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RESEARCH ARTICLE

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Effect of age at first calving and heat stress at parturition on reproductive efficiency and postpartum disorders in Holstein heifers

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Abstract

The objective of this study was to evaluate the impact of age at first calving (AFC) and climatic conditions at calving on peripartum disorders and reproductive performance in Holstein heifers in a hot environment. A total of 3000 reproductive records from a large highly technified dairy farm were used; the variables evaluated were temperature humidity index (THI; <77, 77-83, >83) at calving and AFC (<2.0, 2.0-2.2 and >2.2 years). Across age groups, the cases of dystocic parturition increased ($p<0.05$) when the deliveries occurred with severe heat stress (4.3% *vs.* 3.3% for THI >83 and <83 units, respectively). Across THI, conception rate at the first postpartum artificial insemination (AI) was lower ($p=0.02$) for heifers calving for the first time >2.2 years compared to heifers calving between 2.0 and 2.2 and <2.0 years (9.8, 15.3 and 13.7%, respectively). Conception rate at first AI postcalving was higher ($p<0.01$) in heifers calving with THI less than 83 units than in heifers calving with a THI >83 units (16.8 *vs.* 5.4%). The conception rate considering all services was affected ($p<0.05$) by AFC (42.7, 50.4 and 40.9% for AFC <2.0, 2.0-2.2 and >2.2 years, respectively). The interaction AFC × THI at calving was significant ($p<0.05$). The occurrence of metritis was higher ($p<0.05$) in heifers <2.0 years of age at calving than those calving after 2 years of age. The presence of ovarian cysts was less common ($p<0.05$) in heifers with greater AFC. In conclusion, increasing the AFC in Holstein heifers had no benefits in reproduction and health, compared with heifers calving at <2.0 years. These data in a hot climate suggest that heifers should be selected to begin their first lactation before 2 years of age.

Additional keywords: temperature humidity index; dystocia; conception rate; services per conception; preterm birth.

Abbreviations used: AFC (age at first calving); AI (artificial insemination); BHBA (β-hydroxybutyric acid); DIM (days in milk); NEFA (non-esterified fatty acids); THI (temperature–humidity index).

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Introduction

Age at first calving (AFC) is a greatly important economic trait determining the profitability of intensive dairy operations, therefore, one strategy for reducing the costs of milk yield could be the shortening of the rearing period. In zones with intense and prolonged warm weather calves' growth rate is reduced (López *et al.*, 2018), and therefore, could prolong AFC, an important variable for measuring reproductive behavior in dairy cows (Oikawa, 2017).

The optimum AFC in Holsteins for maximum profit and without negative effects on milk yield and health during their lifetime should be 23 to 24.5 months of age (Ettema & Santos, 2004; Nilforooshan & Edriss, 2004). Moreover, reducing AFC can reduce rearing costs and increase the number of calves per cow (Tozer & Heinrichs, 2001). However, a reduction in AFC in first-parity Holsteins cows has been associated with a lower first-lactation milk production (Ettema & Santos, 2004; Curran *et al.*, 2013) and increased dystocia (Zaborski *et al.*, 2009; Gaafar *et al.*, 2011), which commonly occurs when the heifers are under 22 months old. Thus, dystocia is a negative factor for both reproduction, health and body weight (Moussavi & Mesgaran, 2008). Dystocia is a serious problem in most dairy farms because it reduces milk production by the disorders that result from this reproductive problem (Mee, 2008).

Difficult calvings increase uterine infections (Dubuc *et al.*, 2010) and reduce reproductive efficiency (Gaafar *et al.*, 2011). Thus, complications at birth result in loss of milk production, reproductive behavior (Mokhtari *et al.*, 2016), increased days open (Gaafar *et al.*, 2011), as well as mortality and morbidity in calves (Bicalho *et al.*, 2007; Lombard *et al.*, 2007).

The combined effect of AFC and duration and intensity of heat stress on periparturient disorders and reproduction performance of heifers has not been studied in depth. Also, until now there have been limited studies to assess the impact of AFC on the reproductive tract health postpartum in dairy heifers. Therefore, it was considered relevant to elucidate the impact of AFC and climatic conditions during parturition on the reproductive performance of Holstein heifers. The objective of this study was to assess the impact of AFC and heat stress at calving on disorders derived from parturition as well as the reproductive performance in first calf Holstein heifers in a warm arid environment.

Material and methods

Site of study, animals and feeding

The dairy farm where this study was carried out is located in northern Mexico (26° N, and 103° W) at an altitude of 1140 m above sea level with an annual rainfall of 230 mm, and an average annual temperature of 23.9° C. The minimum and maximum temperature during the study period (2016-2017) was 2.1 and 42.6 °C, respectively (THI range 69.8 to 86.7). A total of 3,000 complete lactation records of Holstein heifers were used from a single commercial large dairy farm from 2016 and 2017. First calf heifers that were included in the study were milked two times a day and received a diet consisting of 49% forage and 51% concentrate (dry basis) to meet the nutritional requirements for a 550 kg lactating Holstein cow with 35 kg of milk per day, with 3.5% fat and 3.2% protein (NRC, 2001). Heifers were housed in large, open-air, dirt-floor pens and water was provided at libitum. Heat stress negatively affects all stages of the heifers' fertility in this area; therefore, involuntary extended lactations are common in this dairy herd.

Reproductive and health management

All heifers were vaccinated against diseases interfering with the reproductive performance such as bovine viral diarrhea, infectious bovine rhinotracheitis, bovine respiratory syncytial virus, para-influenza (CattleMaster Gold FP5®, Zoetis, Mexico D.F., Mexico). Heifers were vaccinated twice a year against leptospirosis using a pentavalent leptospiral vaccine (LEPTAVOID-H®; Merck Sharp and Dohme Corp., Mexico DF) Also, all animals were vaccinated against brucellosis (strain RB51; BRUCEL®, PISA laboratories, Santa Catarina, Mexico). Estrus was detected visually twice daily during approximately one hour early in the morning and late in the evening. A tail head marker (KAMAR Inc., Steamboat Springs, CO, USA) was used as supplement to estrus behavior visual observation and heifers in estrus were inseminated according to the a.m./p.m. rule. After calving, first calf heifers were artificially inseminated for the first time after 50 days of voluntary waiting period. Semen from 12 bulls from accredited companies of USA was used for artificial insemination (AI) and inseminations were performed by eight experienced technicians. Pregnancy diagnoses were performed by palpation of the uterus at 45 ± 2 days from their last AI. Cows not conceiving to the first AI were reexamined for pregnancy after each subsequent insemination until 400 days postpartum.

The all-service conception rate was defined as the number of heifers that conceived after calving, out of the number of first calf heifers detected in estrus and inseminated. First service conception rate was defined as the number of first services resulting in pregnancy as a percentage of the total number of first services given to first calf heifers included in the study.

Case definitions

Abortions were registered by the herd veterinarians and were defined as spontaneous fetal losses before 260 days (Thurmond & Picanso, 1990). Dystocia was defined as a prolonged difficult birth requiring assisted extraction (score 4, according to Hansen *et al.*, 2004). Assistance was provided to heifers without calving progress around 90 min after amniotic sac or feet were outside the vulva (Schuenemann *et al.*, 2011). Premature births were defined as those parturitions between 260 and 270 days of gestation as determined by AI records (Karapinar & Dabak, 2008). All heifers were examined by transrectal palpation between 5 and 15 d after calving by the farm veterinarians who diagnosed, recorded and treated all the periparturient diseases. Cases of retained placenta were defined as the presence of placental debris 24 h after calving. Puerperal metritis refers to inflammation of the uterus (increased uterine volume and tense uterine wall) and purulent fetid, watery, brownish during the first three weeks after calving (Sheldon *et al.*, 2006). Ovarian cysts were detected by rectal palpation between 40 and 60 days postpartum and were defined as follicle-like structures, present on one or both ovaries, with a diameter of at least 2.5 cm for a minimum of ten days in the absence of luteal tissue (Vanholder *et al.*, 2006).

Climatological data and other variables recorded

The ambient temperature and humidity were recorded throughout the study period (2016-2017). The temperature-humidity index (THI) was calculated using the following equation (Thom, 1959):

$$THI = [0.8 \times \text{Temperature} + (\text{Relative humidity} / 100) \times (\text{Temperature}-14.4) + 46]$$

where T is the maximum temperature (ºC) and H is the highest relative humidity value in a given day. Other variables that were recorded were the heifer´s AFC (<2.0 years, 2.0-2.2 years, and >2.2 years), birth variables (abortion rate, occurrence of dystocia and premature births) and reproductive variables.

Statistical analysis

The incidence of dystocic births, premature births, abortion rates, occurrence of metritis and ovarian cysts were determined with the PROC FREQ / binomial procedure of SAS (SAS Inst. Inc., Cary, NC, USA). The conception rate considering all services, the conception rate at first service and the abortion rate, were analyzed using the GENMOD procedure of SAS. The model for these dependent variables contained the effect of AFC, THI at calving, year of service, AI technician and the interaction between these variables.

The occurrence of diseases associated with parturition and culling rate of cows were analyzed using the procedure GENMOD of SAS. The model for these dependent variables contained the effect of AFC, the THI at calving and the interaction between these variables. If the main effects were significant, the means were compared using the DIFF option in SAS. After limiting the number of services per conception to first-calf heifers with a confirmed diagnosis of gestation, the effect of THI and the AFC on the number of services per conception was evaluated by the bivariate Wilcoxon rank sum test (SAS NPAR1WAY procedure) without adjustment for confounding factors. A Kaplan-Meier survival curve for time to culling (sold or died) for heifers was carried out using the statistical program Statgraphics Centurion XV. Records of all cows with lactations lower than 260 days were censored (1.6% of the original data) at that date to avoid bias towards those cows with extended lactations, as very short lactations (<250 days) are usually due to problems of poor recording of the exact culling dates. For all the analyses, the significance was established at $p<0.05$.

Results and discussion

Table 1 shows the effects of AFC and THI on the occurrence of abortions, dystocia and preterm calves in first-lactation heifers. Regarding abortion rate, no significant differences were found ($p>0.05$) between THI levels and AFC. In the area where this study took place, heat stress has been identified as an important risk factor for fetal losses in lactating Holstein cows (Mellado *et al.*, 2016). The discrepancy between these

Table 1. Effects of age and heat stress at first calving on the occurrence of abortion, dystocic parturition and preterm birth in Holstein first calf heifers in a hot environment.

Calving variables[1]	<2 years	2-2.2 years	>2.2 years
Abortion rate (%)			
THI <77	12.2 (71/581)	13.3 (80/600)	13.2 (58/440)
THI 77-83	13.4 (70/523)	14.5 (66/455)	17.1 (59/345)
THI >83	13.8 (81/586)	12.4 (53/426)	14.3 (52/364)
Dystocia rate (%)			
THI <77	4.8 (28/581)	3.3 (32/600)	4.1 (18/440)[a]
THI 77-83	4.2 (22/523)	3.7 (17/455)	2.9 (10/345)[a]
THI >83	4.1 (24/586)	5.2 (22/426)	7.4 (27/364)[b]
Preterm birth rates (%)			
THI <77	3.6 (26/581)	5.0 (30/600)	5.5 (25/440)
THI 77-83	4.6 (24/523)	4.8 (22/455)	3.5 (12/345)
THI >83	4.8 (28/586)	5.2 (22/426)	3.9 (14/364)

[1]THI= temperature-humidity index. [ab]For traits listed, different superscripts within columns denote statistical differences ($p<0.05$).

studies seems to derive from the fact that during heat stress, days in milk (DIM) is barely reduced in heifers (West, 1999) and less metabolic heat is produced compared with pluriparous cows (Baumgard & Rhoads, 2013). In fact, in primiparous cows, severe heat stress does not affect milk production (Avendaño-Reyes *et al.*, 2009). The abortion rate observed in the present study (13.6%) was higher than the 4.8-9.8%% reported for Holstein heifers in temperate climates (Ettema & Santos, 2004; Bach, 2011).

Dystocic parturitions increased when parturitions occurred with severe heat stress (>83 units). Conflicting results have been described in the literature regarding the effect of heat stress on difficult parturitions. On one hand, some authors (Gaafar *et al.*, 2011; Atashi *et al.*, 2012) observed the lowest dystocia frequency with calvings occurring in summer. On the other hand, Neamt *et al.* (2015) observed the highest incidence of this reproductive disorder in summer. The average incidence of dystocia observed in the present study was 4.6%. This value is much lower than the 7-11% registered by other researchers in Holstein cows (Berry *et al.*, 2007; Atashi *et al.*, 2012). The fact that the younger heifers at first calving did not increase the odds of dystocia suggest that with a good nutritional level and consequently a good body development at parturition, dystocia can be reduced in heifers calving earlier than 2 years of age. Gardner *et al.* (1988) also addressed this issue and found that calving difficulty was not different between heifers on an elevated or restricted amount of energy from 6 weeks of age until breeding. Likewise, Berry & Cromie (2009) observed that heifers calving younger for the first time had not a greater likelihood of requiring some assistance at calving.

Preterm births were affected neither by AFC nor by THI >83 at calving. The lack of effect of heat stress at parturition on the occurrence of preterm births came as a surprise because late-gestation heat stress hampers placental growth, which results in fetal malnutrition and hypoxia (Tao & Dahl, 2013) and these are some of the etiologies that contribute to preterm labor (O'Brien & Lewis, 2016).

Premature activation of the fetal hypothalamic–pituitary–adrenal axis may result from fetal physiological stress (for example, insufficient utero-placental blood flow; Laburn *et al.*, 2017). The critical mediator of stress-induced premature births appears to be corticotropin-releasing hormone (Challis *et al.*, 2001), which leads to massive release of prostaglandin F2α (PGF2α), which triggers luteolysis and initiates parturition in the bovine (Attupuram *et al.*, 2016).

Additionally, heat stress results in higher cortisol concentrations in plasma in cows (Chen *et al.*, 2018) and cows giving birth prematurely have 100% higher plasma cortisol levels on the day of parturition than cows giving birth at term (Patel *et al.*, 1996). In fact, exposure of cows to elevated ambient temperature resulted in shorter gestations (Wright *et al.*, 2014).

Despite the fact that premature calves are smaller and weaker than full-term calves, these calves were viable at birth with few signs of prematurity such as reduced ability to stand or suckle. These results indicate that other factors apart from THI at calving and age of heifers at calving triggers the preterm parturition. Thus, preterm birth is not a condition in heat-stressed dams, but there are some other causes behind it, particularly infectious diseases (Kemmerling *et al.*, 2009; McAllister, 2016); however, this was not considered in this study.

Reproductive performance of first calf heifers according to AFC and climatic conditions at first parturition is presented in Table 2. Across THI values, heifers with AFC <2 or 2.0-2.2 years had higher (p<0.01) conception rates at first service than late-calving heifers. These results are partially in line with data of Ettema & Santos (2004) who reported higher conception rates at first AI in heifers calving between 701 and 750 days of age compared to heifers calving outside of this age range. Thus, it seems that heifers in the present study were reared to achieve an adequate body condition score at first calving and were fed a high energetically dense diet in early lactation which allows them to have a better conception rate at first AI compared to older heifers at first calving.

There was an AFC by THI interaction (p<0.05). The conception rate at first service of <2-year-old first calving heifers and 2-2.2-year-old first calving heifers was higher at THI <77 than >2.2-year-old first calving heifers; whereas conception rate at first service was similar for all age groups at THI >83 at calving. Across AFC groups, conception rate at first service was 17% lower in heifers with severe heat stress at calving compared with heifers with THI <77 units at parturition. There exists a wealth of data describing the negative effect of heat stress on fertility of dairy cows (De Rensis & Scaramuzzi, 2003), particularly there are clear seasonal patterns of conception rate in dairy cows (Mellado *et al.*, 2013). Some cows in the present study probably were no longer exposed to heat stress by the time they were bred by the first time, but heat stress was present the following months (Roth, 2008) and this explains the drastic reduction in conception rate in heifers calving under severe heat stress.

Across THI at calving, conception rates for all services were 13% lower (p<0.01) in >2.2-year-old first calf heifers compared with 2-2.2-year-old first-calf heifers. These results are at odds with other

Table 2. Reproductive performance of first calf Holstein heifers according to age at first calving and climatic conditions at first parturition (means ± SD).

Reproductive variables[1]	<2 years	2-2.2 years	>2.2 years
Conception rate 1st service (%)[2]			
THI <77	23.9 (139/581)[a,A]	26.6 (158/595)[a,B]	16.1 (70/436)[a,B]
THI 77-83	11.3 (59/523)[b,A]	9.1 (44/453)[b,A]	6.7 (23/341)[b,B]
THI >83	5.6 (33/586)[c]	5.4 (23/424)[c]	5.1 (18/356)[b]
Conception rate all services (%)[3]			
THI <77	52.8 (307/581)[a,A]	60.0 (360/600)[a,B]	44.6 (196/440)[a,C]
THI 77-83	45.7 (239/523)[b,A]	52.5 (239/455)[b,B]	29.9 (175/586)[b,C]
THI >83	29.9 (175/586)[c]	34.5 (147/426)[c]	34.3 (125/364)[c]
Services per conception[3]			
THI <77	5.4 ± 4.5	4.8 ± 3.6	5.1 ± 3.6
THI 77-83	5.3 ± 4.1	4.9 ± 3.5	5.8 ± 4.0
THI >83	5.1 ± 4.2	5.4 ± 3.7	5.5 ± 3.6

[1]THI= Temperature-humidity index. [2]Effect of the temperature-humidity index × age at first calving interaction (p<0.05). [3]Only pregnant heifers after first calving were included. [ab]For traits listed, different superscripts within columns denote statistical differences (p<0.05). [AB]For traits listed, different superscripts within rows denote statistical differences (p<0.05).

researchers who have found a reduced fertility in younger calving heifers as compared with heifers calving for the first time at an older age (Ettema & Santos, 2004; Berry & Cromie, 2009). Given that first-calving age, growth rate, and body weight at first calving are generally correlated (Le Cozler *et al.*, 2008), these results suggest that the younger heifers at parturition did not present smaller live-weight at calving and adequate nutrients were available for the reproductive function. There was a THI by AFC interaction (p<0.05) for all-service conception rate. All age groups had fairly similar conception rate at THI >83 units. Variation of services per conception was not related to AFC and THI at calving (Table 2). Regardless of AFC and THI at calving the mean ± SD services per conception was 5.3 ± 3.6, which reflects a reduced reproductive performance of this herd during prolonged thermal stress in the study site.

Pregnancy rate of first calf heifers to first service and to all services was not influenced by technician (p>0.10). Average pregnancy rate after calving to all services achieved per technician ranged from 44 ± 3% to 47 ± 2%. These results emphasize the importance of constantly monitoring the performance of the AI technicians to make sure they deliver the best possible results. Significant (p<0.05) variation in all-services conception rate was associated with year of service (42.3% for 2016 and 46.7% for 2017), which could be attributed mainly to differences between years in the quality of feed, management practices, and ambient temperature. Variation of services per conception was not related to year of service.

The occurrence of periparturient disorders and culling rate of first calf heifers as a function of THI at calving and AFC is presented in Table 3. Retained placenta was associated neither with THI >83 units at calving nor with AFC. Other reports have found a greater incidence of retained placenta in summer compared to other seasons (Ahmadi & Mirzaei, 2006; Hossein-Zadeh & Ardalan, 2011). The occurrence of metritis was not associated with heat stress at calving but heifers calving at younger ages had a higher (11.8%; p<0.01) incidence of this reproductive disorder than heifers calving at 2.0-2.2 years (8.0%) and heifers calving >2.2 years of age (8.3%). The reason for the difference in uterus health between the age groups is not known, but differences in physiology, immune status, or both may be of importance. It is, therefore, possible that immune variables may differ between primiparous cows of different AFC.

The occurrence of ovarian cyst was not associated with thermal stress at calving but this reproductive disorder was two-fold higher (p<0.01; Table 3) in heifers calving at <2.2 years of age compared to heifers calving >2.2 years of age. It is no clear why heifers calving before 2 years of age were more susceptible to ovarian cysts. Probably younger heifers at first calving are more prone to disruption of the hypothalamo-pituitary-gonadal axis, by endogenous and/or exogenous factors causing cyst formation (Vanholder *et al.*, 2006).

Culling rate of heifers was associated with both thermal stress at calving and AFC. There was a heat stress by AFC interaction with the higher

Table 3. Occurrence of periparturient disorders and culling rate of first calf Holstein heifers according to age at first calving and climatic conditions at calving.

Periparturient disorders and culling of cows[1]	<2 years	2-2.2 years	>2.2 years
Retained placenta (%)			
THI <77	9.5 (55/577)	8.6 (51/594)	10.4 (45/434)
THI 77-83	10.4 (54/519)	9.4 (42/449)	13.0 (44/339)
THI >83	11.7 (68/580)	11.2 (47/420)	9.4 (34/360)
Metritis (%)[2]			
THI <77	10.4 (60/577)[A]	7.7 (46/594)[B]	8.1 (35/434)[B]
THI 77-83	11.9 (62/519)[A]	8.9 (40/449)[B]	10.6(36/339)[A]
THI >83	13.3 (77/580)[A]	8.3 (35/420)[B]	6.9 (25/360)[B]
Ovarian cyst[2]			
THI <77	9.0 (52/577)[A]	9.4 (56/594)[A]	4.8 (21/434)[B]
THI 77-83	8.7 (45/519)[A]	11.4 (51/449)[A]	5.6 (19/339)[B]
THI >83	9.8 (57/580)[A]	10.7 (45/420)[A]	4.4 (16/360)[B]
Culling rate (%)[2]			
THI <77	47.5 (276/581)[a]	47.8 (287/600)	51.4 (226/440)
THI 77-83	57.2 (299/523)[b,A]	45.9 (209/455)[B]	55.1 (190/345)[A]
THI >83	50.5 (296/586)[a]	50.0 (213/426)	57.1 (208/364)

[1]THI= temperature-humidity index. [2]Effect of the temperature-humidity index × age at first calving interaction ($p<0.05$). [ab]For traits listed, different superscripts denote statistical differences ($p<0.05$) within columns. [AB]For traits listed, different superscripts within rows denote statistical differences ($p<0.05$).

proportion of culled cows for <24 month old first-calving heifers at THI 77-83 units at calving, whereas heifers other than heifers <2.0 years of age presented the highest culling rates at THI >83 units at calving. In agreement with the present study, other researchers (Nilforooshan & Edriss; 2004; Berry & Cromie, 2009) reported a decrease in productive life as AFC increased. Heifers calving at an older age have greater non-esterified fatty acids (NEFA) and β-hydroxybutyric acid (BHBA) blood levels, and lesser insulin, glucose, and urea nitrogen blood values compared with heifers calving at a younger age (Nyman *et al.*, 2008). Thus, heifers calving at an older age are in more severe negative energy balance in early lactation, due to the greater milk production than cows calving at a younger age (Nilforooshan *et al.*, 2004).

Higher culling rates in heifers calving under thermal stress were more likely to have lower conception rates and to be culled due to infertility. The Kaplan–Meier plot showing the relationship between AFC and lactation length is shown in Fig. 1. This suggests a difference in lactation length between heifers calving <2.0 years, 2.0-2.2 years and >2.2 years of age for the first time. Only heifers calving <2.0 years of age were not culled beyond 1000 days of lactation. On the other hand, some heifers calving for the first time at 2.0-2.2 and >2.2 years of age were culled beyond 1200 days of lactation. Given that non-planed extended lactations are associated with infertile cows (Mellado *et al.*, 2016), these data suggest that more late-calving heifers were unable to get pregnant and consequently some of them were milked for over 35 months.

These results yield evidence of a substantial negative effect of delayed age at first calving (>2.2 years) in first calf Holstein heifers in a hot environment on dystocic delivery, pregnancy rate at first-service, conception rate at all services and culling rates. Also, these data showed that, overall, early first calving (<2.0 years) was not associated with reduced reproductive performance, although <2.0 year old heifers at first calving presented a higher incidence of metritis and ovarian cysts. Thus, these results support the view that in order to reduce the total costs of rearing, dairy farmers in intensive system can lower their heifers' first-calving age by earlier inseminations, without compromising assistance at calving and reproductive performance. Finally, these results reaffirm the negative association between heat stress at calving on reproductive performance and culling rates of first calf Holstein heifers.

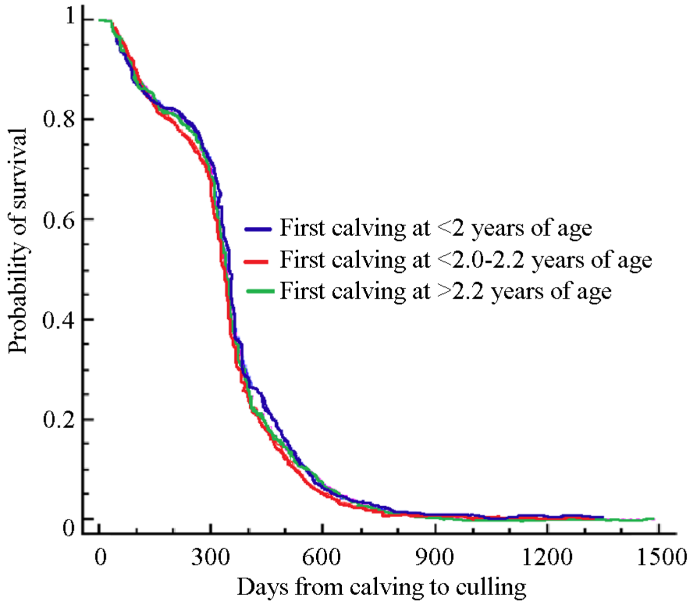

Figure 1. Kaplan-Meier survival curves for the probability of survival of first-lactation Holstein heifers in a hot environment. The probability of survival was lower for heifers calving at 2-2.2 years of age compared with younger or older heifers at first calving (*p*<0.001, Wilcoxon test). Seventy-five percent of heifers <2.0, 2.0-2.2 or > 2.2 years of age at first calving survived 275, 256 and 274 days, respectively.

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