

Risk factors for twinning and effects on the subsequent production and reproductive performances in Moroccan Holstein cows

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ABSTRACT: This study was designed to determine the risk factors for twinning and its effects on the subsequent production and reproductive performances in Moroccan Holstein cows. The data analyzed were from records of 1770 calving events (157 for twins and 1613 for singles) from July 2008 to December 2012 in a private farm. The logistic regression was used to estimate the effect of risk factors comprising parity, calving season and calving year. To analyze the twinning effect on gestation length, days to the first insemination, days open, the number of insemination per conception, 305-day milk and fat yields and fat percentage, the linear model that included fixed effects of parity, calving season, calving year and calving type was used. Out of 1770 calvings, 157 or 8.87% were twins. Risk factors for twin calvings were parity ($p < 0.001$) and calving season ($p < 0.05$), while calving year had no significant effect ($p > 0.05$). The greatest twinning rate was observed at parity 2 and when calving occurred from May to August. Twin calving affected fat percentage in the lactation initiated by twinning, whereas milk and fat yields were not significantly influenced ($p > 0.05$). Twin-calved cows had a greater fat percentage than single-calved cows ($p < 0.01$). Cows delivering twins had shorter ($p < 0.001$) gestation lengths than those giving singles (273.4 vs. 278.2 days), whereas days to the first insemination, days open and the number of inseminations per conception were not affected by twinning ($p > 0.05$). Therefore, intensive management is required for twin calving cows and their calves to achieve maximum production and to diminish losses.

Keywords: Dairy cattle, gestation length, parity, milk yield, twin calving.

INTRODUCTION

Twinning is a phenomenon that occasionally occurs in dairy cattle, with the frequency generally averaging 3 to 5% (Rutledge, 1975; Johanson *et al.*, 2001; Bicalho *et al.*, 2007; Shamiah *et al.*, 2007; Hossein-Zadeh *et al.*, 2008; Andreu-Vazquez *et al.*, 2012). The risk factors for twinning include mainly genetics, age/parity of cows, the season of conception/calving and herd management (Cady and Van Vleck, 1978; Nielen *et al.*, 1989; Kinsel *et al.*, 1998; Fricke, 2001; Johanson *et al.*, 2001; Shamiah *et al.*, 2007; Hossein-Zadeh *et al.*, 2008; Cabrera and Fricke 2021). Twinning may have some positive but numerous

unfavourable effects (Chapin and Van Vleck, 1980; Fricke, 2001; Bicalho *et al.*, 2007; Cobanoglu, 2010; Andreu-Vazquez *et al.*, 2012). It decreased reproductive performance by increasing the average days open and the number of inseminations per conception during the subsequent lactation (Chapin and Van Vleck, 1980; Mostafa, 2009b; Hossein-Zadeh, 2010b). Moreover, it increased dystocia (due to malpresentation), incidence of retained placenta, calf mortality and occurrence of freemartins (Fricke, 2001; Shamiah *et al.*, 2007; Mostafa, 2009b; Hossein-Zadeh, 2010b; Cabrera and Fricke, 2021).

However, effects on milk production related to twinning are controversial. Since twinning has increased milk yield in some studies (Hossein-Zadeh, 2010a; Sawa *et al.*, 2015), but has decreased it in others (Nielen *et al.* 1989; Mostafa 2009a) and had no significant effect in some others (Chapin and Van Vleck, 1980). At present, it is clear that twin calvings reduce the profitability of dairy herds (Cobanoglu, 2010). Thus, based on a summary of economic analyses from several studies, Cabrera and Fricke (2021) reported that the estimated losses due to twinning ranged between \$59 and \$161 per twin pregnancy. In Morocco, there is no information on twinning rate in Holstein dairy cows, the risk factors affecting it, nor its effects on production and reproductive performances.

Therefore, the objectives of this study were to calculate the frequency of twinning and to determine the risk factors affecting it. In addition, the comparison was made between twin and single calvings for the potential effect on production and reproductive performances during the subsequent lactation in a commercial dairy herd located in northern Morocco.

MATERIALS AND METHODS

Herd management

The data analyzed were collected in a private dairy herd in northern Morocco. The mean annual temperature is 18.7°C, varying from 0°C in February to 41°C in August. The mean annual rainfall is 600 mm, mainly from November to March. This herd was studied because of the completeness of its records. All cows in the herd were Holstein. Data were recorded between July 2008 and December 2012. The average age at all calving was 45.5 months, ranging from 21 to 83 months, and the average lactation number was 2.16, ranging from 1 to 3. First-parity cows accounted for 28.6%, whereas second and third parities represented 26.7 and 44.7% of the calving records, respectively. All cows were housed in free stalls and were observed for estrus two times daily, at 0900 h and 1500 h. Artificial insemination is used almost exclusively. Cows detected in estrus were inseminated within 1 h after estrus detection. Pregnancy was confirmed by rectal palpation 55-65 days after artificial insemination. All lactating cows were fed the same diet, formulated to meet the nutrient requirements of a lactating Holstein cow weighing 650 kg and producing 25-35 kg of milk daily. Cows were milked in fully automated milking parlors (Boumatic USA) equipped with automatic milking machines twice to three times daily according to their lactation stage. During the study period, the mean 305-day milk yield was 8110 kg.

Studied variables and statistical analyses

The data available on studied cows were identification of

cow, parity, birth date, conception date, calving date, calving type (single or twin), 305-day milk and fat yields, and fat percentage. From the information available, days to first insemination, days open, the number of inseminations per conception and gestation length were calculated. After editing, 1770 records were analyzed. Calving type was defined as a binary trait (0= single calving, 1= twin calving) based on whether a cow gave one or two calves to assess the effects of risk factors. According to the binary nature of twinning, a binary logistic regression was used to model the odds of calving type. The logistic model, fitted with calving type as the outcome variable (single: 0, twin: 1), included fixed effects of risk factors of parity (3 levels: 1, 2 and 3), the season of calving (3 levels: January - April, May - August and September - December) and year of calving (5 levels: 2008, 2009, ..., 2012). The parameter estimates, standard errors, odds ratios, and 95% confidence intervals were obtained from PROC Logistic (SAS, 2009). Reference classes consisted of parity 3, calving season January - April, and calving year 2011, which had the highest number of observations.

Twinning effects on subsequent cow 305-day milk and fat yields and fat percentage of milk, as well as on days to the first insemination, days open, the number of inseminations per conception and gestation length were determined using the general linear models procedure (Proc GLM) (SAS, 2009). Fixed effects included in the model were parity (3 levels: 1, 2 and 3), season of calving (3 levels: January - April, May - August and September - December), year of calving (5 levels: 2008, 2009, ..., 2012) and calving type (2 levels: single and twin). Interactions between fixed effects were not tested, and hence were assumed negligible.

RESULTS AND DISCUSSION

Mean twinning rate

The incidence of twinning in dairy Holstein cows averaged 8.87% (157/1770). The sex ratio among the twins was 28.7, 23.6 and 47.7% of male pairs (MM), female pairs (FF) and mixed-sex pairs (MF), respectively. These frequencies were similar to 28.0% MM calves, 27.2% FF calves and 44.8% MF calves found by Atashi *et al.* (2012) and to 19.7% MM calves, 27.3% FF calves and 53.0 MF calves reported by Cockcroft and Sorrell (2015), who concluded that they are not statistically different from the expected ratio of 1MM:2MF:1FF. The average twinning rate obtained in the current study is higher than the range (1.4 to 5.6%) reported in previous studies (Kinsel *et al.*, 1998; Bicalho *et al.*, 2007; Shamiah *et al.*, 2007; Mostafa, 2009b; Andreu-Vazquez *et al.*, 2012; Sawa *et al.*, 2015). Nevertheless, it is in the range of the prevalence of twin calvings among farms ranged from 0.3 to 12.0% (Silva del Rio *et al.*, 2007), from 0 to 9.6% (Kinsel *et al.*, 1998), from

2.7 to 7.0% (Bicalho *et al.*, 2007), from 0 to 7.1% (Nielen *et al.*, 1989) and from 2.2 to 5.5% (Hossein-Zadeh *et al.*, 2008). These findings suggest that the incidence of twinning is not a biologically fixed rate but depends on herd management, mainly nutrition (Hossein-Zadeh *et al.*, 2008). In the studied herd, cows were well fed, and the distributed diet covered their nutrient requirements, explaining their high milk yield and twinning rate. Moreover, Wiltbank *et al.* (2000) speculated that high milk production increases steroid metabolism, which slows down the natural decline in follicle stimulating hormone (FSH) so that follicles have more time to undergo physiological changes before they ovulate.

Effects of risk factors

Parameter estimates of the logistic regression and odds ratios for twinning are presented in Table 1. The analysis of risk factors showed that parity and season of calving had significant effects on the incidence of twinning ($p < 0.05$ and $p < 0.001$, respectively). In contrast, the calving year had no significant effect ($p > 0.05$). The twinning increased from parity 1 to parity 2, then decreased at parity 3. Cows at parity 1 and parity 2 had a twinning 12% lower and 58% higher, respectively than cows at parity 3 (reference class). However, the Wald Chi-square test results showed that the difference between parity 1 and the reference class was not significant ($p > 0.05$). The increase in the prevalence of twinning for multiparous compared with primiparous observed in the present study is in agreement with previous studies with Holstein cattle that have shown increases in twinning with parity, with twinning rates ranging between 0.3 and 1.63% in parity 1 and ranging between 3.01 and 6.5% in later parities (Cady and Van Vleck, 1978; Karlsen *et al.*, 2000; Johanson *et al.*, 2001; Bicalho *et al.*, 2007; Hossein-Zadeh *et al.*, 2008; Atashi *et al.*, 2012; Lett and Kirkpatrick, 2018; Cabrera and Fricke, 2021). In addition, the largest change was observed between parity 1 and parity 2, with increases from 1.1 to 4.2% and 0.3 to 4.3%, respectively (Hossein-Zadeh *et al.*, 2008; Lett and Kirkpatrick, 2018). The effect of parity on the twinning rate might be due to the increase in milk production that is the primary factor affecting the incidence of double ovulation in lactating dairy cows (Kinsel *et al.*, 1998; Fricke and Wiltbank, 1999; Wiltbank *et al.*, 2000).

Cows that calved from May to August and from September to December, *i.e.* when conception occurred from September to February, had a twinning rate of 77% and 34%, respectively higher than cows that calved from January to April. Nevertheless, the results of Wald Chi-square test showed that the difference was significant ($p < 0.05$) only between the May to August calvings and the reference class. The present findings are consistent with several studies with Holstein cattle (Cady and Van Vleck, 1978; Nielen *et al.*, 1989; Johanson *et al.*, 2001; Hossein-

Zadeh *et al.*, 2008; Atashi *et al.*, 2012; Lett and Kirkpatrick, 2018), which found that the highest twinning rate occurred in spring and summer. Thus, Nielen *et al.* (1989) reported that cows calving from April to September had higher twinning than those in the October-March period. Likewise, Atashi *et al.* (2012) observed that the twinning rate in the calving season of fall and winter was lower than in spring, but there was no difference between the observed twinning rates in the calving season of spring and summer. Cady and Van Vleck (1978) found the peak of twins in calving between May and July. Johanson *et al.* (2001) reported that the incidence of twins was greatest in April through June and lowest in October through December. Various reasons might explain seasonal variation in twinning, such as nutritional flushing, photoperiod, or both (Nielen *et al.*, 1989). Furthermore, Sartori *et al.* (2002) reported that the seasonal variation in the twinning rate might be due to the highest double ovulation rate and the lowest embryonic loss during warmer months. Contrary to the result of the current study, Kinsel *et al.* (1998) did not observe any detectable association between twinning and season.

The twinning rate did not differ significantly ($p > 0.05$) among calving years, although it was high during years 2011 and 2012. The herd management that did not change across years, especially nutrition, might explain the absence of annual variation in twinning. Similar to this finding, Andreu-Vazquez *et al.* (2012) observed that the twinning rate remained stable over the 11 years period studied. Contradictory to the current finding, Kinsel *et al.* (1998), Atashi *et al.* (2012) and Cabrera and Fricke (2021) observed an annual increase in twinning over the periods examined.

Effects of twinning on reproductive and production performances

Least-squares means for reproductive and production performances of single-calved cows and twin-calved cows are reported in Table 2. The calving type had significant effects on gestation length ($p < 0.001$), but had no significant effect ($p > 0.05$) on days to first insemination, days open and number of inseminations per conception. Gestation lengths of cows calving twins (273.4 days) were 4.8 days shorter than those of cows calving singletons (278.2 days). This result is consistent with those of previous studies (Cady and Van Vleck, 1978; Nielen *et al.*, 1989; Ryan and Boland, 1991; Day *et al.*, 1995; Mostafa, 2009b) that reported gestation lengths of Holstein cows calving twins were 5.2 to 8.5 days shorter than those of single-carrying cows. The main factor explaining the gestation length difference between single and twin pregnancies might be the litter weight carried by cows; as the bearing-twins carried a heavy litter, the short gestation length is a way to get rid of their heavy load. Mostafa

Table 1. Parameter estimates \pm standard errors (SE), p-values, odds ratios and their 95% Wald confidence intervals (CI) from logistic binary regression model for twinning.

Risk factors	Number	Parameter estimate \pm SE	p-value	Odds ratio	95% Wald CI	
					lower	upper
Intercept	1770	-2.65 \pm 0.19	0.0001			
Parity		***				
1	507	-0.13 \pm 0.23	0.5692	0.88	0.55	1.38
2	472	0.46 \pm 0.20	0.0227	1.58	1.07	2.35
3	791	reference		1.00		
Season of calving		*				
January-April	776	reference		1.00		
May-August	302	0.57 \pm 0.24	0.0161	1.77	1.11	2.83
September-December	692	0.30 \pm 0.22	0.1714	1.34	0.88	2.06
Year of calving		NS				
2008	70	0.14 \pm 0.44	0.1038	1.15	0.48	2.76
2009	115	-0.25 \pm 0.36	0.4958	0.78	0.38	1.57
2010	475	-0.16 \pm 0.21	0.5830	0.85	0.56	1.29
2011	970	reference		1.00		
2012	140	0.40 \pm 0.32	1.6404	1.50	0.81	2.78

NS= Not significant at $p > 0.05$, *= significant at $p < 0.05$, ***= significant at $p < 0.001$.

Table 2. Effects of twinning on reproductive and production performances during the subsequent lactation of Holstein cows.

Type of calving	Number	Days to first insemination	Days open	Number of insemination per conception	Gestation length (days)	Milk yield (kg)	Fat yield (kg)	Fat percentage (%)
Single	1613	97.3 \pm 1.63	199.0 \pm 3.62	2.60 \pm 0.06	278.2 \pm 0.22	8067.3 \pm 72.5	338.5 \pm 3.33	4.20 \pm 0.02
Twin	157	98.8 \pm 3.44	194.9 \pm 7.61	2.62 \pm 0.12	273.4 \pm 0.47	8210.8 \pm 151.5	351.1 \pm 6.96	4.30 \pm 0.03
P-value		0.6489	0.5884	0.8082	0.0001	0.3359	0.0665	0.0049

(2009b) speculated that the shorter gestation length for twins compared to single pregnancies might be related to the weight and larger size of twin calves, which represent more pressure stimuli on the wall of the uterus leading to early parturition as compared to singles.

Differences in days to the first insemination, days open and the number of inseminations per conception of twin and single calving cows were insignificant ($p > 0.05$). Similar to the present study, Bell and Roberts (2007) found no significant difference for days open between twin and single bearing cows. However, the results are in direct contrast to other studies (Chapin and Van Vleck, 1980; Nielen *et al.*, 1989; Bicalho *et al.*, 2007; Mostafa, 2009b; Hossein-Zadeh, 2010b; Andreu-Vazquez *et al.*, 2012) on twinning effects for reproductive performance of cows. The twin bearing cows had 15 days (Kay 1978) and 10.47 days (Mostafa, 2009b) to first insemination more than their single bearing counterparts. Days open were detected to

be longer by 11 days (Syrstad, 1974), 11.8 days (Nielen *et al.*, 1989), 15.6 days (Hossein-Zadeh, 2010b), 18.6 days (Mostafa, 2009b) and 22 days (Chapin and Van Vleck, 1980) for cows having twins compared to cows having singles. Additionally, Kinsel *et al.* (1998) observed 6-day shorter days open in the case of cows giving twins in some North American dairy farms. On the other hand, Mahnani *et al.* (2016) reported that twinning increased the number of inseminations per conception by 0.04 and 0.18 units in primiparous and multiparous cows, respectively. He also concluded that the negative reproductive effects of twinning were more severe for primiparous than multiparous cows. Delays and decreases in the studied reproductive performance in cows with twins relative to those with singletons may result from low body reserves and body condition score at the calving time of twin calvers resulting from the short gestation length, and consequently short dry off days, as well as from increased incidence of

dystocia and retained placenta, which had a negative effect on reinitiation of estrus cycle in twin-calving cows (Echternkamp and Gregory, 1999a,b; Bell and Roberts, 2007).

Except for the fat percentage of milk that was significantly higher for twin-calved cows than single-calved cows ($p < 0.01$), twinning had no significant effect on milk and fat yields ($p > 0.05$). The literature is inconsistent on the effect of twin births on milk production. Similar to current results, Bell and Roberts (2007) found no significant difference in 305-day milk yield between twin and single calvers. In addition, Mahnani *et al.* (2016) found that twinning had no significant effect on 305-day milk, fat and protein percentages. Contradictory to the present finding, some studies (Syrstad, 1974; Kay, 1978; Kinsel *et al.*, 1998; Hossein-Zadeh, 2010a) reported a positive association between twinning and milk production in dairy cows. Twin-calved cows had greater 305-day milk production, fat yield and fat percentage of milk than single-calved cows, especially cows with FF twin pairs (Hossein-Zadeh, 2010a). However, Nielen *et al.* (1989) reported that cows bearing twins had an average of 103 kg more milk production than cows bearing singles in the first 100 days of lactation. However, no significant differences were detected when the 305-day milk yields were compared. Moreover, Atashi *et al.* (2012) reported that the number of days to peak yield, persistency and milk yield from calving to lactation peak were higher for cows of twin calves than cows of single calves.

On the other hand, Chapin and Van Vleck (1980) and Fricke (2001) found low milk yields in the following lactation for twinner cows owing to metabolic disorders and the depressive effect observed on cows during the early part of lactation. Moreover, Bicalho *et al.* (2007) reported that primiparous and multiparous cows with singletons produced 1.2 and 0.8 kg/day, respectively more milk than cows with live twins, and 1.9 kg and 1.5 kg/day, respectively more than cows were with at least 1 dead twin. Likewise, Mostafa (2009a) reported that cows having twins had lower daily milk yield (1.6 kg), peak yield (4.39 kg) and 305-day milk yield (487.6 kg) than those delivering single calves. The low body condition at calving time and the greater incidence of metabolic and reproductive disorders of most twinner cows might negatively affect subsequent milk yield (Mostafa, 2009a).

Conclusion

The findings in this study show that under the current management practices, the twinning rate of Moroccan Holsteins is about 9%. It is affected by parity and season of calving, but not by year of calving. Moreover, twinning shortens the gestation length and increases the milk fat percentage. However, it did not affect milk and fat yields, nor on days to the first insemination, days open and the

number of inseminations per conception. Therefore, an intensive management is required for twin calving cows and their calves to achieve maximum productivity and to diminish losses. Nevertheless, it is necessary to determine the twinning effects on other traits, such as dystocia, postpartum problems, perinatal calf mortality and culling, in order to have a clear idea on the herd management to adopt and the real impact of twinning.

CONFLICT OF INTEREST

The author declares no competing interests.

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