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REGULAR ARTICLES

Interrelationship between diseases and calving season and their impact on reproductive parameters and milk production of tropical dairy cows

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Abstract
The interactions between calving season, the occurrence of retained placenta, intrauterine infections (IUI), and early mastitis, and their effects on the reproductive performance and milk yield of Holstein–Friesian cows in a tropical environment were studied using data from 3320 calvings (1948 cows) from two farms in El Salvador. Based on environmental conditions, season of calving was categorized into: quadrimester 1 (November–February), quadrimester 2 (March–June), and quadrimester 3 (July–October) where quadrimester 2 and 3 had the highest ambient temperature and relative humidity, respectively. Cows were classified into 1, 2, and 3 + parities. The effects of quadrimester and of diseases on days to first service, services per conception, days open, interval between services and 305-day milk yield were studied in separated multivariate regressions. The likelihood of experiencing a disease contingent on the calving season and the likelihood of a cow being culled due to poor fertility associated with experiencing a disease were evaluated using logistic regression. Cows calving in quadrimester 2 and 3 were more likely to suffer from IUI and showed poorer reproduction than cows calving in quadrimester 1. Reproduction was more strongly affected by IUI. Mastitis increased the days to first service, days open, and interval between services. Mastitis and IUI also caused a lower 305-day milk yield. Overall, hotter and more humid conditions lead to higher incidence of disease and poorer reproductive performance. The physiological responses that lead to these phenomena should be further studied to understand the interactions between diseases, environmental conditions and reproduction.

Keywords Intrauterine infections · Mastitis · Placenta retention · Fertility · Heat stress · Tropical dairy

Introduction

The sustainability of a dairy farm is strongly linked to a cow's ability to achieve pregnancy and produce offspring. High levels of production, suboptimal nutrition, low body condition, and diseases are common causes for poor fertility (Lucy 2001). Particularly, the occurrence of an inflammatory disease before mating has a strong detrimental effect on reproduction performance, by reducing the fertilization of oocytes and the development to the morula stage (Ribeiro et al. 2016). For example, studies have found a loss in reproductive performance in dairy cows suffering from clinical and subclinical mastitis during early lactation (Campos et al. 2020; Mohammed 2021; Schrick et al. 2001), highlighting the negative impact of infections even if they are not located in the reproductive tract.

The fertility of dairy cattle is further affected by heat stress (Wolfenson and Roth 2019). Heat stress is a common occurrence during hot summer periods in temperate regions and is a yearlong challenge in tropical humid areas, with greater negative impacts over multiparous cows compared with primiparous (Aguilar et al. 2009). Hot and wet conditions are not only conducive of hyperthermia in the dairy cow, but are also favorable conditions for the development of bacterial infections such as metritis and mastitis. Indeed, previous studies have found greater incidences of

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intramammary infections during the rainy season compared with the dry season in Holstein cattle in Brazil (Nobrega and Langoni 2011) and Ethiopia (Kerro Dego and Tareke 2003). Similarly, cows experiencing heat stress during pregnancy tend to calve early, which in turn is a risk factor for retained placenta and intrauterine infections (IUI) (DuBois and Williams 1980), while heat stress itself depresses the immune system increasing susceptibility to diseases (Jacobsen 1996). The hyperthermia-diseases-poor fertility continuum, therefore, becomes a critical challenge for dairy farms that results in poor reproduction, low milk production levels, early culling, and ultimately a less profitable and sustainable herd.

Understanding the relationships between the calving season and the occurrence of diseases can assist the adoption of management strategies to improve the health and reproduction of dairy cattle. The objectives of the current study were to identify the associations between the calving season and the incidence of diseases and to identify how these diseases affect reproductive parameters in tropical dairy cows.

Materials and methods

This study analyzed data from two dairy farms in western El Salvador located at 400 m.a.s.l.. The herds were composed of Holstein cows with average (± SD) milk yield of 17.8 ± 5.10 kg/d (min = 8, max = 31.9 kg/d). Information was available for calving in the years 2010, 2011, 2012, 2015, 2016, and 2017. The parameters recorded for each calving were: cow's ID, cow parity, date of calving, date of first artificial insemination (AI), date of additional AI's, date of successful AI, dates and reasons for culling, occurrence and dates of mastitis, IUI, and retained placenta. Body condition score (BCS) at calving was also recorded when available. The BCS was assessed by the farm veterinarian on a scale ranging from 1(emaciated) to 5 (excessively fat) with 0.5-point increments (Wildman et al. 1982). Moreover, culling due to infertility was recorded and was considered as such if the cow was culled after not becoming pregnant, not suffering any injury or disease and if no other reason was provided. In this dataset, culled cows received 4.17 services in average before being culled. Intrauterine infections were recorded when diagnosed and reported by the farm veterinarian within 21-day postpartum (Sheldon et al. 2006). Retained placenta was diagnosed if the placenta was not expulsed after 24-h postpartum (Giuliodori et al. 2013). Mastitis was diagnosed using the California Mastitis Test (CMT) if a thickening reaction was observed in at least one quarter with a CMT score of at least 2 (Mellenberger and Roth 2000), in which case the farmer would decide to treat a cow. The CMT is routinely used at every milking in both farms. For this study, mastitis was considered if diagnosed during the early stages of lactation (before the median days at first service (72)) and is referred to as mastitis before mating. Only the first occurrence of mastitis was considered for the analysis.

The information was attained from the dairy management programs VAMPP® Bovino 3.0 and Afifarm®. The programs also produced the projected milk yield over the 305-day lactation based on monthly recordings of individual measurements. The collected data were processed in an Excel sheet where days to first service, days open, services per conception, and interval between services were calculated. Incidence of each disease was converted into a binary variable with cattle testing positive to a disease being classified as 1, and no disease as 0.

The observations were further classified into calving seasons according to the date of calving in one of three quadrimesters: quadrimester 1, for parturitions occurring between November and February; quadrimester 2, for parturitions between March and June; and quadrimester 3, for parturitions between July and October. The distribution of calvings into the proposed quadrimesters was based on records available on the temperature and relative humidity, as well as the experience of farmers and researchers in the study region. This separation of quadrimesters was further supported by a recent study from Rosales–Martinez et al. (2021). Moreover, observations were classified according to parity into cows of first (1), second (2), or more (3 +) parities.

Ambient temperature and relative humidity for the experimental years were recorded from historical records on the Comalapa weather station (http://www.worlddata.info). Temperature humidity index was calculated based on Hahn (1999). In El Salvador, the rainy season spans from May to October, in which the relative humidity significantly increases and ranges between 75 and 82% (http://www.worlddata.info) (Fig. 1). Similarly, ambient temperature increased in March averaging above 28 °C throughout August (http://www.worlddata.info) (Fig. 1).

Statistical analysis

A logistic regression was conducted using the inbuilt *glmer* function of R to find the log odds of the occurrence of retained placenta, IUI, and mastitis as influenced by quadrimester (i.e., Nov-Feb, Mar-Jun, Jul-Oct). The model included quadrimester, parity (i.e., 1, 2, 3 +) and their interaction, year within farm as random effect, and cow as repeated measurement. A wald test was used to verify the significance of the effects using the *wald.test* inbuilt function of R. The interaction was removed from the model if $P \geq 0.10$. A confidence interval of the log odds was estimated, and both coefficients and confidence intervals were exponentiated to present results as odds ratios (OR). Differences between levels of a factor were tested with the wald test.

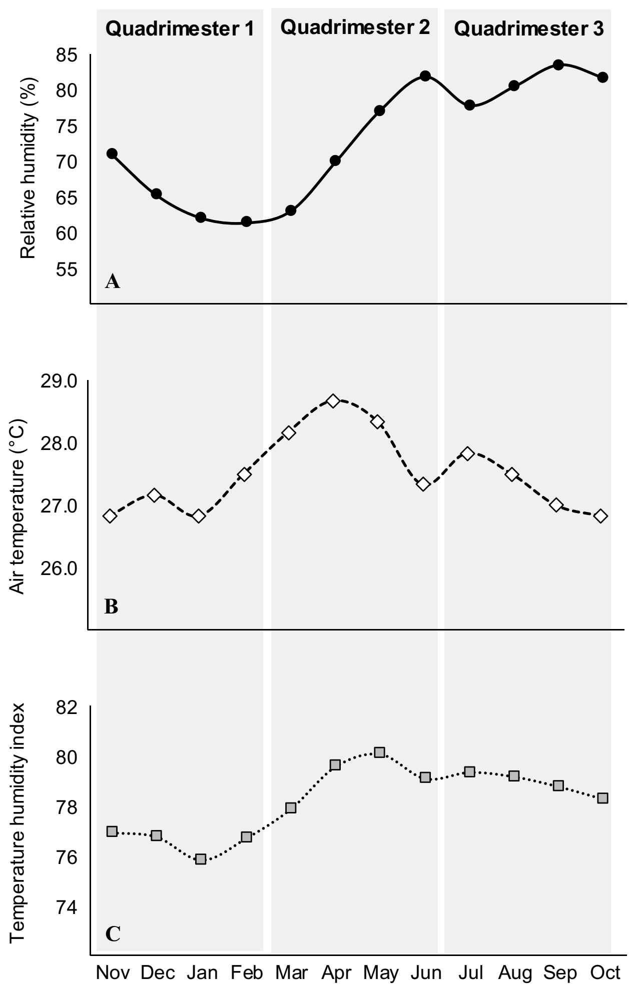


Fig. 1 Average relative humidity (**A**), air temperature (**B**), and temperature humidity index (**C**) in the region of the study by quadrimester (Monthly averages from years 2010, 2011, 2012, 2015, 2016, and 2017) Source: http://www.worlddata.info accessed on 13th July 2020

The OR of the effects of experiencing retained placenta, IUI, or mastitis on being culled due to infertility were estimated conducting a logistic regression as explained above. Year within farm were included in the model as random effect, and cow as repeated measurement, as well as parity and its interaction with each disease. The interaction was removed from the model if $P \geq 0.10$. The likelihood of developing an IUI after experiencing retained placenta was also estimated by logistic regression following the same methodology.

Multiple linear regression was conducted to evaluate the effects of quadrimester on days to first service, services per conception, interval between services, and days open by using a mixed model with quadrimester and parity as fixed effects, year within farm as a random effect, and cow as repeated measurement. A second multiple linear regression was conducted to evaluate the effects of diseases on days to first service, services per conception, interval between services, and days open by using a mixed model with retained placenta, IUI, and mastitis as well as parity as fixed effects, year within farm as a random effect, and cow as repeated measurement.

An additional multiple linear regression was conducted to evaluate the effects of quadrimester on 305-day milk yield using a mixed model with quadrimester and parity as fixed effects, year within farm as a random effect, and cow as repeated measurement. Similarly, the effects of diseases on 305-day milk yield were evaluated with a mixed model with retained placenta, IUI, and mastitis as well as parity as fixed effects, year within farm as a random effect, and cow as repeated measurement.

For all multiple linear regressions, main effects were kept in the model regardless of their significance. Interactions between significant effects were explored and removed if $P \geq 0.10$. The regression was conducted using the *lmer* function from the "lme4" package in R.

Before the multiple linear regression, all response variables were tested for normality by plotting a histogram and by estimating the skewness using the *skewnesss* function from the "moments" package in R. Days to first service, services per conception, interval between services, and days open showed a positive skewness (2.36, 1.28, 2.76, and 1.60, respectively) and were therefore log transformed to approach normality. After log transformation skewness was reduced to 0.488, 0.164, -0.147, and 0.486 for days to first service, services per conception, interval between services, and days open, respectively. The regressions were conducted on the log transformed data. For the interpretation of the data during the results description and discussion, the coefficients of the model were exponentiated to express the effects of the factors as a proportion of the intercept. Skewness of 305-d milk yield was 0.249 and needed no transformation.

A final analysis was conducted to study the effects of BCS. First, a logistic regression was conducted to estimate the OR of the occurrence of retained placenta, IUI, and mastitis as influenced by BCS. Second, a multiple linear regression was performed to find the effects of parity and quadrimester (fixed effects) on BCS using the *lmer* function from the lme4 package in R with year within farm as a random effect and cow as repeated measurement.

For all analyses significances were declared for $P < 0.05$, while tendencies were declared for $0.05 \leq P < 0.10$.

Results

A total of 3320 calving events from 1948 cows were recorded. There were 1107, 681 and 1532 calvings from cows with 1, 2, and 3 + parities, respectively. There were

1066, 1046 and 1208 calvings in Quadrimester 1, 2, and 3, respectively. Retained placenta was observed in 304 cases out of 2685 available records, IUI were observed in 1397 cases out of 3320 records, and mastitis occurrence before the median first service was observed in 511 cows out of 3290 records. Descriptive statistics of parity, days to first service, services per conception, interval between services, days open, and body condition score are presented in Table 1.

Effects of quadrimester and parity on reproductive parameters

Before describing the results, a brief explanation of the interpretation of the output of a regression of factor variables on log transformed data is as follows. The intercept and the regression coefficients must be exponentiated, but each is interpreted in a different manner. First, for a given response, the intercept represents the reference condition (set by the researcher), which in Table 2 was that of a primiparous cow in the first quadrimester. Therefore, after exponentiation, the days to first service for primiparous cows calving in quadrimester 1 (intercept) were 67.3 ($e^{4.21}$). The regression coefficients, after exponentiation, represent a ratio of the intercept: for quadrimester 2 the exponentiated regression coefficient was 1.103 ($e^{0.098}$), which in terms of percentage, indicates that cows calving in quadrimester 2 increased the days to first service by 10.3% compared with cows calving in quadrimester 1 (intercept). Therefore, cows calving in the second and third quadrimester increased days to first service by 10.3 and 10.8%, respectively, without differences between quadrimesters 2 and 3 ($P = 0.64$). Cows with 2 and 3 + parity decreased the number of days to first service by 13.0 and 11.9% without differences between multiparous cows ($P = 0.45$) (Table 2; Fig. 2A). There was no quadrimester × parity interaction on days to first service (Table 2).

When an interaction effect is significant, using Table 2 as an example, the effects “Quadrimester 2” and “Quadrimester 3” represent primiparous cows calving in quadrimester 2 or 3, respectively; the effects “Parity 2” and “Parity 3 + ” represent cows with 2 and 3 + parities, respectively, calving in quadrimester 1; the interaction effects are defined by the parity and quadrimester represented on the table, hence “Quadr 2*Parity 2” represents a cow of second parity calving in quadrimester 2. Figure 2 aids to the interpretation of interaction effects in this study.

Table 1 Descriptive statistics of the variables utilized for the current analysis of reproductive performance of cows under tropical conditions

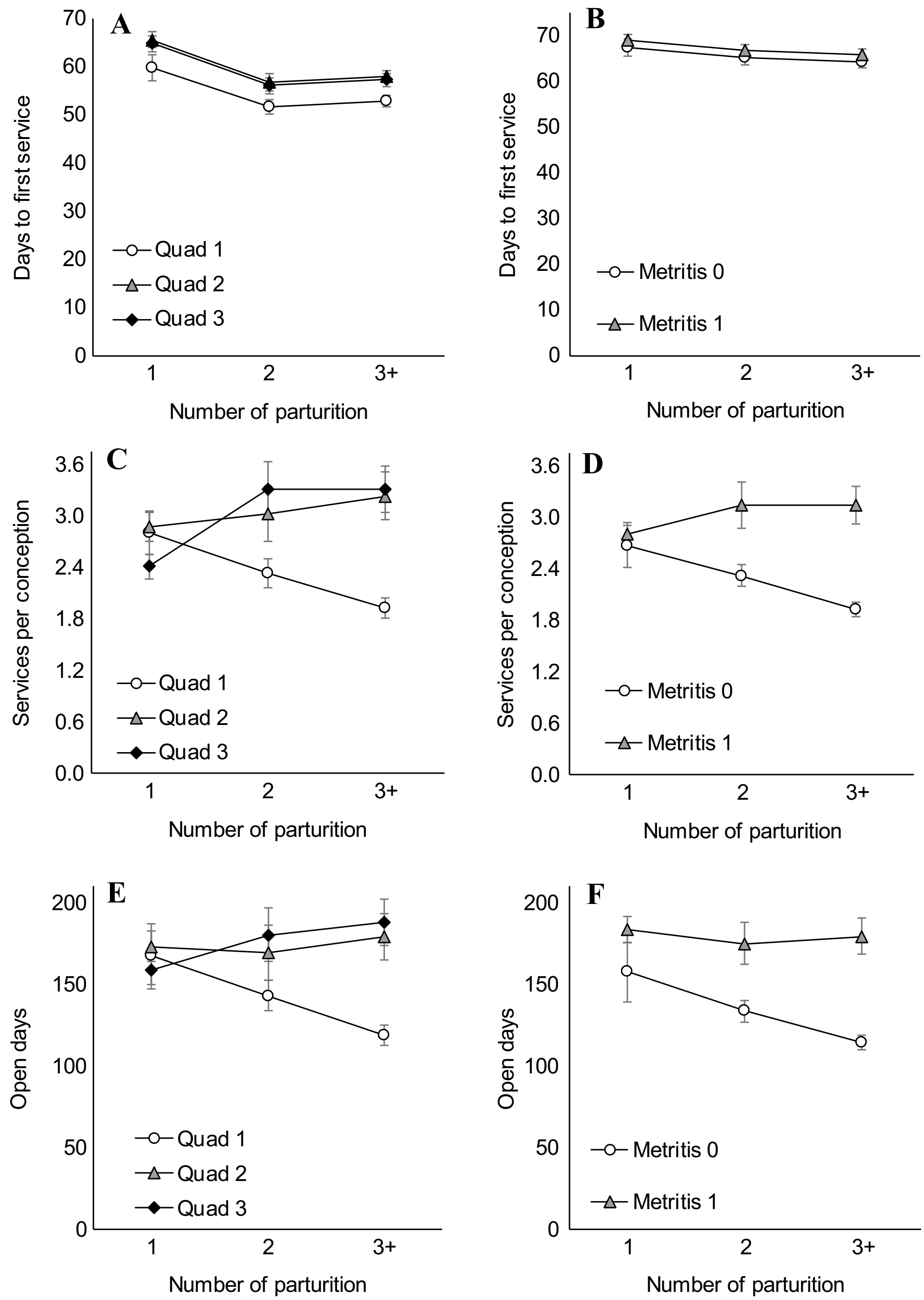
	n	Average	Standard deviation	Median	Minimum	Maximum
Parity	3320	2.82	1.95	2	1	13
Days to first service	2790	76.3	41.0	72.0	28.0	439.0
Services per conception	2194	3.13	2.28	2	1	11
Interval between services	1457	53.5	38.72	43.0	7	351
Days open	2212	187.5	127.5	149.0	35	997
305-day milk yield (kg)	2021	5483	1354	5441	2454	9904
Body condition score	955	2.4	0.48	2.5	1.5	4

Table 2 Effects of quadrimester (Quadr) and parity on the log transformed days to first service, services per conception, days open, and interval between services in tropical Holstein cows

	Days to first service		Services per conception		Days open		Interval between services	
Effect	Coefficients	P	Coefficients	P	Coefficients	P	Coefficients	P
(Intercept)[a]	4.21 (±0.031)	< 0.01	1.03 (±0.086)	< 0.01	5.12 (±0.1001)	0.01	3.78 (±0.092)	< 0.01
Quadrimester 2	0.098 (±0.021)	< 0.01	0.029 (±0.059)	0.62	0.030 (±0.052)	0.56	-0.055 (±0.037)	0.14
Quadrimester 3	0.103 (±0.020)	< 0.01	-0.148 (±0.058)	0.01	-0.059 (±0.052)	0.26	0.020 (±0.036)	0.58
Parity 2	-0.139 (±0.023)	< 0.01	-0.148 (±0.067)	0.02	-0.150 (±0.060)	0.01	0.043 (±0.039)	0.27
Parity 3 +	-0.126 (±0.019)	< 0.01	-0.354 (±0.057)	< 0.01	-0.324 (±0.051)	< 0.01	0.052 (±0.033)	0.12
Quadr 2*Parity 2			0.088 (±0.094)	0.37	0.023 (±0.089)	0.79		
Quadr 3*Parity 2			0.171 (±0.094)	0.07	0.079 (±0.084)	0.35		
Quadr 2*Parity 3 +			0.141 (±0.082)	0.08	0.065 (±0.073)	0.38		
Quadr 3*Parity 3 +			0.159 (±0.079)	0.04	0.112 (±0.071)	0.11		

[a] Reference category: Parity 1, quadrimester 1

Fig. 2 Interactive effects of quadrimester and parity on days to first service (**A**), services per conception (**C**) and on days open (**E**), and interactive effects of metritis and parity on days to first service (**B**), services per conception (**D**) and on days open (**F**) in tropical Holstein cows



Services per conception of primiparous cows calving in quadrimester 1 were 2.80 (intercept). There was a quadrimester × parity interaction where for quadrimester 1 services per conception decreased by 13.8 and 29.8% for parity 2 and 3 +, respectively (Table 2). However, in the quadrimester 2 and 3 services per conception increased with increasing parity with greater effects found in quadrimester 3 (Table 2, Fig. 2C). A similar pattern was observed for open days (intercept = 167 days) (Table 2, Fig. 2E).

The exponentiated intercept of interval between services for those cows with more than one service was 43.8 days and was not affected neither by quadrimester nor by parity ($P > 0.05$) (Table 2).

Effects of quadrimester and parity on the incidence of retained placenta, IUI and mastitis before mating

There was no effect of quadrimester and parity on retained placenta ($P \geq 0.15$) (Table 3). Compared with

Table 3 Odds ratio and confidence interval (CI) of the effects of quadrimesters and parity on the incidence of retained placenta, intrauterine infection, and mastitis before breeding in dairy cows

Effect[a]	Retained placenta		Intrauterine infection		Mastitis	
	Odds ratio (CI)	P	Odds ratio (CI)	P	Odds ratio (CI)	P
Quadrimester 2	1.257 (0.930—1.700)	0.14	1.312 (1.083—1.591)	< 0.01	1.038 (0.810—1.331)	0.77
Quadrimester 3	1.081 (0.801—1.459)	0.61	1.187 (0.990—1.423)	0.06	1.112 (0.880—1.406)	0.37
Parity 2	1.021 (0.724—1.439)	0.91	0.725 (0.587—0.894)	< 0.01	1.804 (1.343—2.423)	< 0.01
Parity 3 +	1.086 (0.827—1.427)	0.55	0.660 (0.557—0.782)	< 0.01	2.619 (2.054—3.341)	< 0.01

[a] Reference category: Parity 1, quadrimester 1

calving in quadrimester 1, the OR of developing an IUI increased when cows calved in the second (1.312; $P < 0.01$) and the third (1.187; by tendency $P = 0.06$) quadrimester ($P < 0.05$) (Table 3), with no differences between the two latter quadrimesters ($P = 0.25$). Moreover, compared with primiparous cows, those in second and 3 + parities were less likely to suffer from IUI (OR = 0.725 and 0.660 for 2 and 3 + parities, respectively) ($P < 0.01$) (Table 3), with no differences between these multiparous cows ($P = 0.40$).

The quadrimester of calving had no effect on the OR of experiencing mastitis before mating ($P \geq 0.37$) (Table 3). Cows with 2 and 3 + parities had higher OR of experiencing mastitis before mating than primiparous cows (1.804 and 2.619 for 2 and 3 + parities, respectively) (Table 3), with cows in 3 + parities being more likely to experience mastitis than those in second parity ($P < 0.01$). No quadrimester × parity interaction was observed on the incidence of retained placenta, IUI's or mastitis.

Effects of retained placenta, IUI, and mastitis before mating on reproductive parameters

After exponentiation of the intercept, that is the condition of primiparous cows developing no disease, days to first service was 69.4 days. Experiencing retained placenta did not influence days to first service ($P = 0.31$), but developing from IUI or mastitis before mating increased the days to first service by 9.1 and 6.0%, respectively ($P \leq 0.01$) (Table 4). Cows in 2 and 3 + parities had 12.7 and 11.7% lower days to first service than primiparous cows, respectively (Table 4), with no differences between those multiparous cows ($P = 0.16$).

For services per conception, after exponentiation, the intercept was 2.69, and no effect of mastitis or retained placenta was observed ($P = 0.43$ and 0.76, respectively) (Table 4). There was an IUI × parity interaction, where IUI increased the services per conception, majorly affecting multiparous cows. In healthy cows, services per conception decreased with increasing parity (2.68, 2.36 and 1.96 for cows in 1, 2 or 3 + parities, respectively) ($P < 0.01$);

Table 4 Effects of the incidence of mastitis, intrauterine infection (IUI), and retained placenta, as well as parity on the log of days to first service, services per conception, days open, and interval between services in tropical Holstein cows

Effect	Days to first service		Services per conception		Days open		Interval between services	
	Coefficients	P	Coefficients	P	Coefficients	P	Coefficients	P
(Intercept)[a]	4.24 (± 0.022)	< 0.01	0.99 (± 0.087)	< 0.01	5.06 (± 0.12)	< 0.01	3.72 (± 0.092)	< 0.01
IUI	0.088 (± 0.018)	< 0.01	0.052 (± 0.049)	0.28	0.151 (± 0.043)	< 0.01	0.097 (± 0.031)	< 0.01
Mastitis	0.058 (± 0.023)	0.01	-0.032 (± 0.041)	0.43	0.074 (± 0.036)	0.04	0.076 (± 0.042)	0.07
Retained placenta	0.030 (± 0.029)	0.31	0.017 (± 0.055)	0.76	0.011 (± 0.049)	0.82	0.075 (± 0.053)	0.16
Parity 2	-0.135 (± 0.023)	< 0.01	-0.128 (± 0.050)	0.01	-0.153 (± 0.045)	< 0.01	0.049 (± 0.039)	0.21
Parity 3 +	-0.124 (± 0.019)	< 0.01	-0.311 (± 0.043)	< 0.01	-0.305 (± 0.038)	< 0.01	0.051 (± 0.034)	0.13
IUI*Parity 2			0.173 (± 0.081)	0.03	0.097 (± 0.071)	0.17		
IUI*Parity 3			0.154 (± 0.066)	0.02	0.116 (± 0.058)	0.04		

[a] Reference category: Healthy cow in parity 1

conversely, in cows experiencing an IUI, services per conception increased from 2.82 in primiparous cows to 3.18 and 3.13 in cows with 2 and 3 + parities, respectively ($P < 0.05$) (Fig. 2D).

The days open of healthy primiparous cows were 155.6. There was no effect of retained placenta ($P = 0.82$) (Table 4). Experiencing mastitis before breeding increased days open by 7.7% ($P = 0.04$) with no interaction with parity. Experiencing an IUI increased the days open ($P < 0.05$) (Table 4), but an IUI × parity interaction appeared, where in healthy cows, days open decreased by 14.2 and 26.3%, for 2 and 3 + parities, respectively, whereas for cows experiencing an IUI, an increase in days open was observed with 3 + parities ($P = 0.04$) (Table 4; Fig. 2F).

Interval between services for cows with more than one service increased by 10.2% if a cow developed an IUI ($P < 0.01$), while experiencing mastitis tended ($P = 0.07$) to increase the interval between services by 7.9% (Table 4). No effects of retained placenta or parity were observed ($P \geq 0.13$) (Table 4).

Effects of retained placenta, IUI and mastitis before mating on cows' culling due to infertility

Experiencing retained placenta ($P = 0.03$) or an IUI (by tendency, $P = 0.08$) increased the OR of a cow being culled for not achieving a pregnancy (Table 5). Developing mastitis before mating did not have any effect on the likelihood of a cow being culled due to infertility ($P = 0.52$) (Table 5). Multiparous cows were more likely to be culled compared with primiparous cows, with those with 3 + parities showing higher OR of being culled than those of cows in second parity ($P < 0.01$) (Table 5). No interaction appeared between parity and the incidence of any disease.

Table 5 Odds ratio of the effect of suffering from a disease and of parity on being culled due to infertility

Effect[a]	OR (Confidence interval)	P
IUI	1.163 (0.979—1.384)	0.08
Mastitis	1.078 (0.857—1.356)	0.52
Retained placenta	1.334 (1.036—1.717)	0.03
Parity (2)	1.402 (1.107—1.776)	< 0.01
Parity (≥ 3)	2.159 (1.767—2.664)	< 0.01

[a] Reference category: Healthy cow in parity 1

Effects of quadrimester on 305-day milk yield

There was an interactive effect ($P = 0.07$) between quadrimester and parity (Fig. 3), where in quadrimester 1, cows of 2 and 3 + parities had greater 305-day milk yields than primiparous cows. However, in quadrimester 3, 305-day milk yield decreased with increasing parity.

Effects of retained placenta, IUI and mastitis before mating on 305-day milk yield

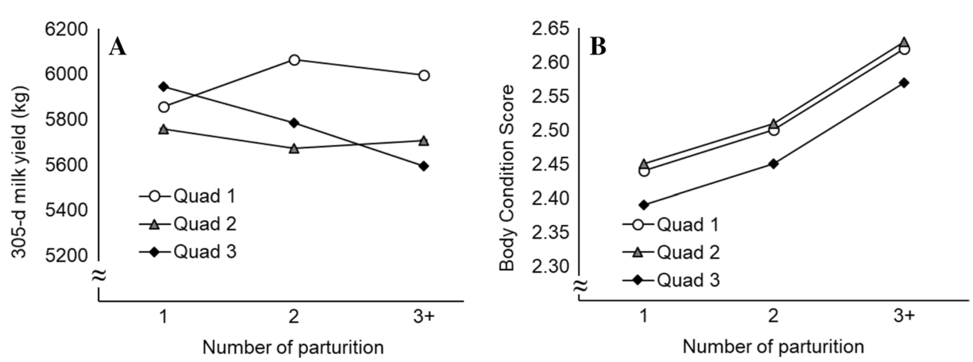
Retained placenta did not have an effect on 305-day milk yield ($P = 0.21$) (Table 6). Suffering from an IUI caused a reduction of 309 kg of milk on the 305-day milk yield compared with healthy cows, without any interaction with

Table 6 Effects of the incidence of mastitis, intrauterine infection (IUI), and retained placenta, as well as parity on the 305-day milk yield in tropical Holstein cows

	305-day milk yield (kg)	
Effect	Coefficients	*P*
(Intercept)[a]	5635 (± 167.3)	< 0.01
Mastitis	-216.1 (± 181.6)	0.23
Retained placenta	-121.7 (± 95.9)	0.21
IUI	-309.3 (± 61.1)	< 0.01
Parity (2)	268.1 (± 85.3)	< 0.01
Parity (≥ 3)	-24.23 (± 70.6)	0.73
Mastitis*Parity 2	-501.7 (± 262.3)	0.06
Mastitis*Parity 3 +	-128.5 (± 218.5)	0.56

[a] Reference category: Healthy cow in parity 1

Fig. 3 Effects of quadrimester and parity on 305-day milk yield (**A**) and on body condition score (**B**) in tropical Holstein cows



the number of parities of the cows (Table 6). Mastitis before mating also had a negative effect on 305-milk yield where ($P=0.05$) reducing it by an average of 282 kg per 305-day lactation, with stronger negative effects on cows of second parity (Table 6).

Effects of body condition score at calving on incidence of retained placenta, IUI and mastitis before mating

From the results of logistic regression, increasing the BCS at calving by one unit decreased the likelihood of developing an IUI (OR = 0.611, CI = 0.414 – 0.893, $P=0.01$) or mastitis before mating (OR = 0.374, CI = 0.253 – 0.546; $P<0.01$). The incidence of retained placenta was not influenced by BCS at calving (OR = 3.56, CI = 0.754 – 16.9; $P=0.11$).

From the results of the multivariate regression (Fig. 3B), BCS was only marginally affected by Quadrimester (2.52, 2.53, and 2.47 for quadrimester 1, 2 and 3, respectively; $P=0.08$). Body condition score slightly increased with increasing parity (2.42, 2.49, and 2.60 for parity 1, 2 and 3 +, respectively; $P=0.01$). There was no quadrimester × parity interaction ($P>0.05$).

Discussion

Overall, the herds included in this study had poor reproductive performance, with an average of more than three services per conception, and median days open of almost five months. The median BCS of those observations available was 2.5, which is considered to be low; however, the causal association between the poor reproduction and the low BCS cannot be concluded from this study. Better reproductive performance can certainly be attained under tropical conditions, nevertheless, reproductive parameters similar to the ones reported in this study are not uncommon for dairy cattle in hot and humid environments with studies in Colombia, Ethiopia, Mexico, and Sudan reporting days open ranging between 160 and 186 (Ahmed et al. 2007; Avendaño-Reyes, et al. 2010; Motta et al. 2012; Wondossen et al. 2018). As such, the conditions of the herds included in this study reflect the conditions of a significant proportion of dairy farms in the tropics.

For the remainder of the discussion is important for the reader to keep in mind that indicators of heat stress or of the presence of pathogenic agents were not measured in this observational study. Rather the inference is made that hotter, more humid climatic conditions are conducive of heat stress in dairy cows, while infectious diseases appear to be more prominent during the rainy season than in the dry season as supported by practical observations and the scientific literature (Kerro Dego and Tareke 2003; Nobrega and Langoni 2011). Predictably, cows calving during quadrimester 1 find the least challenging environmental conditions in the period around calving; those calving in quadrimester 2, experience higher temperatures, and partially higher humidity; and those calving in quadrimester 3 experience the highest humidity but also have spent an extended period under hot and humid conditions, including late pregnancy, and therefore, are expected to experience greater negative impacts of hyperthermia and disease challenges (Fig. 1).

Cows calving in quadrimester 1 had in general better reproductive performance than cows calving in the other two quadrimesters, where the environmental conditions were more conducive of thermal stress. Wilson et al. (1998) found that the second wave dominant follicle was less likely to ovulate in cows undergoing heat stress, while Roth and Wolfenson (2016) stated that hyperthermia can reduce the production of androstenedione and follicular estradiol, inhibiting estrous behavior. This can help to explain the increased days to first service observed in quadrimesters 2 and 3, which could have been the result of both a delayed resumption of ovulation and a depressed estrous behavior that prevents the farmer from detecting the estrous. In this study, days to first service decreased with increasing parities and is in agreement with previous studies (e.g., Meikle et al. 2004; Wathes et al. 2007). The shorter number of days to first service in multiparous cows has been linked to a faster return to the ovarian activity mediated by greater plasma concentrations of IGF-I, which in turn, is linked to a better body condition score (Meikle et al. 2004).

Services per conception in quadrimester 1 decreased with increasing parity. A lower conception rate in primiparous cows can be associated with a lower body condition at calving, as oocytes collected from cows with low body condition have been shown to have lower rates of cleavage (Snijders et al. 2000). However, during quadrimesters 2 and 3, where hyperthermia was more likely to occur, multiparous cows had significantly higher services per conception than primiparous, a tendency that became more obvious in quadrimester 3 (2.42, 3.31 and 3.31 services per conception for cows with 1, 2 and 3 + parities, respectively) (Fig. 2C). Higher levels of production and a lower surface area to body mass ratio make multiparous cows more susceptible to heat stress (Aguilar et al. 2009). Several studies have showed delays in embryonic divisions and reduced numbers of fertilized oocytes in heat stressed cows (Gendelman et al. 2010; Wolfenson and Roth 2019). Therefore, the hyperthermia suffered to a greater extent by multiparous cows could cause the poorer conception rate during quadrimesters 2 and 3. Moreover, the more obvious effects during quadrimester 3 might be related to the long-lasting consequences of heat stress, as these are influenced not only by the exposure, but also by the accumulated influence from previous time periods (Ji et al. 2020; Wilson et al. 1998). Services per conception appeared to determine the days open, but, because of the lower number of days to first service in multiparous cows, the differences in days open between primiparous and multiparous cows were proportionally less than in services per conception.

For those cows with more than one service, the interval between services was much larger than the expected 21 days (e.g., 43.8 days for primiparous cows). In practical terms, this indicates that after the first insemination, one in two estrous cycles did not occur or was not detected. It is well documented that exposure to conditions leading to heat stress several days before and after insemination may result in embryonic death, which can occur even past day 17 after insemination (Hansen 2002), which may delay the occurrence of the following estrous. Moreover, hot and humid environments, which are ubiquitous for these cows the year around, not only inhibit estrous behavior (Wolfenson and Roth 2019), but also reduce its duration (e.g., from 20 h in spring to 14 h in summer, Gangwar et al. 1965), both conditions making it more difficult for the observer to timely detect the estrous. Regardless of the causes, it is clear that an important problem occurs in these herds that significantly increases the interval between services, and further research is required to better understand its causes and provide better management guidelines.

Heat stress has been associated with early parturition (DuBois and Williams 1980; Markusfeld 1984), and early parturition, in turn, has been listed as a probable cause for increased incidence of retained placenta and metritis (DuBois and Williams 1980). This agrees in part, with the findings of the current study, where the incidence of IUI –but not retained placenta—increased in quadrimester 2 and 3 compared with quadrimester 1. However, retained placenta is a common precursor of metritis (Pohl et al. 2015; Sandals et al. 1979), which was also observed in the current study, where cows that experienced retained placenta had higher OR of developing an IUI (2.76, CI = 2.10 – 3.62 $P < 0.01$) (data not showed). Cows with 2 and 3 + parities were less likely to develop an IUI than primiparous cows, in agreement with previous studies and likely as a consequence of more calving difficulties for the first parturition cows (Burfeind et al. 2014; Pohl et al. 2015).

Hyperthermia also affects the health status of the cows and makes them more susceptible to ailments as high circulating levels of stress hormones can interfere with the ability of the immune system to prevent bacterial infections (Jacobsen 1996). Moreover, warm and wet conditions, like those found in quadrimester 2 and 3, are favorable conditions for the proliferation of pathogens, which in the current study reflected in the higher incidence of IUI, albeit not of mastitis before mating. Mastitis was more prominent in cows with 2 and 3 + parities than in primiparous ones.

In agreement with our findings, van den Borne et al. (2010), with high yielding Dutch dairy cows, and Mungube et al. (2004) with crossbred Ethiopian cows, found greater incidence of clinical and subclinical mastitis in multiparous cows compared with primiparous ones. Importantly, the previously cited studies referred to mastitis throughout the complete lactation, and the current study refers to mastitis before the first service. It is not possible to know with certainty the reasons for the higher incidence of mastitis in multiparous cows, but it could be due to a debilitated immune status, for example due to higher levels of production, higher susceptibility to heat stress, and/or to the wider teat canals of multiparous cows compared with primiparous (Klaas et al. 2005).

Body condition score at the time of calving was available only for a subset of all observations in the dataset ($n = 955$). These observations clearly demonstrated that BCS was associated to the incidence of diseases, where a low BCS increased the likelihood of developing mastitis or and IUI, which is in agreement with previous reports (Dubuc et al. 2010; Garnsworthy 2006). Notably, the median BCS at calving reported in the current study was 2.5, which is generally recognized as low (Ribeiro et al. 2016) and is outside the commonly recommended range of 3.0 to 3.75. It can therefore be concluded that these herds are at risk of excessive loss of condition during the first stages of lactation, what could contribute to the poor reproductive performance found in the current study.

Suffering from a disease itself can further limit the fertility of a cow (Pérez-Báez et al. 2019). Previous studies have found that the incidence of IUI, retained placenta or mastitis can increase the days open, days to first service, or calving interval (DuBois and Williams 1980; Kumari et al. 2016; Nava-Trujillo et al. 2010; Ribeiro et al. 2016; Sandals et al. 1979). An alteration in the cow's hormonal profile, poor oocyte competence, fertilization failure, and unfavorable uterine environment for embryonic development have been listed as possible reasons for the reduced fertility when suffering one of these conditions (see review of Kumar et al. 2017). In the current study IUI and mastitis—both inflammatory conditions—caused a decrease in fertility in the cows, but not retained placenta.

Days to first service increased when a cow contracted an IUI or mastitis, indicating difficulties to return to a normal ovarian activity in these affected cows. Developing uterine diseases can decrease the number of ovarian follicles, increase the incidence of ovarian cysts, and reduce the luteal size (Molina-Coto and Lucy 2018). Moreover, the inflammation of tissues when an animal contracts either mastitis or IUI causes a release of inflammatory mediators, such as endotoxin or the cytokine interleukin 1 (IL-1), which disrupts the follicular phase and can cause premature luteolysis due to increase levels of prostaglandin F2α (Santos et al. 2004; Sheldon et al. 2002). Services per conception and interval between services increased with the occurrence of IUIs, and multiparous cows were more affected by IUIs than primiparous cows (Fig. 1D). Previous studies have reported that even when an egg is fertilized, experiencing an inflammatory disease before mating reduced the ability of the zygotes to develop and survive (Giuliodori et al. 2013; Ribeiro et al. 2016). Therefore, it appears that developing a disease not only increases the time needed to return to a normal ovarian activity and achieving a pregnancy, but also decreases the success in maintaining a pregnancy once that egg has been fertilized. The overall result would be an increase in days open, as was observed in the current study.

In this study, during the quadrimester 1, multiparous cows produced more milk than primiparous ones, as it is would be expected in regular dairy cows farming. However, when cows calved under more humid and hot conditions, like those of qudrimesters 2 and 3, their 305-day milk yield decreased, in accordance with the findings of Collier et al. (1982) and Almoosavi et al. (2021). The decreased production from cows that have calved or spent their last part of the gestation under thermal stress is related to an altered endocrine status of the cow that carries into subsequent milk yield (Collier et al. 1982). Even though heat stress was not directly measured in the cows included in this study, this study confirms the effects of the environmental conditions on the milk production over the course of the subsequent lactation.

Moreover, a poor reproduction and poor health management is typically associated with a net economic loss in the farm (Inchaisri et al. 2010; Kossaibati and Esslemont 1997), mainly associated with the culling costs and a lower return in milk production. Indeed, in this study, we found a decrease milk yield over the course of 305-day adjusted lactation when animals suffer from mastitis before mating or IUI, in agreement with the findings of Inchaisri et al. (2010) and Kossaibati and Esslemont (1997). The economic loss due to a reduced lactation milk yield was not calculated in this study, but the negative impact of the diseases directly on productivity becomes obvious and adds to the costs associated with a poor reproduction and the treatment of diseases itself.

An obvious consequence of infertility is the culling of the cow. Together with udder-related problems and low productivity, poor reproduction is a main reason for culling in dairy farms (Compton et al. 2017). In the current study, developing an IUI (by tendency) and retained placenta lead to a higher likelihood of a cow being culled due to infertility. Mastitis has been previously associated with increased culling (Santos et al. 2004), but those results refer more to udder-related problems. Despite its obvious effects on reproductive parameters, mastitis before mating did not appear to play a major role on culling in the current study.

Conclusion

There was a clear influence of calving season on reproductive parameters and incidence of diseases, where hotter, more humid conditions led to poorer reproduction as well as higher occurrence of infectious diseases like IUI's and mastitis. Calving under hotter and more humid conditions also led to lower milk yields over the lactation. Developing either mastitis before mating, or an IUI, led to a decreased in milk yield over the lactation, as well as to a poor reproductive performance, possibly related to problems such as a later return to ovarian activity, failure to achieve a pregnancy after insemination, and longer intervals between estrous, likely as a result of silent estrous and embryonal death. The low body condition score observed for the cows could be associated with the reproductive problems of the herds included in the study. Management strategies should be directed toward minimizing the effects of high temperature and high humidity, particularly during the hottest and more humid months of the year, creating more hygienic conditions, and ensuring an appropriate body condition at calving to improve the reproductive efficiency of tropical dairy cows.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Conflicts of interest/Competing interests The authors of this paper declare that there is no conflict of interest.

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