An Observational Analysis of Twin Births, Calf Stillbirth, Calf Sex Ratio, and Abortion in Iranian Holsteins

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ABSTRACT

Calving records of Holstein dairy cows from April 1998 to September 2006 comprising 16 herds with 104,572 calving events representing 4,045 twin births were used to evaluate reported twinning rate, calf sex ratio, stillbirth, and abortion rates in single and twin births. Overall, the reported twinning rate was 3.9%, and twinning increased with parity [1.1% for primiparous cows vs. 5.7% for cows in their fourth or greater lactation; odds ratio (OR) = 5.50]. Regardless of parity, the greatest twinning rate was observed when conception occurred in fall season from September to December (OR = 1.17). Calf stillbirth was greater after twin births, with 18.8% of twin calving events resulting in one or both calves as stillborn, compared with 4.0% for singleton births (OR = 7.58). Calf stillbirth for multiparous cows was 2.9% for single births and 18.0% for twin births (OR = 7.08), whereas for primiparous cows, stillbirth was 6.6% for singletons and 27.7% for twins (OR = 5.85). Calf sex ratios (male, M; female, F) were 52.4% M and 47.6% F for singleton calves and 28.2% MM, 48.9% MF, and 22.9% FF for twin calves. The mean abortion rate was 13.4%, with 13.8% for single births and 4.2% for twin births (OR = 1.22). Abortion rate for multiparous cows was 15.9% for single births and 4.0% for twin births (OR = 4.31), whereas for primiparous cows, abortion rate was 9.4% for single births and 5.4% for twin births (OR = 1.89). Although milk production, as a causative factor associated with twinning, increased in recent years, twinning rate decreased over the years.

Key words: twinning, calf stillbirth, calf sex ratio, abortion rate

INTRODUCTION

Cattle (*Bos taurus*) are uniparous species, meaning that, in most cases, females produce only 1 offspring per

pregnancy (Komisarek and Dorynek, 2002). Twinning occurs relatively rarely, with the frequency generally not exceeding 1% in most beef herds. However, in dairy herds, the incidence of twin births is greater (on average 4 to 5%) and is strongly affected by age and parity of the dam (Day et al., 1995; Komisarek and Dorynek, 2002; Silva del Río et al., 2007).

The incidence of double births may have both positive and negative effects, which mainly depends on the purpose for which cattle are raised (Komisarek and Dorynek, 2002). A beef cow can wean more total calf weight by raising twins. Twin births offer the potential for increased beef production efficiency, if sustainable changes in management can be made to accommodate problems inherent with twinning (Kirkpatrick, 2002). However, twin birth is disadvantageous for most beef and dairy producers because of its association with several unfavorable effects, including lower potential calf survival and poorer cow reproductive performance (Fricke, 2001). The risk of pregnancy loss increases and the profitability of the herd diminishes drastically as the frequency of twin births increases (Lopez-Gatius et al., 2002; Lopez-Gatius and Hunter, 2005). Regarding cow performance, twinning is associated with increased dystocia (due to malpresentation), increased incidence of retained placenta, greater mortality rates, frequent occurrence of freemartins, and longer interval from parturition to first estrus (Kirkpatrick, 2002; Echtenkamp et al., 2007; Silva del Río et al., 2007). Cows that had stillbirth had significantly increased risk of culling-death throughout the lactation and increased median days open by 88 d compared with cows that had live calves (Bicalho et al., 2007). Also, economic losses from stillbirth include not just the lost calf, the decreased survival, and increased days open and increase in days to first breeding but also the decreased milk yield (Maizon et al., 2004; Bicalho et al., 2008). Abortions represent a loss of reproductive efficiency in normal bovine populations, and spontaneous abortion of dairy cows is an increasingly important problem that contributes substantially to low herd viability and production inefficiency by decreasing the number of potential female herd replacements and lifetime milk production and by increasing costs associated with

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breeding and premature culling (Thurmond et al., 2005).

Increased frequency of twinning would increase the potential for obtaining more progeny from a genetically superior female, thereby allowing those females to play a larger role in a selection program (Cady and Van Vleck, 1978). Low heritability, a long generation interval for progeny testing, sex-limited expression, and an unfavorable correlation with milk yield make the control of twinning difficult by selection (Komisarek and Dorynek, 2002). But, the genetic potential for improving twinning is large, and this highlights the importance of increased knowledge on the inheritance of twinning (Gregory et al., 1997).

The objective of this study was to analyze incidences of twin births recorded in Iranian Holstein cows to make comparisons with single births regarding several factors influencing the odds of twinning. Comparisons were also made for the incidences of abortion, stillbirth, and calf sex ratio between twin and single births.

MATERIALS AND METHODS

Data Set

Calving records from April 1998 to September 2006 comprising 104,572 calving events from 16 dairy herds of Iran were included in the data set. Twin calvings accounted for 4,045 of the total observations. Information for individual calving events, including herd identification, cow identification, successful insemination date, calving date, parity, multiple births, calf sex, calf stillbirth, abortion, and calving difficulty, were included in the data set. The number of calves born per calving event was coded as a dichotomous variable [0 = singleton (C1); 1 = twin (C2)]. Calf sex for singleton births was coded **M** for males and **F** for females, whereas calf sex for twin births was coded as **MM** for male pairs, **FF** for female pairs, and **MF** for mixed-sex pairs. Stillbirth was defined as the loss of calves from d 260 until calving. Stillbirth for singleton calves was coded as **A** for calves born alive and **D** for calves born dead, whereas for twin calves was coded as **AA** when both calves were born alive, **DD** when both calves were born dead, and **DA** when only one of the twin calves survived. Abortion was defined in the data set as loss of the fetus between 45 and 260 d of gestation. Abortion was coded as **Y** for the cows aborted and **N** for the cows that did not abort. Previous twinning was defined as a binary variable where 0 is allocated to a cow that did not have twins in the previous lactation and otherwise 1. Months of birth were grouped into 4 seasons: January through March (winter), April through June (spring), July through September (summer), and October through December

(fall). Also, calving years were grouped into 3 classes: 1998 through 2000 (**Y1**), 2001 through 2003, and 2004 through 2006 (**Y3**). First-parity cows represented 31.2%, whereas second, third, and fourth and greater parities accounted for 24.9, 17.6, and 26.3% of the calving records, respectively.

Characteristics of the Herds

The modern dairy industry in Iran is about 50 yr old. A majority of the Iranian cattle population consists of several domestic breeds and their crosses with Holstein cows. Only about 800,000 heads are purebred Holsteins. The herds used in this study are among the purebred Holsteins that are managed under conditions similar to most other developed countries. The herds are under official performance and pedigree recording. Cows are milked 3 times a day. The main components of the dairy ration consist of corn silage, alfalfa, cotton seed, cotton seed meal, barley grain, canola meal, wheat bran, fat powder, beet pulp, and feed additives, and cows were fed by total mixed ration. Herringbone milking parlors are common, and well-known milking machines are used. Artificial insemination is used for almost all of the herds, and 60 to 80% of semen is usually of US and Canadian proven sires. Rolling herd average for these herds is 9,000 to 10,000 kg per cow per year, and average days open is 137 d. However, the national herd average for all Iranian Holsteins is around 8,000 kg per lactation.

Statistical Analyses

A multivariable logistic regression model was used to analyze twinning, calf stillbirth, and abortion using the maximum likelihood method of the LOGISTIC procedure of SAS (SAS Institute, 2002). Model specification was based on the backward elimination method, and the fit of all statistical models was evaluated by using the Hosmer and Lemeshow goodness-of-fit test of SAS (Hosmer and Lemeshow, 2000) by including the lackfit option in the model statement. Variables (main effects or interaction terms) that were significant by the Wald statistic at $P < 0.05$ were included in the model.

The general equation of the logistic regression model was defined as follows:

$$\text{Logit}(\pi) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_n X_n$$

where π = either of the odds of twin births, stillbirth, or abortion; α = the intercept parameter; β_1 to β_n = the logistic regression coefficients (parameter estimates) for the explanatory effects (X_1 to X_n) included in the statistical model.

Table 1. Estimated odds ratios, parameter estimates, and their 95% confidence intervals (CI) for the effects of calving season, parity of dam, previous twinning, and calving year on reported twinning rates of Holstein cows from 1998 to 2006

Effect	Class	Estimate $\pm$ SE	95% CI	Odds ratio	95% CI	P-value
Intercept		-3.4 ± 0.03	-3.40 to -3.30	—	—	—
Season	Spring	—	—	1	—	<0.001
	Summer	0.2 ± 0.03	0.13 to 0.23	1.17	1.07 to 1.27	—
	Fall	0.0 ± 0.03	-0.06 to 0.05	0.98	0.89 to 1.07	—
	Winter	-0.2 ± 0.03	-0.26 to -0.13	0.80	0.73 to 0.89	—
Year	1	—	—	1	—	<0.001
	2	0.1 ± 0.02	0.01 to 0.10	0.94	0.87 to 1.02	—
	3	-0.2 ± 0.02	-0.22 to -0.13	0.75	0.69 to 0.81	—
Previous twinning	0	—	—	1	—	0.005
	1	0.1 ± 0.04	0.03 to 0.17	1.22	1.06 to 1.40	—
Parity of dam	1	—	—	1	—	<0.001
	2	0.2 ± 0.03	0.13 to 0.25	4.02	3.56 to 4.54	—
	3	0.5 ± 0.03	0.45 to 0.56	5.51	4.88 to 6.23	—
	≥ 4	0.5 ± 0.03	0.45 to 0.56	5.50	4.89 to 6.18	—

The model used to analyze twinning across time included the fixed class effects of herd, calving year, calving season, parity of dam, previous incidence of twinning, and parity by year interaction. The model used to analyze stillbirth for calves born as singleton included the fixed class effects of herd, parity of dam, sex of the calf (M vs. F), calving year, and parity by year interaction. The model used to analyze stillbirth for calves born as twins included the fixed class effects of herd, parity of dam, and sex of the calf (MM, MF, and FF). Also, the model used to analyze stillbirth for all calves included the fixed class effects of herd, parity of dam, calf number (2 vs. 1), calving year, parity by year, and calf number by parity.

The model used to analyze abortion for single births included the fixed class effects of herd, season of calving, parity of dam, calving year, parity by year, and the covariate of gestation length. But, the abortion for twin births was analyzed in a model in which the main effects of herd, season of calving, and the covariate of gestation length were included. The model fitted to the analysis of abortion for single and twin births was similar to the model used for the analysis of abortion

for twin births, but the effects of calving year, parity of dam, and calf number were added to the statistical model. Reference categories for the comparison of odds ratios (OR) for each effect were as follows: spring, calving year Y1 (1998 to 2000), parity 1, cows that did not have twins in the previous lactation (previous twinning = 0), singleton births for analysis of calf stillbirth and twin births for analysis of abortion, interaction effect between single births and parity 1, F for singleton births, and FF for twin births.

RESULTS AND DISCUSSION

Reported Twinning Rates in Holstein Dairy Cows

Herd. The incidence of twinning across farms ranged from 2.2 to 5.5% ($P < 0.001$). Similarly, previous reports with twinning rates of 3.2 and 2.4%, respectively, reported twinning rates ranging from 0 to 9.6% among farms (Nielen et al., 1989; Kinsel et al., 1998). Silva del Río et al. (2007) reported that the incidence of twinning across farms ranged from 0.3 to 12.0%. This range suggests that the incidence of twinning is not a biologically

Table 2. Estimated twinning rate of Holstein cows by year of calving and parity

Calving year	Estimated twinning rate				
	Parity 1	Parity 2	Parity 3	Parity ≥ 4	Total
1998	1.40	4.31	6.65	6.37	4.68
1999	1.40	4.43	6.00	6.56	4.60
2000	0.87	5.12	7.33	6.47	4.95
2001	1.29	5.14	6.01	6.95	4.85
2002	1.11	5.22	5.94	5.58	4.46
2003	1.29	4.19	5.04	5.41	3.98
2004	0.88	3.62	5.08	5.41	3.75
2005	0.90	3.46	5.30	4.79	3.61
2006	0.88	3.10	5.23	4.74	3.49

Table 3. Estimated odds ratios, parameter estimates, and their 95% confidence intervals (CI) for the effects of parity of dam, calf number, and calving year on reported calf stillbirth for singleton and twin births of Holstein cows from 1998 to 2006

Effect	Class	Estimate $\pm$ SE	95% CI	Odds ratio	95% CI	P-value
Intercept		-2.4 ± 0.03	-2.44 to -2.33	—	—	—
Parity of dam	1	—	—	1	—	<0.001
	2	-0.2 ± 0.03	-0.26 to -0.15	0.43	0.40 to 0.47	—
	3	-0.3 ± 0.03	-0.32 to -0.19	0.41	0.37 to 0.45	—
	≥ 4	-0.2 ± 0.03	-0.24 to -0.12	0.44	0.41 to 0.48	—
Year	1	—	—	1	—	0.048
	2	0.0 ± 0.02	-0.05 to 0.05	1	0.98 to 1.02	—
	3	0.0 ± 0.02	-0.02 to 0.07	1.02	0.99 to 1.05	—
Calf number	1	—	—	1	—	<0.001
	2	1.0 ± 0.02	0.91 to 1.04	7.58	6.92 to 8.29	—
Calf number $\times$ parity	C1-P1	—	—	1	—	<0.001
	C2-P2	-0.5 ± 0.06	-0.65 to -0.43	0.58	0.45 to 0.76	—
	C2-P3	-0.5 ± 0.06	-0.59 to -0.35	0.62	0.49 to 0.79	—
	C2-P4	-0.7 ± 0.04	-0.83 to -0.67	0.47	0.34 to 0.65	—

fixed rate and that differences in herd management including genetic makeup may allow some control of the incidence of twin births.

Parity. Overall, the reported frequency of twinning was 3.9%. The percentages of twin births at parity 1, parity 2, parity 3, and parity ≥ 4 were 1.1, 4.2, 5.7, and 5.7%, respectively. The odds of twinning increased from parity 1 to parity 4 and beyond ($P < 0.001$; OR = 5.50; parity ≥ 4 vs. parity 1). The odds of twinning not only increased from first to second parity (OR = 4.02; parity 2 vs. parity 1) but also from second to third parity (OR = 1.37; parity 3 vs. parity 2; 95% confidence interval = 1.21 to 1.55) to reach a plateau for the remaining parities (Table 1). Previous studies reported an increasing trend for twinning with parity (Fricke and Wiltbank, 1999; Johanson et al., 2001; Silva del Río et al., 2007). In this study, a greater increase in reported twinning rate at each calving year was observed for lactating cows compared with nonlactating heifers (Table 2). Lactating dairy cows are at greater risk for twinning because of their greater occurrence of multiple ovulations compared with nonlactating heifers (Silva del Río et al., 2007). Fricke and Wiltbank (1999) and Kinsel et al. (1998) concluded that milk production was the

primary factor affecting the incidence of double ovulation in lactating dairy cows and may explain the effect of parity on twinning rate.

Parity by Year. Twinning rate decreased over the years from 4.7% in calving year Y1 to 3.6% in calving year Y3 (OR = 0.75; $P < 0.001$; Y3 vs. Y1). Interestingly, there was parity by year interaction for twinning rate ($P < 0.001$). For primiparous and multiparous cows, the twinning rate decreased from 1.2 and 5.9% during calving year Y1 to 0.9 and 4.5% during calving year Y3, respectively. Previous studies have reported an increase in twinning rate over time (Kinsel et al., 1998; Johanson et al. 2001; Silva del Río et al., 2007).

Season of Calving. Independent of parity, the greatest odds of twinning were observed in summer, when conception occurred from September to December (OR = 1.17; Table 1; $P < 0.001$; summer vs. spring), and the lowest odds of twinning were for winter calvings (OR = 0.80; $P < 0.001$; winter vs. spring). Silva del Río et al. (2007) reported that the greatest twinning rate was observed in summer months compared with other seasons. Johanson et al. (2001) reported that the incidence of twins was greatest in April through June, at 5.88%, and lowest in October through December, at 4.23%.

Table 4. Estimated odds ratios, parameter estimates, and their 95% confidence intervals (CI) for the effects of calving year, parity of dam, and sex of calf on reported calf stillbirth for singleton births of Holstein cows from 1998 to 2006

Effect	Class	Estimate $\pm$ SE	95% CI	Odds ratio	95% CI	P-value
Intercept	—	-3.4 ± 0.03	-3.44 to -3.33	—	—	—
Parity of dam	1	—	—	1	—	<0.001
	2	-0.2 ± 0.03	-0.26 to -0.13	0.45	0.41 to 0.49	—
	3	-0.3 ± 0.04	-0.33 to -0.18	0.42	0.38 to 0.47	—
	≥ 4	-0.2 ± 0.03	-0.24 to -0.11	0.45	0.42 to 0.50	—
Sex	Male	0.4 ± 0.02	0.34 to 0.41	2.10	1.96 to 2.25	<0.001
	Female	—	—	1	—	—
Year	1	—	—	1	—	0.001
	2	0.05 ± 0.02	0.01 to 0.10	1.16	1.06 to 1.27	—
	3	0.05 ± 0.02	0.00 to 0.09	1.15	1.06 to 1.26	—

Table 5. Reported twin stillbirth for dairy cows from 1998 to 2006 by sex of calf and parity

		Parity of dam at calving								
		1		2		3		≥4		
Status ¹	Sex ²	n	%	n	%	n	%	n	%	Total (%)
AA	MM	53	15.9	230	22.2	250	24.6	322	21.6	80.4
	FF	77	23.1	206	19.8	187	18.4	277	18.6	
	MF	105	31.5	410	39.5	401	39.4	601	40.3	
DD	MM	22	6.6	25	2.4	21	2.1	34	2.3	7.8
	FF	9	2.7	11	1.1	14	1.4	15	1.0	
	MF	18	5.4	45	4.3	31	3.0	58	3.9	
DA	MM	9	2.7	37	3.6	46	4.5	46	3.1	11.8
	FF	7	2.1	21	2.0	21	2.1	44	2.9	
	MF	33	9.9	53	5.1	46	4.5	94	6.3	

¹AA = both calves born alive; DD = both calves born dead; DA = only 1 of the calves survived.

²MM = male pairs; FF = female pairs; MF = mixed-sex pairs.

The seasonal variation in reported twinning rates could be explained by variation in the ambient temperature. Sartori et al. (2002) reported that double ovulation rate was greater during warmer months. During late summer until early autumn, a reduction in early embryonic loss attributable to decreased heat stress during a time of increased double ovulations and conception of twins may cause the seasonal variation in twinning rate. Photoperiod, nutritional flushing, or both have been speculated to affect the seasonal variation in twinning (Nielen et al., 1989).

Previous Twinning. Among cows that had twin birth once, the chance of delivering twins again was 6.9% (Table 1; OR = 1.22; $P < 0.01$; class 1 vs. class 0), and 9.6% of those having twin birth twice delivered twins again. Silva del Río et al. (2007) and Kinsel et al. (1998) reported that cows that delivered twins previously have been shown to be at a greater risk of delivering twins subsequently. Similarly, Nielen et al. (1989) reported that 9.3% of the cows that had twin birth once and 12.5% of those having twin birth twice delivered twins again. These results may underestimate the true recurrence of twinning in the data set, because cows calving twins are of greater parity and at a greater risk

of culling during the subsequent lactation (Nielen et al., 1989).

Reported Stillbirth in Holstein Calves

Herd. The overall incidence of calf stillbirth was 4.9% although it varied among herds from 2.9 to 9.8%. There was an increase in singleton calf stillbirth as herd size increased ($P < 0.05$). Caution must be taken when interpreting the effect of herd size on calf stillbirth. One explanation for these results may be that individual cows are less intensively managed during calving in larger herds; however, larger herds represented in this data set may have more accurately reported calf stillbirth than smaller herds, or perhaps the effect is due to herd expansion rather than the effect of herd size itself.

Parity. Greater odds of calf stillbirth existed for calves born from primiparous cows than from multiparous cows ($P < 0.001$) and for calves born as singletons (7.2 vs. 3.4%) or twins (29.4 vs. 18.7%; Tables 3 to 6). Bicalho et al. (2007) and Meyer et al. (2001) reported a significant decreasing trend in the incidence of stillbirth by parity in Holstein dairy cows.

Table 6. Estimated odds ratios, parameter estimates, and their 95% confidence intervals (CI) for the effects of parity of dam and sex of calf on reported calf stillbirth for twin births of Holstein cows from 1998 to 2006

Effect	Class ¹	Estimate ± SE	95% CI	Odds ratio	95% CI	P-value
Intercept		−1.3 ± 0.10	−1.49 to −1.21	—	—	—
Parity of dam	1	—	—	1	—	<0.001
	2	−0.2 ± 0.10	−0.31 to −0.02	0.53	0.40 to 0.71	—
	3	−0.2 ± 0.10	−0.39 to −0.10	0.49	0.37 to 0.66	—
	≥4	−0.1 ± 0.10	−0.19 to 0.06	0.59	0.45 to 0.77	—
Sex	FF	—	—	1	—	0.002
	MF	0.1 ± 0.10	−0.07 to 0.19	1.37	1.10 to 1.69	—
	MM	0.2 ± 0.10	0.07 to 0.28	1.51	1.20 to 1.90	—

¹MM = male pairs; FF = female pairs; MF = mixed-sex pairs.

Table 7. Estimated odds ratios, parameter estimates, and their 95% confidence intervals (CI) for the effects of parity of dam, season of calving, calf number, gestation length, and calving year on reported abortion rate of Holstein cows from 1998 to 2006

Effect	Class	Estimate $\pm$ SE	95% CI	Odds ratio	95% CI	P-value
Intercept		12.0 $\pm$ 0.15	11.67 to 12.26	—	—	—
Season	Spring	—	—	1	—	<0.001
	Summer	0.0 $\pm$ 0.03	−0.04 to 0.09	0.84	0.75 to 0.93	—
	Autumn	−0.1 $\pm$ 0.03	−0.15 to −0.03	0.74	0.67 to 0.83	—
	Winter	−0.1 $\pm$ 0.04	−0.22 to −0.07	0.71	0.63 to 0.79	—
Year	1	—	—	1	—	<0.001
	2	−0.1 $\pm$ 0.03	−0.13 to −0.01	1.11	0.99 to 1.24	—
	3	0.2 $\pm$ 0.03	0.19 to 0.29	1.51	1.36 to 1.67	—
Parity of dam	1	—	—	1	—	<0.001
	2	0.1 $\pm$ 0.02	0.06 to 0.15	1.59	1.44 to 1.77	—
	3	0.1 $\pm$ 0.02	0.09 to 0.17	1.64	1.51 to 1.78	—
	≥ 4	0.1 $\pm$ 0.03	0.08 to 0.19	1.65	1.47 to 1.84	—
Calf number	1	0.1 $\pm$ 0.05	0.01 to 0.19	1.22	1.02 to 1.47	0.032
	2	—	—	1	—	—
Gestation length		−0.06 $\pm$ 0.00	−0.06 to −0.06	0.944	0.943 to 0.945	<0.001

Parity by Year. Stillbirth over time was defined by a significant parity by year interaction for singleton births ($P < 0.001$; Tables 3 and 4), but not for twin births (Table 6). Meyer et al. (2001) reported that the percentage of stillborn calves in primiparous cows increased from 9.5% in 1985 to 13.2% in 1996 and increased from 5.0 to 6.6% from 1985 to 1996 for multiparous cows.

Calving Year. The odds of stillbirth increased over years from 4.4% in calving year Y1 to 4.6% in calving year Y3 (OR = 1.02; $P < 0.05$; Table 3; Y3 vs. Y1). Hansen et al. (2004) reported that the overall frequency of stillbirth in Danish Holsteins increased from 0.071 to 0.090 during 1985 to 2002.

Birth Type (Twin vs. Single). The odds of calf stillbirth were greater after twin births, with 18.8% of the twin calving events reporting calves as DD or DA compared with 4.0% for singleton births (OR = 7.58; $P < 0.001$; Table 3; twins vs. singletons). Also, there was a significant interaction effect between calf number and parity ($P < 0.001$). Previous studies of Holstein herds reported stillbirth rates for singleton births between

3.2 and 5.4% and for calves born as twins between 12.9 and 15.7% (Mee, 1991; Day et al., 1995). Guerra-Martinez et al. (1990) reported that the frequency of stillborn calves was 3.4 and 7.9% for single- and twin-calved heifers and was 8.7 and 5.4% for single- and twin-calved cows, respectively.

Sex of Calf. The odds of singleton stillbirth differ significantly based on sex of the calf, with 6.1 vs. 3.0% for M and F calves, respectively ($P < 0.001$; OR = 2.10; Table 4; M vs. F). For same-sex twin pairs, the odds of calf stillbirth were greater ($P < 0.01$; OR = 1.51; MM vs. FF) for M (21.9%) than for F (16.0%) twin pairs, whereas calf stillbirth for MF twins was 19.9% (Tables 4 and 5). Similarly, previous studies reported greater stillbirth rates among M twins (Mee, 1991). This effect is probably due to greater body size of M calves than F ones at birth. Also, Heins et al. (2006) reported that M calves had significantly more stillbirths than heifer calves. Meyer et al. (2001) reported sex of the calf had different associations with stillbirth incidence in primiparous and multiparous cows.

Table 8. Estimated odds ratios, parameter estimates, and their 95% confidence intervals (CI) for the effects of calving year, season of calving, gestation length, and parity of dam on reported abortion rate for singleton births of Holstein cows from 1998 to 2006

Effect	Class	Estimate $\pm$ SE	95% CI	Odds ratio	95% CI	P-value
Intercept		11.8 $\pm$ 0.14	11.49 to 12.04	—	—	—
Season	Spring	—	—	1	—	<0.001
	Summer	0.0 $\pm$ 0.03	−0.03 to 0.10	0.85	0.76 to 0.94	—
	Fall	−0.1 $\pm$ 0.03	−0.17 to −0.04	0.74	0.66 to 0.82	—
	Winter	−0.1 $\pm$ 0.04	−0.21 to −0.06	0.71	0.63 to 0.80	—
Year	1	—	—	1	—	<0.001
	2	−0.1 $\pm$ 0.03	−0.13 to −0.01	1.11	1 to 1.24	—
	3	0.2 $\pm$ 0.03	0.20 to 0.30	1.53	1.38 to 1.70	—
Parity of dam	1	—	—	1	—	<0.001
	2	0.1 $\pm$ 0.02	0.06 to 0.15	1.63	1.46 to 1.81	—
	3	0.13 $\pm$ 0.02	0.10 to 0.17	1.67	1.54 to 1.81	—
	≥ 4	0.1 $\pm$ 0.03	0.09 to 0.19	1.68	1.50 to 1.88	—
Gestation length		−0.06 $\pm$ 0.00	−0.06 to −0.06	0.945	0.944 to 0.946	<0.001

Table 9. Estimated odds ratios, parameter estimates, and their 95% confidence intervals (CI) for the effects of calving season and gestation length on reported abortion rate for twin births of Holstein cows from 1998 to 2006

Effect	Class	Estimate $\pm$ SE	95% CI	Odds ratio	95% CI	P-value
Intercept		64.9 $\pm$ 11.22	42.91 to 86.90	—	—	—
Season	Spring	—	—	1	—	0.039
	Summer	0.0 $\pm$ 0.13	−0.25 to 0.27	0.72	0.48 to 1.09	—
	Fall	0.1 $\pm$ 0.14	−0.14 to 0.40	0.82	0.54 to 1.25	—
	Winter	−0.5 $\pm$ 0.19	−0.85 to −0.10	0.45	0.25 to 0.78	—
Gestation length		−0.3 $\pm$ 0.02	−0.31 to −0.22	0.76	0.73 to 0.80	<0.001

Reported Abortion Rates for Holstein Dairy Cows

Herd. The overall abortion rate was 13.4%. In the present study, the abortion rate among herds ranged from 9.5 to 18.6% ($P < 0.001$).

Parity. Greater odds of abortion existed for cows in their fourth and greater lactations than other parities ($P < 0.001$; OR = 1.65; Table 7 and Figure 1; parity ≥ 4 vs. parity 1). Abortion rate for multiparous cows was 15.9% for single-bearing cows and 4.0% for twin-bearing cows (OR = 4.42; singleton vs. twin), whereas for primiparous cows, abortion rate was 9.4% for single-bearing cows and 5.4% for twin-bearing cows (OR = 1.82; singleton vs. twin).

Calving Year and Season There was increasing trend for the odds of abortion from calving year Y1 to calving year Y3 ($P < 0.001$; OR = 1.51; Tables 7 to 9; Y3 vs. Y1). Also, there was parity by year interaction for abortion rate of singleton births (Table 8; $P < 0.01$). Greater odds of abortion occurred during spring (April through June) compared with other seasons ($P < 0.001$; OR = 1.41; Tables 7 to 9; spring vs. winter).

Birth Type (Single vs. Twin). The odds of abortion were greater for singleton births, with 4.2% of the twin births compared with 13.8% for singleton ones ($P < 0.01$; OR = 1.22; Tables 7 to 9; singleton vs. twin). Previous studies reported the greater abortion rates for twin calves than singleton calves (Guerra-Martinez et al., 1990; Day et al., 1995; Echterkamp and Gregory, 1999), but Mee (1991) reported the abortion frequencies being 1.81 and 1.20% for single and twin births, respectively. Gestation length had significant associations with abortion incidence for singleton and twin births ($P < 0.001$; Tables 7 to 9).

Reported Calf Sex Ratio

The percentage of M births in singletons was 52.4% and varied among herds (from 50.6 to 53.9%). The percentage of M calves in twin births was 52.7%, which was not significantly different from that of single births. Our results were consistent with the results of Echterkamp et al. (2007) and Silva del Río et al.

(2007), who reported that the calf sex ratio favored M calves for singleton and twin births. In the present study, same-sex twin pairs (28.2% MM and 22.9% FF) occurred more frequently than MF pairs (48.9% MF). Although this finding could be interpreted as a greater occurrence of monozygotic twins, a previous study reported that only 5% of all twins in cattle are monozygotic (Silva del Río et al., 2006). Several factors have been hypothesized to influence sex ratio, including vaginal and uterine pH and maturity of the oocyte and time of AI (Pursley et al., 1998).

CONCLUSIONS

The overall average twinning rate was 3.9%. Parity of the dam and season of calving were identified as effective factors and had significant effects on the incidence of twinning. Lactating dairy cows are at greater risk for twinning because of their greater occurrence of multiple ovulations compared with nonlactating heifers. Even though milk production, as a causative factor associated with twinning, increased in recent

Figure 1. Reported abortion rates of Holstein dairy cows from 1998 to 2006 by parity. Black bars = primiparous cows; open bars = cows in their second lactation; gray bars = cows in their third lactation; diagonal bars = cows in their fourth and greater lactations. A greater abortion rate existed for cows in their fourth and greater lactations than other parities ($P < 0.001$; odds ratio = 1.65; parity ≥ 4 vs. parity 1).

years, twinning rate decreased over the years. There was an increase in singleton calf stillbirth as herd size increased. Calf stillbirth was greater for M than for F calves and for twin than for single births. Greater abortion rate was observed for calves born as singles, and there is an increasing trend for abortion over the years. The secondary calf sex ratio favors M calves for both singleton and twin births.

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