stress. Overall, across *in utero* heat stress one, two or three months before calving, pregnancy rate to all services was higher ($p<0.05$) for first-calf heifers gestated under maternal conditions of no heat stress during delivery, compared with heifers gestated under maternal conditions of heat stress (66.7 *vs.* 51.1%). Median days for getting pregnant was higher (140 d) for heifers whose dams were exposed to THI >83 at calving than heifers whose mothers were exposed to <76 or 76-83 (117 and 114 d) at calving.

Research highlights: These data suggest that in utero heat stress during the last three months of gestation negatively affects the reproductive performance of first-calf Holstein heifers.

Additional keywords: conception rate; twining rate; services per pregnancy; age at first calving; fetal losses.

Abbreviations used: AI (artificial insemination); AFC (age at first calving); BCS (body condition score); DM (dry matter); FTAI (fixed-time artificial insemination); IgG (immunoglobulin G); THI (temperature-humidity index).

Authors' contributions: Data acquisition: MIC, LG. Study design and drafted the manuscript: MM. Analyzed the results: JEG, MM. Revised the manuscript and reviewed the pertinent literature: FGV, AS. All authors read and approved the final version of the manuscript.

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Correspondence should be addressed to Miguel Mellado: mmellbosq@yahoo.com

Introduction

High body temperature during late pregnancy causes distresses in the intrauterine environment, which can induce functional alterations in the fetus that could persist at maturity (Skibiel *et al.,* 2018). A positive consequence of maternal heat stress during late gestation on calf function during postnatal life is the enhancement thermo-tolerance at maturity by increasing the ability to dissipate body heat which reduces their core body temperature, which is reflected in lower sweating rate and rectal temperature when exposed to high ambient temperatures (Ahmed *et al.,* 2017).

However, other potential physiological processes which alter fetal programming in cows born from heat-stressed cows have a detrimental effect on productive and reproductive response in dairy cows. Lower body weight at 12 months of age has been observed *in utero* heat-stressed cows, although no difference in this trait at maturity was detected, but the productive life of these cows in the herd was reduced (Monteiro *et al.,* 2016). Also, maternal heat stress in late gestation depress birth weight of calf at parturition and hampers mammary growth and the absorption of IgG, which lower serum IgG concentration in young calves (Tao *et al.,* 2012, Tao & Dahl, 2013; Monteiro *et al.,* 2014; Laporta *et al.,* 2017).

While the negative effects of *in utero* heat stress on birth weight, weaning weight, passive immune transfer and milk yield in dairy cattle have been well documented, information on the exposure of developing fetuses to heat stress *in utero* in late gestation on subsequent reproductive performance in dairy cattle is incomplete. Likewise, the stages of pregnancy in which heat stress has the most adverse effects on fetuses have not been well established. Therefore, this study was carried out to determine if bovine fetal exposure to heat stress during the last three months of pregnancy negatively alters the subsequent reproductive performance of these animals. It was hypothesized that first-calf heifers born to cows exposed to heat stress during late gestation would decrease reproductive performance by increasing age at first calving, decreasing pregnancy rate, and requiring greater services per pregnancy.

Material and methods

Animal management and facilities

The experimental procedures and animal care conditions were approved by the Ethics Committee of the Research Department of the Autonomous Agrarian University Antonio Narro. This study included 4976 first-lactation Holstein heifers from a large commercial dairy operation in a hot arid environment (26° N, elevation 1140 m, mean annual rainfall 230 mm, mean annual temperature 23.7 ºC). The dairy operation consisted of approximately 3000 lactating Holstein cows housed in open-dirt pens equipped with a fixed metal framework shade in the center of pens that provided 4 m^2 of shade per cow. Shades also covered feed alleys. The study took place from 2015 to 2018.

All diets were offered as total mixed rations (49% forage and 51% concentrate; DM basis) and were served at 07:00 and 14:00 h. Feed was offered calculating 35 g refusals for each kg offered. Diets were formulated to meet the net energy requirements (1.62 Mcal/kg net energy of lactation and 18% crude protein) for lactating Holstein cows weighing 650 kg and producing 42 kg of milk containing 35 g/kg of fat and 30 g/kg of protein when consuming 24 kg/d of DM (NRC, 2001). Cows in this study were born to dams not exposed to heat stress (temperature-humidity index, THI <76), exposed to moderate heat stress (THI 76-83), or exposed to high heat stress (THI >83) during one, two or three months before delivery and at parturition.

The THI value of 76 was chosen because it was deemed as the upper critical temperature, as hyperthermia (based on increased rectal temperature) would be expected at THI above this value (Dikmen & Hansen, 2009). Kaufman *et al.* (2018) found that THI of 76.4 practically did not alter rectal temperature of Holstein cows. It is worth mentioning that the upper critical THI used in the current study is higher than values of 72 for dairy cows in Georgia and 74 for cows in Arizona (Bohmanova *et al.,* 2007), although these values derived from studies relating THI with losses in milk production.

Reproductive management

All cows in this herd were vaccinated against diseases impairing fertility, such as brucellosis (*Brucella abortus* strain RB51 vaccine), infectious bovine rhinotracheitis, bovine respiratory syncytial virus, bovine viral diarrhea, para-influenza, *Campylobacter fetus* and leptospirosis (5-serovars). Herd veterinarians examined fresh cows to identify and treat cows with postpartum infection and inflammation in the reproductive tract such as retained placenta, metritis, and clinical endometritis.

Cows became eligible for artificial insemination (AI) after 50 days postpartum. Technicians kept trying to achieve pregnancy until 360 days in milk or when milk yield dropped below 25 kg/d; this practice leads to more than 12 services per cow in some animals, and consequently to extended lactations (>500 days). First-calf heifers not pregnant around 200 post-partum and with more than 3 services were submitted for fixed-time artificial insemination (FTAI) using the Ovsynch protocol.

Nonpregnant first-calve heifers were observed for estrus three times a day, for approximately 30 min. Estrus detection was reinforced with the aid of pedometers, and AI was conducted based on visual observation of estrous behavior, according to the a.m. - p.m. guideline. Commercial non-sexed frozen-thawed semen from multiple sires was used across all months of the year. Pregnancy was detected by palpation of the uterus per rectum about 45 days post-AI.

Data collection

Data from reproductive examinations and AI were collected by the herd veterinarians during daily herd health revision for cows included in this study. For each animal, the following variables were recorded: age at first calving (AFC), occurrence of dystocia (prolonged assisted extraction), retained fetal membranes (retained fetal membranes for more than 24 h), occurrence of twin pregnancies, metritis, premature calves (<265 days, as determined by AI records) and fetal losses (expulsion of a fetus or presence of extraembryonic membranes and vaginal discharges; return to service after being confirmed pregnant). Also, pregnancy rate at first service, pregnancy rate to all services, and time from calving to pregnancy were recorded. Cows experiencing calving-related disorders were appropriately treated by the herd veterinarians, so that they were healthy by the time the first breeding postpartum occurred. Body condition scores (BCS) were determined at calving using the scoring system suggested by Edmonson *et al.* (1989).

Additional variables were THI, the day dams delivered the first-calf heifers included in the study, THI one, two or three months before calves were born. Climatic data were obtained from a meteorological station situated 3 km away from the dairy operation for the duration of the study. Information recorded was daily maximum temperatures (mercury thermometer under full shade) and relative humidity. The following equation was used for calculation of THI (highest daily temperature in Celsius degrees; RH refers to maximum relative humidity; Mader *et al.* 2006):

$$THI = (0.8 \times temperature) + (\%RH/100) \times (temperature - 14.4)] + 46.4$$

Statistical analyses

To analyze the combined effect of maximum ambient temperature and humidity contributing to pregnancy (binary outcome), fetal losses, twin births, premature births and dystocic parturition, a logistic regression model of SAS (SAS Inst. Inc., Cary, NC, USA) was used. The model included the following potentially explanatory variables of interest: THI when first-calf heifers were born, THI one, two or three months before the first-calf heifers were born and one-way interactions. Interactions were only retained if significant at the 0.15 level. The SAS model included fixed effects of THI groups with first-calf heifers within the THI groups as a random effect. Covariates used in the model included BCS at calving and year of breeding.

AFC was analyzed using the MIXED procedure of SAS. The model included the main effects of THI at various stages of fetal life and one-way interactions; year and BCS at calving were included as covariates. The number of services per pregnancy was evaluated by the bivariate Wilcoxon rank-sum test (non-parametric; proc npar1way of SAS). The effect of THI at various periods in late gestation on the interval from calving to pregnancy was analyzed using survival plots generated by Kaplan-Meier survival analysis performed with Statgraphics Centurion version XVII software (Statgraphics Technol., Inc., The Plains, Virginia). The final models were assessed for collinearity using the variance inflation factor (VIF option) in SAS; collinearity did not exist. Statistical significance was set at $p<0.05$.

Results

The percentage of fetal losses (16%), twining rate (0.7%), dystocic parturition (10%), and premature calves (5%) were not affected ($p>0.10$) by the THI prevailing during the last three months of the uterine life of first-calf heifers included in this study. THI when calves were born affected ($p<0.05$) AFC of these first-calf heifers. THI one, two, or three months previous to delivery did not affect this trait. There was a THI at parturition by THI one month before calving interaction ($p<0.05$). When heat stress was moderate or high for two months previous to parturition, the age at first calving was shorter ($p<0.05$) in cows born to dams not exposed to heat stress the day of parturition (Table 1). Likewise, heifers born to cows not exposed (THI <76) or exposed to severe heat stress (THI>83) three months before parturition but with no heat stress at parturition of their dams presented shorter AFC than heifers whose mothers experienced mild or severe heat stress at parturition.

Pregnancy rates at first service are summarized in Table 2. Pregnancy rate at first service was severely depressed ($p<0.05$) in first-calf heifers born to dams exposed to moderate (THI 76-83) heat stress one month before calving and severe heat stress (THI >83) the day of parturition. Regardless of the magnitude of heat stress exposure of dams in late gestation, first-calf heifers born to dams exposed to heat stress the day of calving presented a marked reduction in first-service pregnancy rates compared with heifers born to dams not exposed to severe heat stress. There was a THI at calving of dams by THI one month before calving interaction ($p<0.05$).

Pregnancy rates to all services are summarized in Table 3. THI at calving and during the first, second, and third month before parturition of dams affected ($p<0.01$) this reproductive variable. THI at calving of dams × THI one month before calving was an interaction term significantly associated with pregnancy rates to all services. Overall, across THI one, two or three months before calving of dams, pregnancy rate was depressed ($p<0.05$) when parturition of their dams coincided with severe heat stress.

The number of services per pregnancy confirmed at d 45 after the last AI was smaller in first-calf heifers from dams experiencing moderate and severe heat stress one, two and three months before calving and THI 76-83 at

Table 1. The effect of the temperature-humidity index (THI) experienced by first-calf Holstein heifers at delivery and previous to delivery *(in utero)* on the subsequent age at first calving in a hot environment (mean annual temperature 23.7 °C).

Variable	THI 1-month before delivery		
	<76	76-83	>83
THI at delivery <76[1]	732 ± 55	737 ± 61	740 ± 68
THI at delivery 76-83	737 ± 56	740 ± 60	740 ± 62
THI at delivery >83	737 ± 64	746 ± 66	741 ± 62
	THI 2-months before delivery		
	<76	76-83	>83
THI at delivery <76	732 ± 58	736 ± 57^{a}	733 ± 57^{a}
THI at delivery 76-83	736 ± 57	738 ± 63^{ab}	745 ± 63^{b}
THI at delivery >83	739 ± 64	745 ± 65^{b}	741 ± 61^{b}
	THI 3-months before delivery		
	<76	76-83	>83
THI at delivery <76	729 ± 55^{a}	735 ± 59	735 ± 58^{a}
THI at delivery 76-83	735 ± 56^{ab}	737 ± 58	747 ± 65^{b}
THI at delivery >83	743 ± 67^{b}	742 ± 62	742 ± 62^{ab}

[1]THI at parturition × THI one month before calving interaction (p<0.05). [ab]Within groups, values in columns with different superscript letters differ (p<0.05).

Table 2. Pregnancy rate at first service of first-calf Holstein heifers gestated under maternal conditions of no heat stress (temperature-humidity index; THI<76), moderate heat stress (THI 76-83), or severe heat stress (THI>83) at delivery and one, two or three months before calving in a hot environment.

Variable	THI 1-month before delivery		
	<76	76-83	>83
THI at delivery <76 [1]	149/1130 (13.2)^{a}	37/343 (10.8)^{a}	9/105 (8.6)^{a}
THI at delivery 76-83	46/477 (9.6)^{b}	118/909 (13.0)^{a}	73/651 (11.2)^{b}
THI at delivery >83	10/85 (11.8)^{a}	28/416 (6.7)^{b}	71/860 (8.3)^{a}
	THI 2-months before delivery		
	<76	76-83	>83
THI at delivery <76	99/840 (11.8)^{a}	61/ 437 (14.0)^{a}	35/301 (11.6)^{a}
THI at delivery 76-83	104/874 (11.9)^{a}	57/449 (12.7)^{a}	76/714 (10.6)^{a}
THI at delivery >83	13/233 (5.6)^{b}	48/520 (9.23)^{b}	48/608 (7.9)^{b}
	THI 3-months before delivery		
	<76	76-83	>83
THI at delivery <76	51/386 (13.2)^{a}	77/693 (11.1)a	67/499 (13.4)^{a}
THI at delivery 76-83	87/780 (11.2)^{a}	75/567 (13.2)a	75/690 (10.9)^{a}
THI at delivery >83	18/300 (6.0)^{b}	57/597 (9.6)b	34/464 (7.3)^{b}

[1]THI at calving of dams × THI one month before calving interaction (p<0.05). [ab]Within groups, values in columns with different superscript letters differ (p<0.05).

Table 3. Pregnancy rate to all services of first-calf Holstein heifers gestated under maternal conditions of no heat stress (temperature-humidity index; THI<76), moderate heat stress (THI 76-83), or severe heat stress (THI >83) at delivery and one, two or three months before calving in a hot environment.

Variable	THI 1-month before delivery		
	<76	76-83	>83
THI at delivery <76[1]	787/1130 (69.7)[a]	223/343 (65.0)[a]	42/105 (40.0)[a]
THI at delivery 76-83	292/477 (61.2)[b]	565/909 (62.2)[a]	378/651 (58.1)[b]
THI at delivery >83	30/85 (35.3)[c]	212/416 (51.0)[b]	453/860 (52.7)[c]
	THI 2-months before delivery		
	<76	76-83	>83
THI at delivery <76	585/840 (69.6)[a]	292/437 (66.8)[a]	175/301 (58.1)
THI at delivery 76-83	293/ 581 (66.5)[a]	260/449 (57.9)[b]	394/714 (55.2)
THI at delivery >83	103/233 (44.2)[b]	276/520 (53.1)[b]	316/608 (52.0)
	THI 3-months before delivery		
	<76	76-83	>83
THI at delivery <76	290/386 (75.1)[a]	480/693 (63.3)[a]	282/499 (56.5)[a]
THI at delivery 76-83	537/780 (68.9)[b]	335/567 (59.1)[b]	363/690 (52.6)[a]
THI at delivery >83	158/300 (52.7)[c]	317/597 (53.1)[c]	220/464 (47.4)[b]

[1]THI at calving of dams × THI one month before calving interaction ($p<0.05$). [ab]Values in rows with different superscript letters differ ($p<0.05$).

Table 4. Services per conception in first-calf Holstein heifers gestated under maternal conditions of no heat stress (temperature-humidity index; THI<76) moderate heat stress (THI 76-83) or severe heat stress (THI>83) at delivery and one, two or three months before calving in a hot environment.

Variable	THI 1-month before delivery		
	<76	76-83	>83
THI at delivery <76[1]	4.6 ± 3.3[a]	5.4 ± 3.7[a]	4.7± 3.8[a]
THI at delivery 76-83	4.5 ± 3.1[a]	4.2 ± 3.1[b]	4.2 ± 3.3[b]
THI at delivery >83	3.3 ± 3.0[b]	4.6 ± 3.1[b]	5.0 ± 3.6[a]
	THI 2-months before delivery		
	<76	76-83	>83
THI at delivery <76	4.8 ± 3.4	4.8 ± 3.4	5.0 ± 3.7[a]
THI at delivery 76-83	4.3 ± 3.1	4.3 ± 3.5	4.2 ± 3.1[b]
THI at delivery >83	4.7 ± 3.2	4.8 ± 3.5	4.9 ± 3.4[a]
	THI 3-months before delivery		
	<76	76-83	>83
THI at delivery <76	4.7 ± 3.5[ab]	5.0 ± 3.4[a]	4.5 ± 3.5[ab]
THI at delivery 76-83	4.3 ± 3.1[a]	4.1 ± 3.2[b]	4.2 ± 3.3[a]
THI at delivery >83	5.0 ± 3.3[b]	4.8 ± 3.5[a]	4.6 ± 3.4[b]

[ab]Values in rows with different superscript letters differ ($p<0.05$).

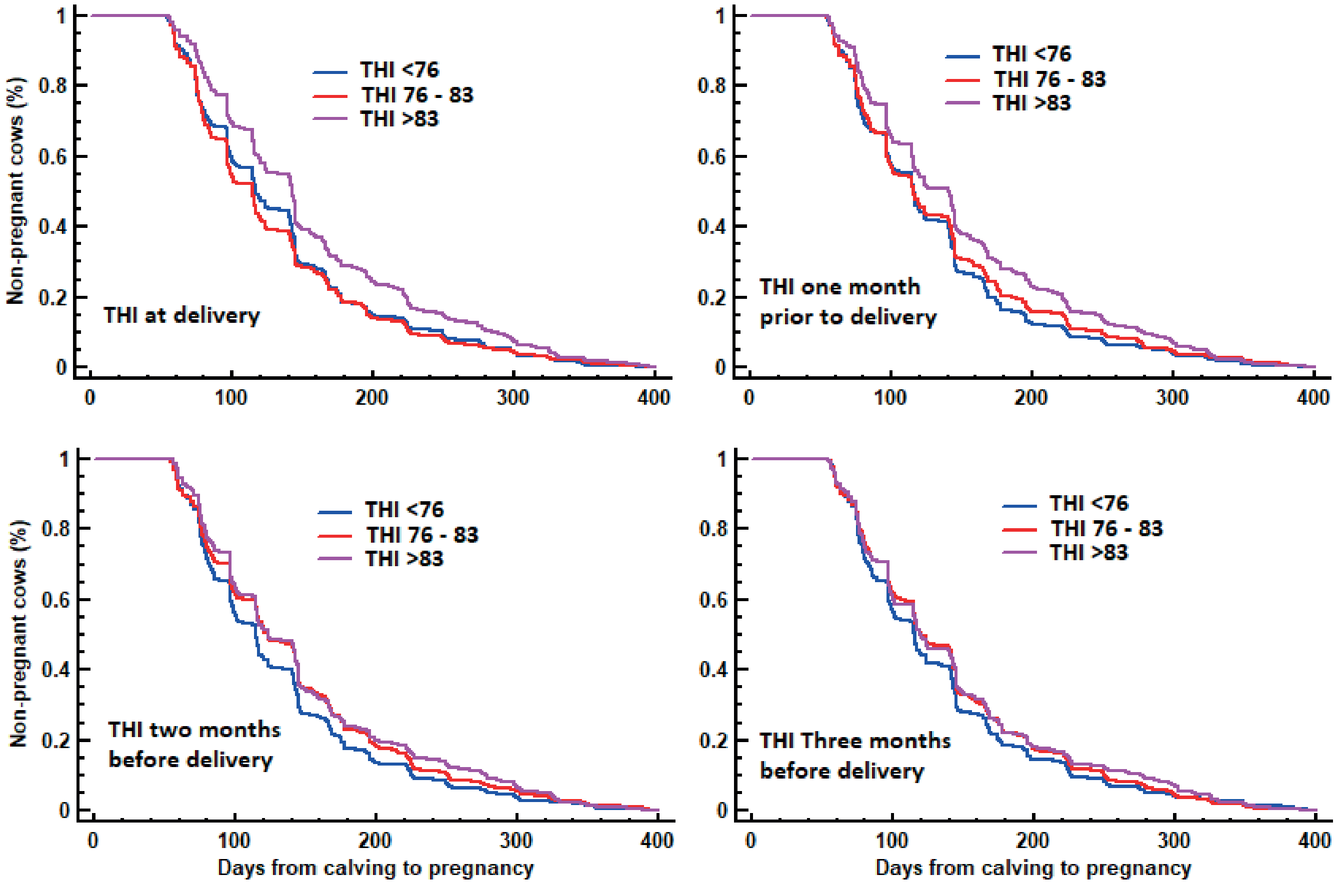

Figure 1. Kaplan-Meier survival curves for the proportion of first-calf heifers gestated under maternal conditions of heat stress in late gestation. Days open were lower for heifers whose dams were exposed to THI <76 and those born at 76-83 and >83 three months before calving (p<0.001; Wilcoxon test). Median days for getting pregnant were 117, 114, and 140 for heifers whose dams were exposed to THI <76, 76-83, and >83 at calving, respectively. Median days for getting pregnant were 115, 115, and 120 for heifers whose dams were exposed to THI <76, 76-83, and >83 one month before calving, respectively. Median days for getting pregnant was 114, 119 and 117 for heifers whose dams were exposed to THI <76, 76-83 and >83 two months before calving, respectively. Median days for getting pregnant were 115, 117, and 117 for heifers whose dams were exposed to THI of <76, 76-83, and >83 three months before calving, respectively.

calving (Table 4). For first-calf heifers born to dams exposed to different THI categories at birth, one, two and three months before calving, the median days open for heifers undergoing severe heat stress *in utero* was larger (p<0.05) than that of heifers exposed to moderate or no heat stress *in utero* (Fig. 1).

Discussion

The prevalence of fetal losses in the present study corresponds well with literature reports in hot environments (Pontes *et al.,* 2015; Mellado *et al.,* 2016), but it is much higher than the 4.8-9.8%% reported for Holstein heifers in temperate climates (Ettema & Santos, 2004; Bach, 2011). This reproductive disorder was not affected by the exposure of dams to heat stress at parturition or various periods before calving. The same was true for twining rate, dystocic parturition, and premature calves. Thus, there was no evidence of the negative effects of maternal heat stress on these periparturient disorders on offspring. Dystocia, premature deliveries, stillbirths, and abortions, are linked, in part, to endocrine changes in late pregnancy which impair pregnancies (Kindahl *et al.,* 2002; Sandman *et al.,* 2003); thus, these results do not support the view that *in utero* heat stress altered hormones related to calves delivery.

An important finding of this study was that *in utero* heat stress was associated with an increase in the AFC of heifers. This response seems to be linked to the fact that *in utero* heat-stressed calves are lighter at birth compared to non-heat stressed animals (Tao *et al.,* 2012; Monteiro *et al.,* 2014). Additionally, these lighter calves born from heat-stressed cows in late pregnancy do not have any compensatory growth before puberty, as they remained smaller and lighter up to one year of age (Monteiro *et al.,* 2016). Thus, heifers suffering heat stress *in utero* apparently do not achieve rapid growth rates due to reduced fetal secretion of insulin-like growth factor-I, prolactin, and insulin (Guo *et al.,* 2016), which did not enable them to achieve breeding size earlier, lessening AFC which increases costs of raising replacement heifers (Davis Rincker *et al.,* 2011).

Overall, first-service pregnancy rate was very low in first-calf Holstein heifers (10.9%) in this hot environment, but is in line with values observed in dairy operations in this hot environment (Flores *et al.,* 2019). In temperate and tropical zones the percentage success at first service in dairy cattle has ranged between 26.7% and 49.6% in previous studies (Tillard *et al.,* 2008; Fodor *et al.,* 2018), which is much higher than the figure found in the present study, even when careful attention was given to reproductive management. This poor response is due to chronic heat stress suffered by cows in this zone which causes impaired fertilization (Sartori *et al.,* 2002; Moghaddam & Karimi, 2009), oocyte quality (Hansen, 2009; Souza-Cácares *et al.,* 2019), sperm viability and hampers embryonic development (Sakatani *et al.,* 2015; Wolfenson & Roth, 2019).

First-calf heifers born to cows with moderate (THI 76-83) and severe (THI >83) heat stress, one, two or three months before calving and severe heat stress at calving consistently presented a marked reduction in pregnancy rate at first service. The rise of internal body temperature in high-milk yielding dairy cows subjected to heat stress (Wolfenson & Roth, 2019) causes a hyperthermic uterine environment which seems to influence the phenotypic variation of offspring mediated by epigenetic modifications regulating gene expression (Skibiel *et al.,* 2018). Thus, heat stress in late pregnant Holstein cows seems to produce indirect developmental and post-natal effects in bovine offspring, adversely impacting reproduction function as evidenced by a noticeable reduction in pregnancy rate at first service. Reports suggest that epigenetic mechanisms are the link between early-life environment and fitness later in life (Gabory *et al.,* 2011; Jammes *et al.,* 2011); however, the underlying mechanisms involved in the association between the uterine environment of developing fetuses and postnatal reproductive function remain unclear.

In general, regardless of THI in the last trimester of gestation, pregnancy rate to all services was reduced in first-calf heifers gestated under maternal conditions of heat stress compared to heifers gestated with no heat stress in late gestation. The associations between late gestational exposure to heat stress and subsequent heifer reproductive function are complex and have yet to be elucidated but could be attributed, in part, to the fact that late-gestation heat stress compromises placental growth due to reductions in secretion of progesterone and placental lactogen (Bell *et al.,* 1989), which leads to fetal malnutrition, hypoxia, and fetal growth retardation (Tao & Dahl, 2013). Additionally, heat stress during late gestation depresses cell proliferation in tissues, thereby reducing cell number and restricting tissue growth (Harding & Johnson, 1995; Fowden *et al.,* 1998). *In utero* heat stress also reduces immune function and alters systemic metabolism (Flouris *et al.,* 2009; Tao *et al.,* 2012). Thus, *in utero* acute heat-stressed calves probably presented alterations in their reproductive organs which was reflected in lower reproductive capacity in adulthood. Insults during late gestation alter cell proliferation and program their morphology in postnatal life, which seems to contribute to the reduced reproductive performance of *in utero* heat-stressed heifers (Skibiel *et al.,* 2018).

Services per pregnancy in the present study were extremely high (4.6 ± 3.4; Table 4). This value was far higher than the 2.7 services per conception in Holstein herds in southeastern United States (Norman *et al.,* 2009), but is close to the mean number of services per pregnancy > 6 (Flores *et al.,* 2019) typical of dairy herds in this zone. To put this value into perspective, services per pregnancy for the whole herd (only pregnant cows) was 6.7 ± 3.4. High ambient temperature for the most of the year was responsible for the impaired reproduction in this dairy herd (Mellado *et al.,* 2013). The present study provides no clear evidence that services per pregnancies were reduced in first-calf heifers gestated under maternal conditions of heat stress (THI >83) compared to heifers born to dams not exposed to heat stress in late pregnancy. Therefore, measurement of ambient and humidity during late pregnancy in Holstein dams do not help to predict services per pregnancy in their first-calf offspring under the management and ambient conditions of the present study.

However, the calving-to-conception interval was longer in first-calf heifers gestated under maternal conditions of heat stress compared to heifers gestated with no heat stress the last trimester of pregnancy. It is worth mentioning that, the effect of *in utero* heat stress on calving-to-conception interval was not adjusted for milk yield and this variable could have an important impact on this variable. Given that animals in this dairy operation received adequate diets throughout lactation, deficient dietary protein or energy early in lactations were not responsible for this response. Intervals from calving to first ovulation neither seemed to be involved in this response as most first-calf heifers presented behavioral estrus repeatedly before getting pregnant. Thus, the greater struggle to become pregnant in first-calf heifers born to cows exposed to heat stress in late pregnancy apparently derive from induced structural and functional changes of the reproductive tract or disruption of the endocrine systems controlling the reproductive organs in the fetus that apparently persisted through adulthood (Skibiel *et al.,* 2018), and possibly hindered the production of viable ovum, its fertilization and implantation in the uterus.

These data support the hypothesis that exposure of high-milk producing dairy cows to heat stress during the third trimester of pregnancy and at calving is negatively associated with reproductive function of their first-calf heifers, but the causes of this fertility depression are unclear. Thus, intervention strategies for minimizing the duration and degree of heat stress at the end of gestation could result in offspring with enhanced reproductive ability.

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