

Risk Factors for Twinning in Dairy Cows

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ABSTRACT

Records for 52,362 lactations over a 10-yr period from 260 dairy farms in North America that used a common commercial software for record keeping were evaluated for potential risk factors for twinning. Records were evaluated for the associations of reproductive disease, parity, production, drug therapy, and the occurrence of subsequent twins. The rate of twinning on these farms steadily increased over the observation period from 1.4% in 1983 to 2.4% in 1993. The rate of twinning also increased as parity of the cow increased, from 1.0% for cows in their first lactation to >4.1% for cows in their fifth or higher lactation. No association between twinning and season of year was detected. A multivariate logistical regression analysis found that the rate of twinning increased with increases in milk production, incidence of cystic ovarian disease, and the use of common pharmaceuticals, including GnRH, PGF_{2α}, and antibiotics. Results of the regression model also indicated that the single most important reason for the recent increase in the rate of twinning was a concurrent increase in milk production.

(**Key words:** twinning, dairy, epidemiology)

INTRODUCTION

Growing concern has focused on the increased incidence of twin births in dairy cattle. Twin births are undesirable because the majority of mixed sexed twins are freemartins and because twinning has been associated with the increased incidence of dystocia (2), retained placenta (9), metritis (3, 9), and abortion (6, 9); the increased need for antibiotic and hormone therapy (9); and the increased occurrence of calf mortality (9). Although twin births result in more calves per dam, the number of available replace-

ments does not increase. Dams of twins produce less milk and are more likely to be culled than are dams of single calves (9). In economic analyses of the expenses of twinning, each set of twins has been estimated to cost the farmer between \$100 and 250 (1, 3, 4).

Previous studies (2, 9) have suggested potential risk factors for twinning that include hereditary factors in breed lines, parity, season of conception, previous twinning, cystic ovarian disease, use of somatotropin, use of GnRH, use of PGF_{2α}, intrauterine therapy, antibiotic therapy, and high milk production. The objective of this study was to determine the risk factors for twinning and their relative importance through multivariate regression analysis. These results could then be used to conduct economic studies regarding the appropriate use of disease control and hormone therapy.

MATERIALS AND METHODS

Records for 260 farms of unknown breeds served as the initial data file. All farms used the same software for record keeping (DairyCHAMP® 2.0; University of Minnesota, St. Paul). The Database Applications report in DairyCHAMP® was used to export data that detailed four different reproductive management practices prior to conception (use of PGF_{2α}, use of GnRH, uterine infusion, and use of systemic antibiotics), two disease complexes prior to conception (uterine infection and cystic ovarian disease), previous twinning, parity, season of conception, peak milk production, and the subsequent number of calves born. The final data file consisted of data extracted from 52,362 lactations that ended in a birth. A computer program was then written in Pascal to convert the data into a format that could be loaded into the statistical analysis package. All disease or treatment events were coded as dichotomous variables; 1 designated the presence of at least one event prior to conception, and 0 indicated the absence of the event

Received June 25, 1997.

Accepted November 24, 1997.

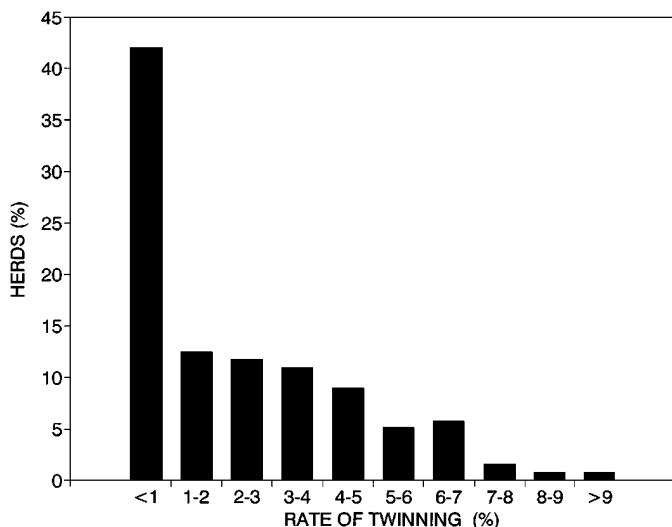


Figure 1. Distribution of the rate of twinning in 260 study herds.

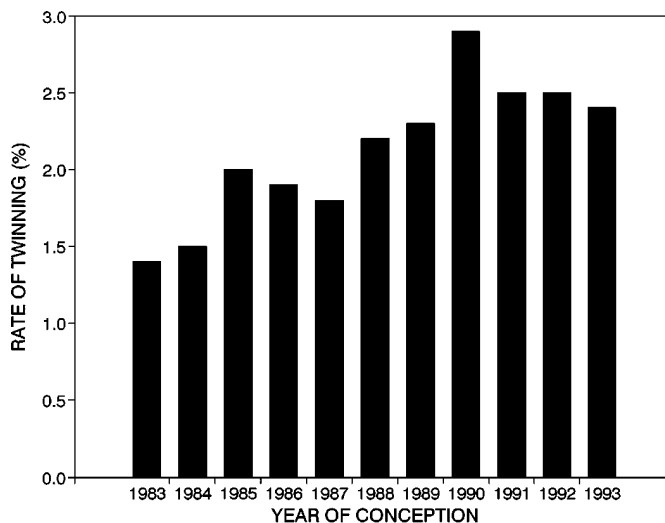


Figure 2. Distribution of the rate of twinning by year of conception.

prior to conception. Peak milk production, days open, and days to the diagnosis of pregnancy were coded as continuous variables for analysis. This data file was then imported into the statistical analysis software (Statistix 4.1; Analytical Software, Tallahassee, FL) for the construction of a multivariate logistic regression model of the relative contribution of each independent variable to the probability of twinning. The regression analysis followed the method of Hosmer and Lemeshow (5). Briefly, this method consists of five steps: preliminary screening of all variables for univariate associations, construction of a full model from all significant variables on the univariate analysis, stepwise removal of nonsignificant variables from the full model and comparison of the reduced model to the previous model for model fit and confounding, evaluation of the interaction among variables, and assessment of model fit using the Hosmer-Lemeshow statistic. Variables with univariate associations that had P values that were <0.25 were included in the initial model. Modeling continued until all main effects or interaction terms were significant by the Wald statistic at $P < 0.05$.

RESULTS

Of the 52,362 lactations evaluated, 1276 (2.44%) ended with a twin birth. Twinning rates at the herd level ranged from 0 to 9.6%; the mean rate was 2.24% (Figure 1).

Figure 2 shows twinning rate by year of conception. Risk of twinning increased from a low of 1.4% for cows conceiving prior to 1983 to 2.4%, the most recent percentage for cows conceiving in 1993. Incidence of

twin births by parity is shown in Figure 3. Twinning rate increased ($P < 0.01$) as parity increased from 1.0% for heifers delivering their first calves to $>4.1\%$ for cows in their fifth or higher lactation. Rate of twinning by quarter of conception is shown in Figure 4. No detectable association ($P = 0.17$) between twinning and season was observed.

The crude association between twinning and the categorical variables evaluated in the logistic regression analysis is presented in Table 1. Crude associations for the continuous variables used in the logistic regression analysis are presented in Table 2.

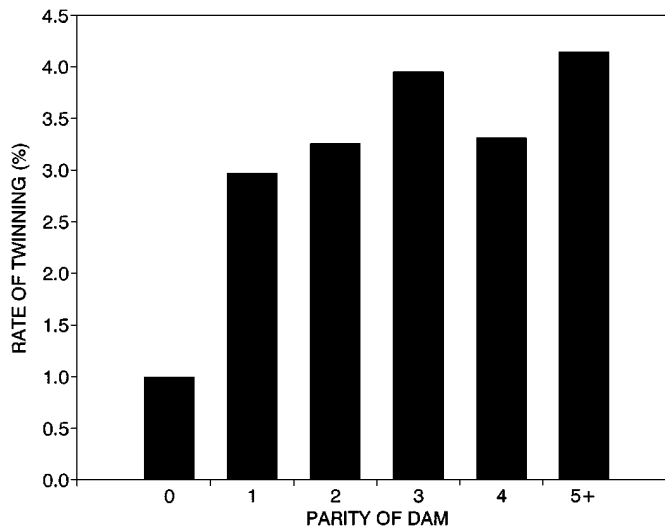


Figure 3. Distribution of the rate of twinning by parity of dam.

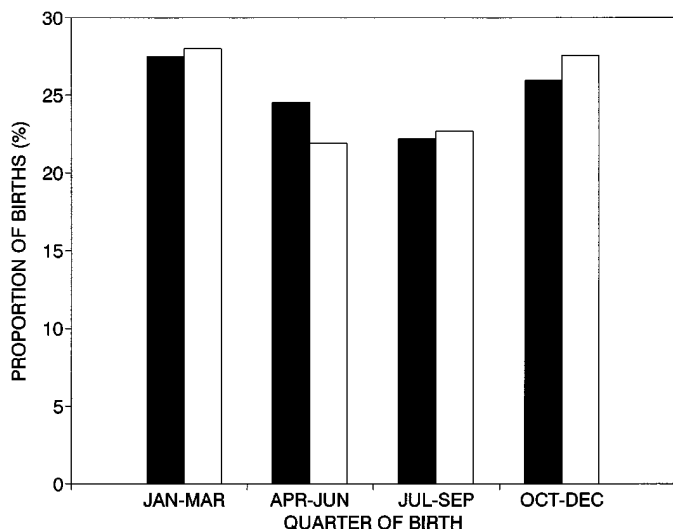


Figure 4. Distribution of single (■) and twin (□) births by season.

The final logistic regression model for the 12,046 lactations that had complete data (all categorical variables, days open, days to diagnosis of pregnancy, and production data) is presented in Table 3.

DISCUSSION

The incidence of twinning that was observed in this study (2.4%) was well within the normal range (1 to 5%) reported by Sreenan and Diskin (11) but was somewhat lower than the range (2.5 to 4.1%) reported in more recent studies (3, 4, 7, 9, 10). Figure 2 shows a somewhat steady increase in inci-

TABLE 1. Percentage of occurrence, crude odds ratios, and 95% confidence intervals for categorical variables.

Factor	Twins ¹	Single calves	Odds ratio	95% Confidence interval
	———— (%) ————			
Ovarian cysts	5.4	2.4	2.33	1.47–3.65
Metritis	4.9	2.4	2.10	1.40–3.12
Retained placenta	5.7	2.4	2.49	1.56–3.80
Treatment				
Antibiotics	5.2	2.5	2.26	1.79–2.85
GnRH	5.5	2.2	2.39	1.83–3.11
PGF _{2α}	4.4	2.3	1.97	1.68–2.35
Previous twinning	4.4	2.4	1.85	1.06–3.18
Maturity ²	3.8	2.1	1.85	1.63–2.09

¹Calves: 1276 twins; 51,086 single calves.

²Parity >2.

TABLE 2. Two sample *t* tests for continuous variables.¹

Factor	Twins	Single calves	<i>P</i>
Interval from calving to conception, d	113.4	119.4	0.0045
Interval from conception to pregnancy, d	56.2	64.3	0.0001
Peak milk production, kg	33.9	31.2	0.0001

¹Calves: 1276 twins; 51,086 single calves.

²On a mature equivalent basis.

dence from a low of 1.4% for cows that conceived prior to 1983 to 2.4% for cows that conceived in 1993. This difference in rate supports findings in recent studies (4) that the incidence of twinning in dairy cows has increased over time.

The range of the incidence of twinning on a herd basis was 0 to 9.6% (Figure 1), which is consistent with the report of Nielsen et al. (9), who reported a range of 0 to 7.1%. This range suggests that the incidence of twinning is not a biologically fixed rate and that differences in herd management may allow some control of the incidence of twin births. The incidence of twinning increased as parity increased (Figure 3), which was consistent with results of several previous studies (3, 9, 10) that described a consistent, near linear increase from <1% incidence

TABLE 3. Final multivariate logistical regression model.

Variable	Coefficient	Odds ratio	95% Confidence interval	<i>P</i>
Constant	-3.545	<0.0001
Herd	0.001	0.0281
Treatment				
Antibiotics	0.261	1.30	0.99–1.69	0.0547
GnRH	0.448	1.56	1.09–2.24	0.0144
PGF _{2α}	0.175	1.19	0.94–1.52	0.1528
Untreated cysts	0.921	2.51	1.28–4.93	0.0074
Cysts plus GnRH treatment	-1.018	0.36	0.14–0.95	0.0392
Cysts plus PGF _{2α} treatment	-1.383	0.25	0.06–1.11	0.0691
Maturity ¹	-0.109	0.90	0.74–1.09	0.2822
Days open ²	-0.019	0.98	0.97–1.00	0.0106
Days until diagnosis ³	-0.021	0.98	0.96–1.00	0.0489
Peak milk production ⁴	0.077	1.08	1.04–1.12	<0.0001

¹Parity >2.

²A 10-d increase in the interval from calving to conception.

³A 10-d increase in the interval from conception to diagnosis of pregnancy.

⁴A 4.5-kg increase in mature equivalent peak milk production.

for first parity heifers to >5% for cows in their highest parity. Results of this study did not indicate a significant difference in the incidence of twinning by season of conception (Figure 4), which is consistent with the findings of Eddy et al. (3) but in contrast to the report by Nielen et al. (9).

Findings of the multivariate logistic regression analysis suggested that several risk factors existed for twinning. Cows that received antibiotics prior to conception were 1.30 (95% confidence interval = 0.99 to 1.69) times more likely to deliver twins, which is consistent with findings reported by Nielen et al. (9) who found that cows that received antibiotics were 1.8 (95% confidence interval = 1.0 to 3.1) times more likely to deliver twins.

The evaluation of the reproductive hormones, GnRH and PGF_{2 α} , was more complex because a significant interaction of cystic ovarian disease, the use of these hormones, and the risk of twinning existed. Cows that received prostaglandin F_{2 α} prior to conception and that did not have cysts were 1.19 (95% confidence interval = 0.94 to 1.52) times more likely to deliver twins. Cows that received GnRH prior to conception and that did not have ovarian cysts were 1.56 (95% confidence interval = 1.09 to 2.24) times more likely to deliver twins. In contrast, cows that had ovarian cysts and received PGF_{2 α} or GnRH, respectively, were 0.36 (95% confidence interval = 0.14 to 0.95) and 0.25 (95% confidence interval = 0.06 to 1.11) times more likely to deliver twins. The odds ratio found in the present study for the use of PGF_{2 α} in the absence of cysts was very similar to the odds ratio of 1.1 (95% confidence interval = 0.8 to 1.6) for the use of PGF_{2 α} , FSH/LH, or GnRH and the odds ratio of 1.3 (95% confidence interval = 0.9 to 1.9) for PGF_{2 α} treatment alone as reported by Nielen et al. (9); however, the odds ratio for GnRH use was substantially higher. One explanation for the higher odds ratio associated with the use of GnRH was that Nielen et al. (9) did not investigate reproductive diseases as risk factors and did not control that potential interaction. In addition, GnRH treatment might be used for several reasons, and the two populations are likely to have different distributions of both timing and reasons for hormone use.

Evaluation of the association between previous reproductive diseases and the risk of twinning produced mixed results. Although a significant crude association was found between uterine infection and twinning, this association was not detectable following adjustment for other risk factors in the multivariate analysis. Suggestions from previous reports that uterine infusion is a risk factor for twinning might have been postulated because the use of antibiotics was associated with a 1.3-fold increase in the risk of twinning, which is similar to the 1.6-fold (95% confi-

dence interval = 1.0 to 2.5) increase in the risk of twinning that was described by Nielen et al. (9) for intrauterine therapy. Unfortunately, too few farms recorded uterine infusion events to allow us to evaluate this risk factor directly in this study. An examination of a possible interaction of uterine infection, PGF_{2 α} treatment for the infection, and subsequent twinning did not detect a significant association.

Untreated cystic ovarian disease had the highest degree of association with twin births; cows that had untreated cysts were 2.51 (95% confidence interval = 1.28 to 4.93) times more likely to deliver twins. The association of cystic ovarian disease and subsequent twinning has been described previously. In a case referent study by Bendixen et al. (2), cows with cysts prior to conception did not have a significant (odds ratio = 1.1; 95% confidence interval = 0.8 to 1.5) increased risk of delivering twins compared with Swedish Friesian herdmates (controls); however, the increase (odds ratio = 1.5; 95% confidence interval = 1.1 to 2.0) in the incidence of twinning was significant compared with the incidence in the Swedish Friesian population (controls). One explanation for the difference in odds ratios between herd and population controls is that cystic cows in the case herds might have been misdiagnosed, leading to a bias toward an odds ratio of 1.0. The apparent difference between the odds ratio in the present study and that in the study by Bendixen et al. (2) may be attributed to the apparent lack of adjustment for confounding treatment effects in the latter study because odds ratios prior to adjustment for treatment effects in this study were similar (odds ratio = 1.23; 95% confidence interval = 0.75 to 2.00).

Cows that delivered twins previously have been shown to be at a higher risk of delivering twins subsequently (2, 7, 9). Although a significant association existed in the crude analysis in this study, twinning in a previous lactation was not a significant risk factor after adjustment for confounders in the multivariate analysis. Because twinning is associated with increased disease (i.e., cystic ovaries) and treatment, which are problems associated with subsequent twinning, it is possible that previous findings of a positive association with the recurrence of twins are confounded by these problems.

The significant negative association between the interval from calving to conception (days open) and the risk of twinning that was found in this study was in contrast to the findings of Nielen et al. (9). In the Dutch study, cows that subsequently twinned had, on average, a longer (11.8 d) interval from calving to conception (104.1 vs. 92.3) compared with the shorter (6.0 d) interval (113.4 vs. 119.4) in the current study. The interval from calving to conception is a summary measure that is influenced by several

factors (i.e., voluntary waiting period, estrus detection rate, conception rate) such that it is an intermediate factor and should not be considered a control point in the management of the incidence of twins.

The potential association between the risk of twinning and the interval from conception to the diagnosis of pregnancy has not been investigated previously. A hypothesis as to how the interval from conception to diagnosis of pregnancy may be associated with the risk of twinning is that twin pregnancies may be more fragile and both pregnancies may be lost if cows are palpated earlier. Under this hypothesis, a positive association between twinning rate and the length of this association would be found because cows pregnant with twins and palpated earlier would be more likely to abort. This hypothesis was not supported in this study because cows carrying twins were diagnosed to be pregnant 8.1 d earlier than were cows carrying single calves ($P < 0.05$). Use of this finding as a control point for the management of the incidence of twinning is unlikely.

Results of this study suggest that the risk of twinning increases as milk production increases. Cows that delivered twins had, on average, 2.7 kg greater peak milk production ($P < 0.05$). The association between twinning and higher producing cows has been previously described. In the study by Nielen et al. (9), cows carrying twins averaged 103 kg more milk by 100 DIM (a period close to peak milk production) than did cows carrying single calves; however, no significant difference was noted in 270- or 305-d milk production.

The population attributable rate is an estimate of the rate of an outcome in the population at large attributable to the presence of a risk factor or risk factors (8). The population attributable rate is calculated by multiplying the odds ratio by the prevalence of the factor. This calculation can be extended to the calculation of the expected change in the risk of twinning over time by multiplying the odds ratio by the change in prevalence of the risk factor as shown in Table 4.

According to the model parameters estimated in this study and the estimated change in the prevalence of the risk factors between 1983 and 1993, the rate of twinning would be expected to increase 1.7%. In other words, based on the rate of twinning in 1983 (1.4%), the expected rate of twinning in 1993 would be 3.1% (1.4 + 1.7%) after adjustment for the increase in prevalence of risk factors that were found to be significant in this study. The expected rate based on the present model is within the confidence limits of the 1993 twinning rate calculated in this study and is quite similar to the range (2.5 to 4.1%) reported for studies published between 1989 and 1994 (3, 4, 7, 9, 10). The population attributable rates shown in Table

TABLE 4. Projected increase in the rate of twinning based on the change of prevalence of risk factors over the observation period.

Risk factor ¹	Change in prevalence	Odds ratio	Increase in twinning (%)
Treatment			
Antibiotics	+0.058	1.30	+0.08
GnRH	+0.052	1.56	+0.08
PGF _{2α}	+0.148	1.19	+0.18
Untreated cysts	+0.023	2.51	+0.06
Peak milk production	+1.200	1.08	+1.30
Expected increase in the rate of twinning			+1.70

¹Risk factors for which prevalence did not change between 1983 and 1993 (i.e., days open and treated cysts) were omitted because their contribution to the change in the rate of twinning was 0.

4 suggest that the single largest contributor (>50%) to the recent increase in the rate of twinning is the increase in peak milk production. Peak milk production is influenced by several factors, including energy density of the diet. One possible explanation of this association is the enhancement of double ovulation that is produced by high energy diets. To our knowledge, this finding has not been reported previously. Further research should reveal the mechanisms involved in this relationship.

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