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ORIGINAL ARTICLE

REASONS AND RISK FACTORS FOR THE INCIDENCES OF PERINATAL CALF MORTALITY AND TWIN BIRTHS IN LOCALLY-BORN HOLSTEIN DAIRY COWS UNDER HOT ENVIRONMENTAL CONDITIONS

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published in July 14, 2020 (https://doi.org/10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Perinatal calf mortality (PCM) and multiple pregnancies (twins) present significant challenges in dairy cattle breeding, impacting both economic viability and reproductive performance. This study examined risk factors for stillbirths and twin births in a dairy farm located in Algeria's Northern Sahara, analyzing 1047 full-term calving records of locally-born purebred Holstein cows. Binary logistic regression models were employed to identify factors influencing PCM and twin births. During the study period (1995 – 2017), 7.07 % of calvings resulted in stillbirths, and 3.15 % resulted in twins. PCM was strongly associated ($p = 0.0001$) with severe dystocia, calf gender, and retained placenta, as well as gestation length ($p = 0.023$), dam parity ($p = 0.019$), and temperature-humidity index (THI) at breeding ($p = 0.025$). The likelihood of twin births was higher in multiparous cows and significantly associated ($p = 0.034$) with heat stress conditions at parturition (THI-P). These findings highlight the need for targeted management strategies during the peripartum period to mitigate reproductive disorders and reduce financial losses in dairy herds.

Key words: dystocia; heat stress; Holstein; stillbirth; twinning births

INTRODUCTION

Perinatal calf mortality or stillbirth (SB), has been defined by many researchers as the mortality of full-term calves (normal pregnancy >260 days), within 24 to 48 h after parturition [31, 37, 39]. During the last decade, several studies have been reporting an incessant increase in the frequency of PCM in many countries [38]. Therefore, the high stillbirth rates are a serious problems in cattle breeding, characterised by a high economic and genetic loss and increased costs for replacements [9]. Recently, in necropsy studies internationally defined dystocia (35 %) and anoxia (30 %) as major causes of PCM, to a lesser degree, other causes (15 %), infections (5 %) and congenital defects (5 %) [43]. Besides, the greater risk of perinatal mortality is in primiparous compared to multiparous cattle [40].

Hence, the primary causes of dystocia in primiparae cows are relative fetal oversize and in pluriparae cows there are maldispositions [44].

Otherwise, some findings indicated that maternal diet during different stages of gestation could induce physiological and epigenetic changes in fetal tissues of different species, which could have serious postnatal consequences [30]. Evidence suggests that dairy cows under heat stress, during late gestation, in hot environmental conditions, have been related to lower birth weight, decreased total plasma protein concentration and haematocrit, and compromised immune function of the new-born calf [57].

Multiple pregnancies (twins) are undesirable in dairy cattle herds as they compromise the reproductive performance such as increased abortion, dystocia, retained placenta, calf mortality, occurrence of freemartins, postpartum therapy, and longer rebreeding intervals, and an economic burden of up to $225 per twin pregnancy [14, 33]. Despite this, cows are predominantly a monovular species. The incidence of multiple ovulations and thus twinning in dairy cows has increased considerably alongside milk production in the last 30 years [32]. In effect, global warming is likely already having a negative impact on reproductive functions in mammals [4]. Thus, López-Gatius et al. [33] indicated that the warm climate is the main factor compromising the fate of multiple pregnancies. In addition, the breeding synchronization protocols for fixed-time artificial insemination (FTAI), have become routine components of the dairy herd reproductive management. Therefore, the hormone combinations used for FTAI can increase the risk of double ovulation [14]. Nevertheless, some short protocols appear to reduce the rate of double ovulation compared to spontaneous estrus [11]. Nevertheless, the risk of twin pregnancy is much more common in older cows [15]. In effect, it has been observed by Garcia-Ispierto and López-Gatius [15] that the twin pregnancies may account for 25 % of all pregnancies on day 90 of gestation in cows in their third lactation or more.

This study sets out to explore the influence of risk factors of the incidences of stillborn calves and twinning births in locally-born purebred Holstein dairy cows raised under Saharan climate in Ghardaia region.

MATERIALS AND METHODS

Herd management

The study population was a commercial dairy herd of native purebred Holstein-Friesian lactating dairy cows in Algerian Northern Sahara (32°41'06.7 "N latitude and 4°44'10.8 "E longitude). During the study period (January 1995 to December 2017), the mean number of lactating cows in the herd was 312 and mean annual milk yield was 7030.3 kg per cow. Cows were milked three times daily and fed total mixed ration (roughage: 40 %, concentrate feed: 60 %). All cows were artificially inseminated (n = 2512) 12 h after the expression of signs of estrus (EDAI). Alternatively, by programmed-timed artificial insemination (TAI) using the (GnRH - PGF2α – GnRH) protocol, (OVSYNCH®, CEVA Santé Animale, France). Pregnancy was diagnosed by rectal palpation and transrectal ultrasound using "My's-A001-N", with 1164 pregnancy cases, i.e. a mean pregnancy rate in herd (PR % = 46.33 %). A 1047 full term calving were recorded by farm manager and personnel for each calving event in Excel sheet. Information recorded included dam parity, date of calving, occurrence of dystocia (unassisted or assisted calving), calf gender, calve mortality at 24 h to 48 h post-calving and placental expulsion status (spontaneous expulsion or placenta retention). Calf mortality during calving and after parturition (within the first 24-48 h after birth) is termed as perinatal mortality and it mainly occurs due to dystocia [19].

Climatic factors

Climatic factors were obtained from the regional meteorological station which reflects the weather conditions on the farm. It includes the maximum daily temperatures in °C (T) and the maximum relative humidity (% RH). These were used to calculate the temperature-humidity index (THI) [36]: $THI = (0.8 \times T) + [(\% RH / 100) \times (T - 14.4)] + 46.4$.

Date of breeding and calving were used to allocate the THI values at breeding and late gestation (7th, 8th and at parturition), hence, it were divided into 4 ranges, according to Hahn et al.' [20] classification. They evaluated the intensity of heat stress, as follows: normal, alert, danger and emergency, which corresponds with the following values, THI < 74, [75–78], [79–83] and THI > 84, respectively.

Statistical analysis

To investigate the association between PCM, twinning births and the potential risk factors, the statistical analyses were performed for Holstein cows. According to the binary nature of PCM and twin birth as the response variables, a logistic regression model was constructed in Minitab® 18.1 (Minitab, Inc., in the United States and other countries) as the following models:

Model 1: Logit (π) = $\alpha + AFC_i + Bi\text{-}Y_j + Br\text{-}Y_k + Ca\text{-}Y_l + CS_m + GLR_n + DP_o + BT_p + CE_q + PR_r + THI\text{-}B_s + THI\text{-}7_t + THI\text{-}8_u + THI\text{-}P_v$

Model 2: Logit (π) = $\alpha + AFC_i + Bi\text{-}Y_j + Br\text{-}Y_k + Ca\text{-}Y_l + DP_m + BT_n + THI\text{-}B_o + THI\text{-}P_p$

Where: π = the probability of PCM and twin birth; t α = the intercept parameter; AFC = fixed effect of age at first calving (day) (1 = <720, 2 = [720–900], 3 = [900–1080],

Table 1. Odds ratios (OR) and 95 % confidence intervals (CI) of the animal factors associated with the incidence of stillbirth.

Variable	N	PCM (%)	OR	95 % CI	p-value
Calf sex					
Female	24	32.43	Referent		
Male	35	47.30	1.43	(0.83; 2.44)	0.0001
Twin	15	20.27	16.42	(7.39; 36.49)	
Parity of dam					
Multiparous	34	45.95	Referent		
Primiparous	9	12.16	0.54	(0.25; 1.16)	0.019
Nulliparous	31	41.89	1.51	(0.91; 2.52)	
Gestation length range					
[270-282]	41	55.41	Referent		
[251-269]	12	16.22	2.85	(1.42; 5.70)	0.023
[283-300]	21	28.38	1.18	(0.68; 2.04)	
Age at first calving					
<720	6	8.11	Referent		
[720-900]	43	58.11	0.8	(0.32; 1.95)	
[900-1080]	16	21.62	0.76	(0.28; 2.01)	0.666
[1080-1260]	7	9.46	1.34	(0.42; 4.23)	
>1260	2	2.70	1.71	(0.31; 9.46)	
Breeding type					
EDAI	71	95.95	Referent		0.279
TAI	3	4.05	0.27	(0.02; 3.25)	
Calving ease					
Unassisted	13	17.57	Referent		
Little intervention	1	1.35	0.07	(0.007; 0.67)	0.0001
Difficult	15	20.27	2.63	(0.85; 8.08)	
Very difficult	45	60.81	76.19	(18.76; 309.46)	
Placental expulsion status					
Spontaneous expulsion	9	12.16	Referent		0.0001
Placenta retention	65	87.84	65.97	(31.85; 136.66)	
Twinning birth					
Singleton	59	79.73	Referent		0.0001
Twin	15	20.27	13.49	(6.47; 28.10)	

N = number of cases; OR = Odds ratios; CI = 95 % confidence intervals; PCM = perinatal calf mortality; EDAI = artificial insemination by estrus detection; TAI = timed artificial insemination.

4 = [1080–1260], 5 = >1260); Bi-Y = fixed effect of dam's birth year (1992-2014); Br-Y = fixed effect of dam's breeding year (1994–2017); Ca-Y = fixed effect of dam's calving year (1995-2017); CS = fixed effect of calf sex: (1 = male, 2 = female, 3 = twin); GLR = fixed effect of gestation length range (day) (1 = [251–269], 2 = [270–282], 3 = [283–300]); DP = fixed effect of dam's parity (1 = Nulliparous, 2 = Primiparous, 3 = Multiparous); BT = fixed effect of breeding type (1 = EDAI (artificial insemination by estrus detection), 2 = TAI (timed artificial insemination); CE = fixed effect of calving ease (1 = unassisted, 2 = little intervention, 3 = difficult, 4 = very difficult); PR = fixed effect of placental expulsion status (1 = spontaneous expulsion, 2 = placenta retention); THI-B; THI-7; THI-8; THI-P = fixed effect of temperature-humidity-index (THI) at breeding, at 7th month of gestation, 8th month of gestation and at parturition (1 = <74, 2 = [75–78], 3 = [79–83], 4 = >84).

RESULTS

Perinatal calf mortality occurrence

The analysis of binary regression indicate that out of 1047 full term calving studied, 74 stillborn calves (7.07 %) were recorded. Table 1 provides the odds ratios (OR) and the prevalence rates for SB. It is apparent that the PCM incidence was significantly (p = 0.0001) higher, 13.49 times higher in twins than singleton births. Similarly, in the pregnant dams that had dystocia during the calving process and placenta retention, cases were 76.19 higher and 65.97 times more susceptible to having stillborn calves than unassisted calving and spontaneous expulsion of placenta at the parturition, respectively.

Moreover, females with a short [251–269] and prolonged [283–300] gestation lengths had a significant increased risk (p = 0.023) of stillborn calves, 2.85 and 1.18 times higher than females with average pregnancies [270–282]. Whereas, the calf gender was significantly (p = 0.0001) associated with the occurrence of PCM incidence, as bull calves were 1.43 times more likely to be stillborn than heifer calves. In the same way, there was a significant association between dam's parity and stillbirths (p = 0.019), hence, the incidence of PCM in nulliparous dams was 1.51 times higher compared to the multiparous dams. Otherwise, there was no linking between the AFC (p = 0.666) and the BT (p = 0.279) and SB occurrence.

As shown in Figure 1, the possibility to having a stillborn calf with assisted calving (dystocia) was 92.43%

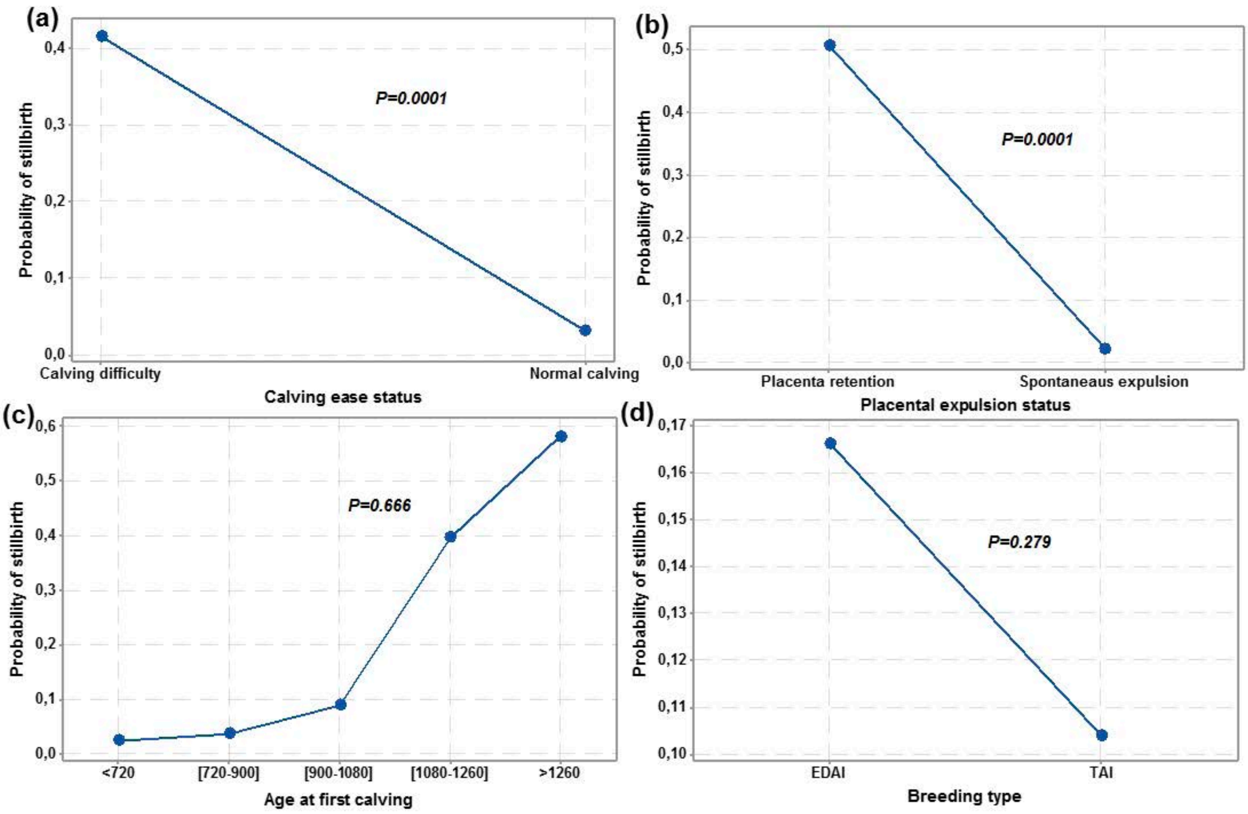

Fig 1. Main effects plot of calving ease (a), placental expulsion (b), AFC (c), and breeding type (d) for the incidence of PCM.

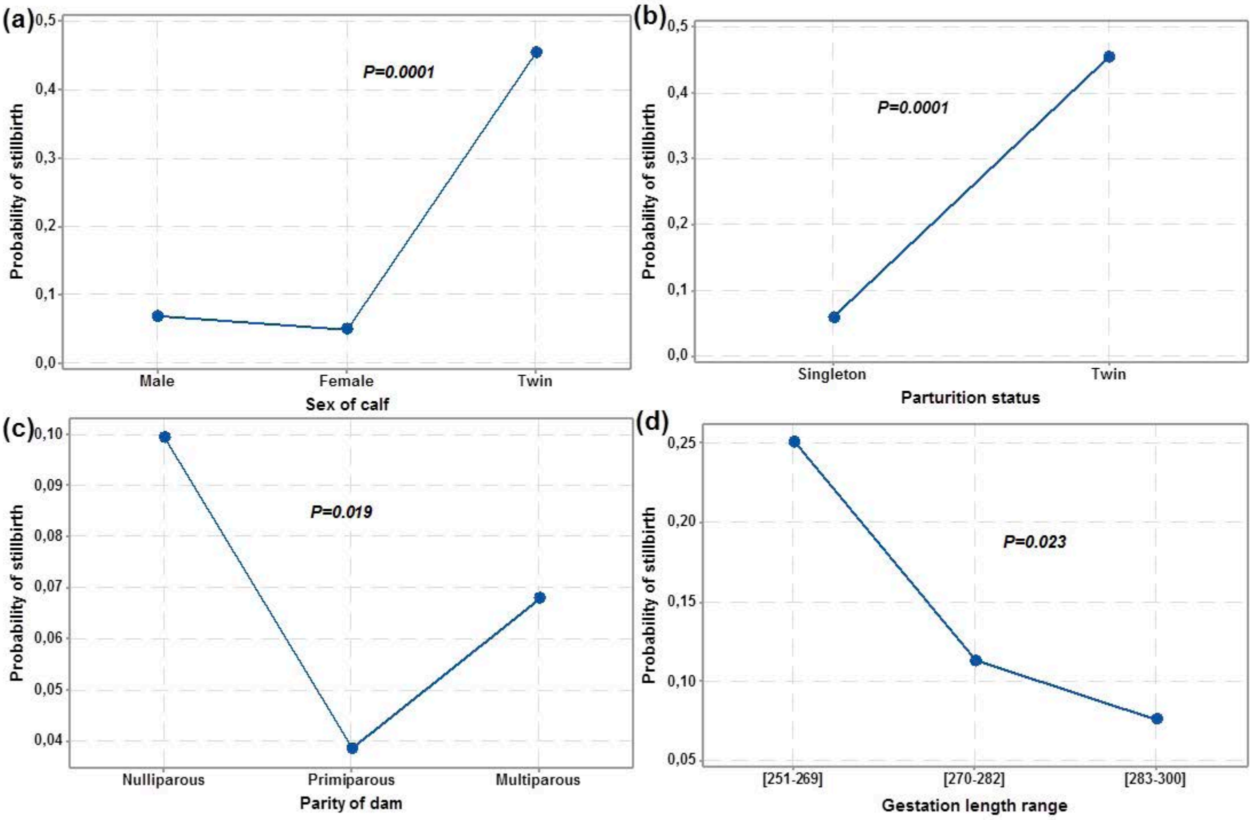

Fig 2. Main effects plot of sex of calf (a), parturition status (b), parity of dam (c), and gestation length range (d) for the incidence of PCM.

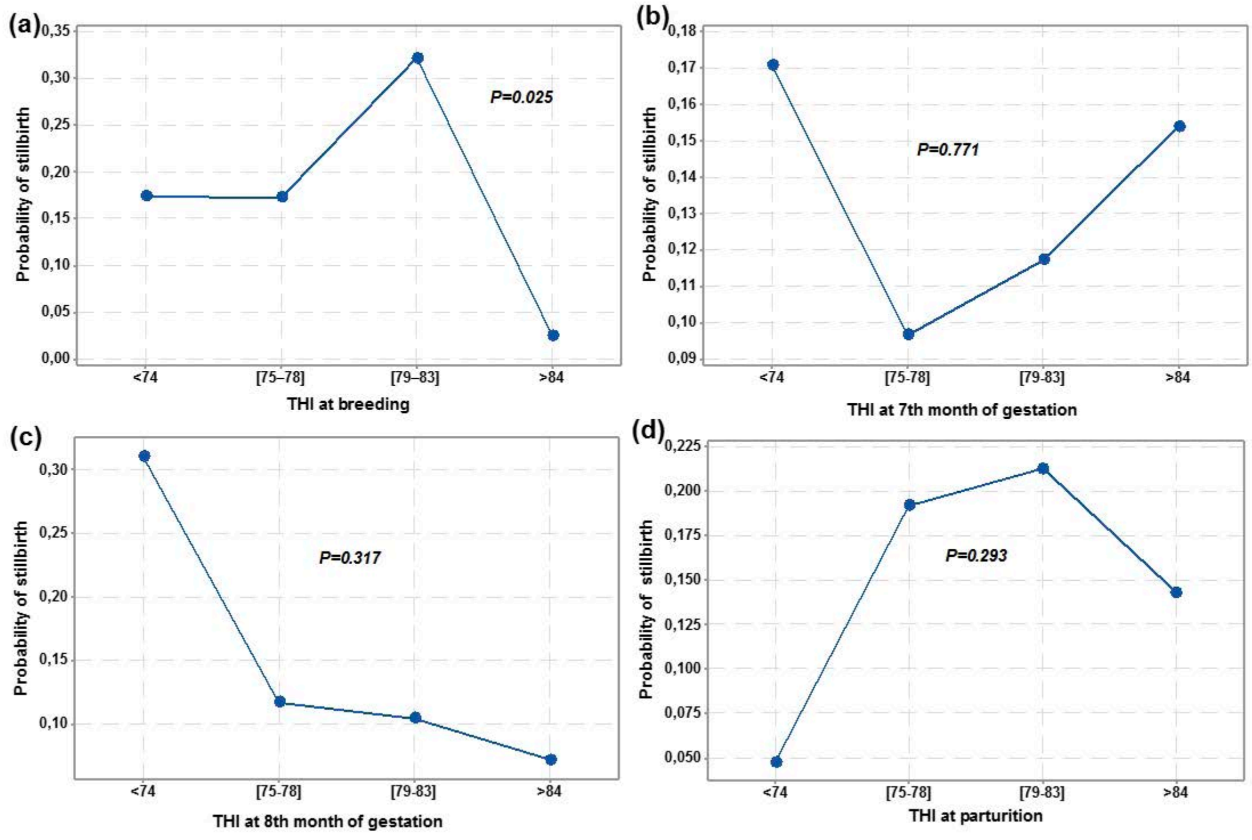

Fig 3. Main effects plot of THI at breeding (a), THI at 7th month of gestation (b), THI at 8th month of gestation (c) and THI at parturition (d) for the incidence of PCM.

higher than normal calving. In addition to the retained placenta cases, which had a high significant linking ($p = 0.0001$) with the incidence of SB (probability: 50.06 % vs. 2.1 %). On the contrary, no significant ($p = 0.279$) differences were found between PCM and BT. On the other hand, when AFC >1260d, the risk of SB is 58.1 %, although the association between the two variables is not significant ($p = 0.666$).

Looking at Figure 2, it is apparent that gestation length (GL) ranges had a significant relationship ($p = 0.023$) with incidence of SB, especially in shorts GL (251 to 269 days), with a probability of 25.1 %. Another significant aspect of mortality risk in twin births (45.45 %). In addition, the gender calf showed a high significant difference ($p = 0.0001$) between bull calves (6.7 %) and heifer calves (3.8 %) in terms of the probability of PCM. Interestingly, stillborn possibility the calves issued from nulliparous dams (9.9 %) differ significantly ($p = 0.019$) from the calves from multiparous (6.7 %) and primiparous dams (3.8 %).

The results of the main effects plot of THI values at conception and late gestation were summarized in Figure 3. Hence, no existence of a significant connection was found between the incidence of SB and THI at the 7th month of gestation ($p = 0.771$), at the 8th month of gestation ($p = 0.317$) and at the parturition ($p = 0.293$). Nevertheless, with THI at breeding, there is a significant difference ($p = 0.025$) in the probability occurrence of PCM, with different THI values.

It can be seen from the data in Table 2 that none of these differences were statistically significant concerning THI values at the 7th ($p = 0.771$), and at the 8th ($p = 0.317$) month of gestation, at the parturition ($p = 0.293$), dam's birth, conception and calving year ($p = 0.617$; $p = 0.879$; $p = 0.868$) with the stillborn calves. Interestingly, the THI [79–83] value at breeding was 3.04 times higher compared to THI < 74 in the manifestation of PCM.

Table 2. Odds ratios (OR) and 95 % confidence intervals (CI) of the environmental factors associated with the incidence of stillbirth.

Variable	N	PCM (%)	OR	95 % CI	p-value
THI at breeding					
<74	39	52.70	Referent		
[75–78]	14	18.92	0.93	(0.22; 3.91)	0.025
[79–83]	16	21.62	3.04	(0.79; 11.62)	
>84	5	6.76	0.1	(0.01; 0.98)	
THI at 7th					
<74	31	41.89	Referent		
[75–78]	18	24.32	0.52	(0.10; 2.70)	0.771
[79–83]	9	12.16	1.25	(0.15; 9.91)	
>84	16	21.62	0.66	(0.04; 9.09)	
THI at 8th					
<74	32	43.24	Referent		
[75–78]	9	12.16	0.14	(0.01; 1.22)	0.317
[79–83]	17	22.97	0.44	(0.02; 6.93)	
>84	16	21.62	0.25	(0.01; 9.98)	
THI at parturition					
<74	30	40.54	Referent		
[75–78]	10	13.51	2.81	(0.52; 15.04)	0.293
[79–83]	16	21.62	4.87	(0.66; 35.84)	
>84	18	24.32	1.79	(0.11; 29.10)	
Year of birth	74	100	0.91	(0.64; 1.30)	0.617
Year of breeding	74	100	0.94	(0.42; 2.09)	0.879
Year of calving	74	100	1.07	(0.47; 2.44)	0.868

N = number of cases; OR = Odds ratios; CI = 95% confidence intervals; PCM = perinatal calf mortality; THI = temperature-humidity index

Twinning birth occurrence

Analyzing the gender ratio in multiple calving, the opposite sex twins were the most common, with the proportion being 45.45 %. Heifer calves were the least frequent (18.18 %) and the proportion of bull calves was around 36 %.

From the Table 3 below, we can see that no significant differences were found between THI values at conception ($p = 0.416$), BT ($p = 0.437$), dam's AFC ($p = 0.638$), dam's birth, conception and calving year ($p = 0.678$; $p = 0.099$; $p = 0.172$) and the appearance of twins, respectively.

Whereas, dam's parity had a significant relationship ($p = 0.013$) with incidence of twinning birth, especially in multiparous dairy cows, which were more susceptible to having twin calves, 10.02 times more than heifers. Following the addition of the link with THI values at parturition, a significant increase ($p = 0.034$) in the occurrence of twins was recorded. The susceptibility to getting the twins were 4.10 and 2.32 times higher in heat stress conditions at calving (from 75 to > 84), respectively.

The results of the probability analysis of the main variables affecting the event of twinning birth were summarized in Figure 4. None of these differences were statistically significant excepting the dams' parity, hence, the probability of having multiple calves was 3.05 %; 1.42 %; 0.31 % in multiparous, primiparous and nulliparous cows, respectively. Similarly, concerning the heat stress conditions at the calving (THI-P), the possibilities of twins were 1.72 %; 6.72 %; 3.93 % and 3.93 % following the variation of THI values.

DISCUSSION

The current study found that stillborn calves were represented by 7.07 % of total calving events. This finding is consistent with that of Mellado et al. [45] in North-eastern Mexico (7.3 %). Also, El – Tarabany [9] under subtropical conditions, showed that the prevalence rate for SB was 15.4 %. This differs from the findings presented here.

Table 3. Odds ratios and 95 % confidence intervals (CI) of the independent factors associated with the incidence of twinning birth.

Variable	N	Twinning birth (%)	OR	95 % CI	p-value
Parity of dam					
Nulliparous	2	6.06	Referent		
Primiparous	6	18.18	4.61	(0.88; 24.01)	0.013
Multiparous	25	75.76	10.02	(1.77; 56.60)	
THI at breeding					
<74	22	66.67	Referent		
[75–78]	4	12.12	0.45	(0.15; 1.38)	0.416
[79–83]	6	18.18	1.01	(0.38; 2.65)	
>84	1	3.03	0.45	(0.05; 3.62)	
THI at parturition					
<74	9	27.27	Referent		
[75–78]	8	24.24	4.10	(1.54; 10.86)	0.034
[79–83]	7	21.21	2.32	(0.85; 6.34)	
>84	9	27.27	2.32	(0.91; 5.94)	
Breeding type					
EDAI	32	96.97	Referent		0.437
TAI	1	3.03	0.48	(0.06; 3.71)	
Age at first calving	33	100	0.99	(0.99; 1.00)	0.638
Year of birth	33	100	1.05	(0.80; 1.39)	0.678
Year of breeding	33	100	0.59	(0.30; 1.15)	0.099
Year of calving	33	100	1.58	(0.79; 3.16)	0.172

N = number of cases; OR = Odds ratios; CI = 95% confidence intervals; THI = temperature-humidity index; EDAI = artificial insemination by estrus detection; TAI = timed artificial insemination.

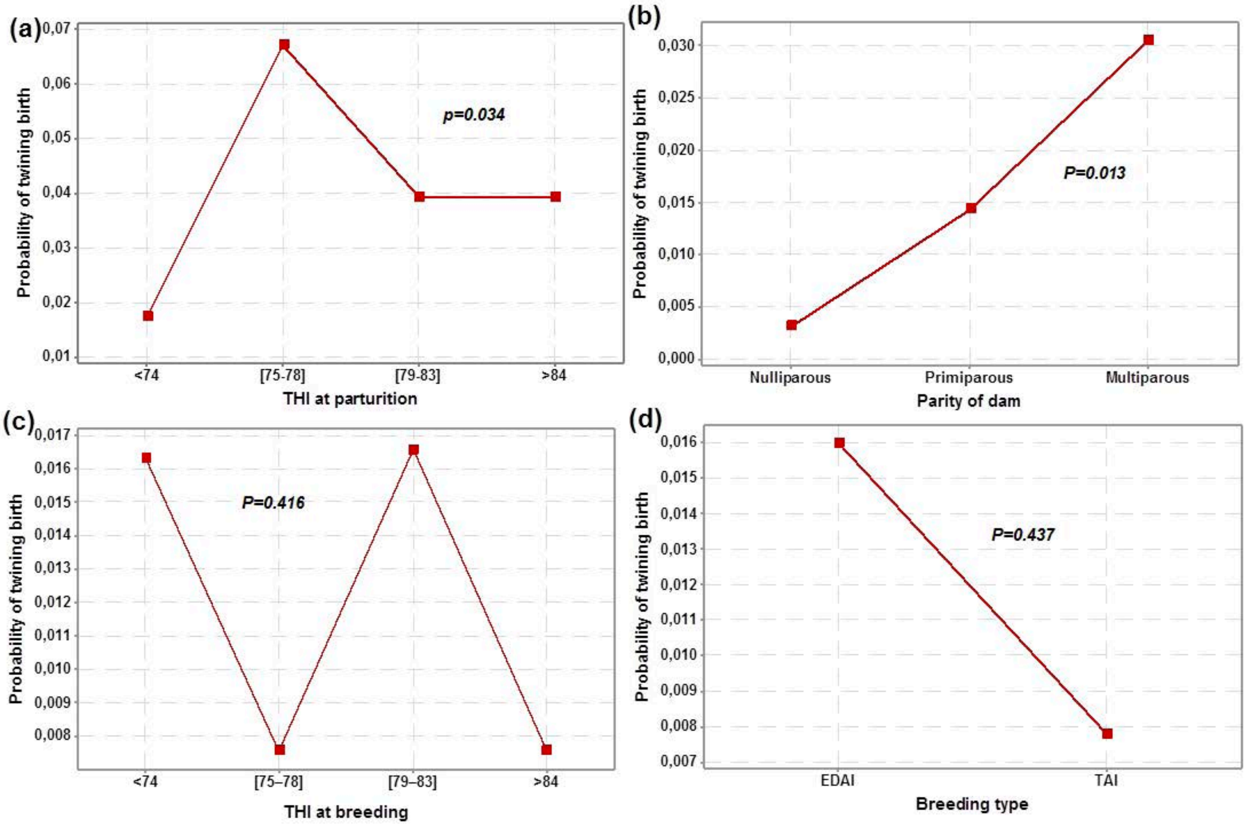

Fig 4. Main effects plot of THI at parturition (a), parity of dam (b), THI at breeding (c) and breeding type (d) for the incidence of twinning birth.

The average incidence of PCM in cows and heifers varies between 2 to 20 % across dairy industries internationally with the majority of countries between 5 to 8 % [37]. In fact, the PCM are related multifactorial complexes [24].

Dystocia

This study supports evidence from previous observations by e.g. Hohnholz et al. [24], Mellado et al. [45], Hoedemaker et al. [23], Lombard et al. [31] indicating that the greater odds of SB was in assisted calving. This consistency may be due to the dystocial stillbirths that usually result from internal and external trauma [2], but also from deprivation of adequate oxygen supply (prolonged hypoxia) [42]. Another possible explanation for this is the timing of the movement of pregnant animals to the calving unit that can influence the risk of PCM [37]. Furthermore, Kristula and Smith [29] showed that the lower rates of stillbirth were in cows moved to the maternity unit during stage 2 (presence of feet at the vulva) compared to stage 1 of calving. Whereas, the parturition in tie stalls has been associated with a lower stillbirth rate than in free stalls [18]. According to Carrier [5], who demonstrated that every additional hour in stage 2 of calving increases the odds of stillbirth by 30 %. Although this may reflect an interaction with calving supervision [23]. Nonetheless, Vernooy et al. [58] found no association between individual or group maternity accommodation and the risk of SB. Anyway, relatively simple interventions have the potential to significantly reduce the impact of dystocia on calf mortality and morbidity on dairy farms [31].

Environmental conditions

Our outcome is contrary to that of Mellado et al. [45] who found the cows in a severe state of heat stress prenatally and at birth (THI > 83 units) had 1.3 higher risk of SB than cows suffering reduced heat stress. As a matter of fact, the occurrence of SB on this dairy farm was associated with either moderate or extreme heat stress during the peripartum period, with negative impacts from the seventh month of pregnancy until calving [45]. Meyer et al. [47] showed that the prevalence of SB in summer were 27 % higher than in winter. These differences can be explained in part by increasing of ambient temperature, especially in calves housed outdoors proved to be a risk factor for early calf mortality in veal calves [54]. As others showed, mortality of 1 to 21 days old Holstein calves was higher in moderate conditions than in the hot season [46]. Yet, the acute brief heat stress during late gestation did not alter passive antibody transfer capacity in calves [56]. However, D a h l et al. [6] concluded that calves experiencing heat stress *in utero* are prone to develop a smaller mature body size and more fat reserves than counterparts in thermoneutrality. All the same, M i s a k a et al. [48] reported that was no effect of interactions between herd size and calving season on the SB rate.

Calf sex and twin pregnancies

A strong relationship between calf gender and PCM has been reported in the literature. Hence, many studies show that male calves have a higher risk of perinatal mortality than females [18, 23, 3]. In fact, male calves have twice the odds of being a stillborn calf than female calves [31]. Whereas, when calves experienced a dystocic calving, female calves were more likely to die than male calves within 24 hours after calving [31]. Also, female calves born from a multiparous cow had 12 % higher odds of being stillborn than male calves [47]. Despite this, the higher percentage of female calves led to reduced dystocia for both heifers and cows, especially for twin births [51]. Provided where perinatal mortality occurs following a normal parturition, there was very little difference between genders of calves [52].

Otherwise, twin pregnancies have a higher risk of fetal death or abortion in the final months of gestation [7, 8, 25]. The limited energy reserves and vitality of twin calves during and after pregnancy are seen as the primary reasons for the elevated proportion of stillbirths [53].

Gestation length

Prior studies have noted the importance of gestation length as the third factor affecting the prevalence of SB, of which GL was the most influential factor for SB in multiparous dams [47]. As a result, extreme birth weight and GL increase PCM rates, implying that premature calves should be given special care at birth [45]. Besides, the both short and prolonged GL were associated with an increased risk of perinatal mortality, when were deviated from the mean [3]. According to N o g a l s k i and P i w c z y ń s k i [50], the lowest PCM rate (3.23%) was observed in respect to GL values in the range of 275–277 days, whereas the share of calves that were born dead and died within 24 h after birth increased three-fold (to 10.48 %), when GL exceeded 283 days. On the whole, the optimal gestation length in Holstein cows was determined in the range of 275–277 days based on calving ease and SB values. In other words, the average GL values optimized lifetime productivity, calving ease, SB rates and calving-to-first-service interval [25]. Overall, the relative risk is much higher for calves born after a short gestation than a prolonged gestation, probably due to organ immaturity [26, 3]. To conclude, GL values should be analyzed in conjunction with calving ease, SB rates and placental expulsion [21].

Placenta retention

In the cow the fetal membranes are normally expelled between 30 minutes and 8 hours after stage 2 of calving. Premature placental separation has been associated with “weak calf syndrome” in heifers [40]. It has been associated with premature birth [1] and maldisposition in multiparous cows [37]. Due to the significant relationship between SB and placental retention may be attributed to the fact that deceased births are often from dystocia, leading to the occurrence of retention of the placenta in dams [8]. Furthermore, E n d l e r et al. [10] found an increased association between placental under perfusion disorders (such as preeclampsia, small for gestational age, and SB) and retained placenta. Besides, S o r g e et al. [55] reported that the level of serum estradiol-17β was lesser in cows with stillbirths, which might be an indicator of placental malfunction or abnormal hormone signals from the calf to the placenta prior to parturition. These relationships may be partly explained by the homeokinetic changes that regulate body temperature of cows suffering heat stress and provoke a redistribution of blood flow from the body core to the periphery, which reduces perfusion of the placental blood vessels [22]. In this respect, heat stress during gestation can compromise placental development, which results in fetal hypoxia, malnutrition, and eventually fetal growth impedance [57]. The condition is adversely affected by hyperthermia-induced placental insufficiency, which results in a reduction in both placental size and functional capacity. This impairment restricts the exchange of oxygen and nutrients between the maternal and fetal systems. Furthermore, even a modest reduction in gestational length, frequently observed during periods of heat stress, exacerbates these detrimental effects [48].

Dams' parity and age at first calving

This also accords with our earlier observations, which showed that calves from first-parity dams had a higher risk ($p < 0.01$) of being stillborn than calves from dams in later parities [24]. In fact, H o h n h o l z et al. [42] and M e e et al. [40] reported that PCM were more frequent for heifers than primiparous and multiparous, especially males, with sexed semen use [51]. Due to the interaction between type of dystocia and parity that has been postulated, whereby a prolonged second stage of calving is a significant risk factor for perinatal mortality in primiparae, whereas maldisposition and twins are more common risk factors in pluriparae [41, 19].

Surprisingly, no differences were found in AFC's of dams and PCM in the current study. Conversely to M e e et al. [40] found that in primiparous cattle, a younger age of calving is associated with an increased risk of perinatal mortality [40]. This includes that the highest risk seen in cattle calving at less than 24 months old is inadequate pelvic size [21, 40]. Stillbirths during the first calving had detrimental effects on subsequent performance of the dam under subtropical conditions [9]. Another point is that prepartum maternal dietary energy content can alter calf birth weight and hence the risk of fetal oversize [13]. Relative fetal oversize is a risk factor for SB [26].

Risk Factors for twinning

Several reports have shown that the incidences of multiple ovulations are much more frequently observed in Holstein Friesian cows than in other dairy cattle breeds [15]. The most interesting finding was that the parity of dam and season of calving were potential risk factors for twinning in dairy cows [25]. Besides, the increase in incidence of double ovulations is approximately linear with increasing calving numbers [12]. J o h a n s o n et al. [27] reported that increasing parity does the chance of twinning, with the largest increase happening between the first and second parities.

Contrary to expectations, this study did not find a significant difference between the incidence of twinning birth when following some FTAI protocols [35, 16]. As mentioned in the literature review, although the heat stress at AI (maximum temperature-humidity index > 72) had not effects on follicular dynamics, cows with two unilateral follicles showed a higher double ovulation rate (48.6 %) than cows with two bilateral follicles (34.8 %) [34]. Furthermore, increased energy content in the diet and increased metabolism of steroid hormones by dairy cows may lead to a higher number of doubled ovulations in lactating dairy cows [59]. Otherwise, the estrus during the warm season decreased the likelihood of double ovulation by a factor of 0.86 [35]. As a matter of fact, K o m i s a r e k and D o r y n e k [28] found the percentage of twin pregnancies were 2.41 % in summer, 2.04 % in spring, and falling to 1.79 % in autumn.

Generally, twinning in dairy cattle is associated with higher milk yields, an increased incidence of abortion, dystocia, retained fetal membranes, freemartinism and an extended calving to conception interval [49]. Over 95 % of female calves twinned to a male are sterile [17].

CONCLUSION

This paper has argued that the PCM is a multifactorial complex, at the same time, twinning births are affected by some factors. Consequently, the binary regression analysis revealed that the rates of stillbirth were significantly higher in nulliparous cows (42 %) than multiparous cows, especially at short and long length of gestation. Besides, dystocia was the most important variable affecting the PCM rate (> 80 %), with the large occurrence (88 %) of the retained placenta cases.

In fact, under the Saharan conditions of the current study, in which prolonged heat loads prevail for most of the year, these data did not support the concept that sustained high ambient temperatures increases PCM of Holstein calves. Otherwise, the overall average twinning rate was 3.15 %. Dams' parity and heat stress at calving were identified as important factors and had significant effects on the incidence of twinning birth.

These results suggest that, in general, possible preventive measures should include improving the peripartum environment and maternal ration, improving genetic selection for calving ease and optimising heifer rearing. The study has contributed to a better understanding of the factors responsible for twin pregnancy in Holstein cattle and the establishment of effective management strategies for pregnant cows. Further research and experimentation on the effects of perinatal mortality and reproductive disorders associated with twins under warm environmental conditions are strongly recommended.

DATA AVAILABILITY STATEMENT

The original datasets used in this research and, if applicable, supporting information files are available.

ETHICAL STATEMENT

No ethical approval was obtained because this study only involved data collection from the farm manager.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have influence the work reported in this paper.

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The authors declare that no Gen AI was used in the creation of this manuscript.

AUTHORS CONTRIBUTIONS

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