

# Association of Twin Pregnancy Diagnosis and Parturition with Days Open, Days Pregnant at Diagnosis, Parity, and Milk Production in Dairy Cattle

John D. Day,<sup>1a</sup> Leon D. Weaver<sup>1</sup> and Charles E. Franti<sup>2</sup>

<sup>1</sup>Veterinary Medicine Teaching and Research Center,  
University of California, Davis, Tulare, CA

<sup>2</sup>Department of Epidemiology and Preventive Medicine,  
School of Veterinary Medicine, University of California, Davis, CA

## Abstract

Associations between twin pregnancies diagnosed by either rectal palpation or observation at calving and days open, days in gestation at pregnancy diagnosis, parity, and milk production in the lactation preceding the birth of twins were studied on 5493 cows on 14 California dairies. A total of 196 bicornual (3.57%) and 74 unicornual (1.35%) twin pregnancies were diagnosed, but the actual twinning rate at parturition was 6.86%. The incidence of twins at diagnosis and at calving was dependent on parity and independent of days open. Detection of twins at diagnosis by rectal palpation was dependent on days pregnant at diagnosis. Cows in parities >2 diagnosed with twin pregnancies and calving with twins had higher peak milk production than cows diagnosed with single pregnancies and calving with singles, respectively. However, when data were stratified by dairy, no significant difference occurred in peak milk production between cows diagnosed with singles or twins, and no significant difference occurred between cows calving with singles and twins. The 305 day mature equivalent milk production (ME) did not differ between cows diagnosed or calving with twins and singles. Optimal time for diagnosis of twins was at 51 to 60 day postbreeding.

## Introduction

Many producers and veterinarians have clinical impressions that twinning rates in Holstein cows are increasing in association with improved genetics and management. Abortion rates in cows diagnosed with twins are higher.<sup>5</sup> Furthermore, cows calving with twins are at greater risk of many postparturient diseases,<sup>7,11,13</sup> including stillbirth, retained placenta, metritis, displaced abomasum, ketosis, and consequently, days open<sup>3,13</sup> and number of inseminations per conception.<sup>13</sup>

Prior investigations examining risk factors associated with twinning have been retrospective analysis of data from cows calving with twins. Several researchers<sup>2,7,11,13</sup> have reported increased incidence of twinning with advancing parity; the difference was greatest between first and second lactation cows. Nielen *et al.*<sup>13</sup> found that cows that subsequently calved with twins

had 103 kg more cumulative milk production for the first 100 day, but the difference did not persist as the lactation progressed. However, Chapin and Van Vleck<sup>3</sup> found that ME increased in the lactation preceding the birth of twins (TB) in comparison with herdmates. Also, Kay<sup>10</sup> found that total milk production increased and that average production per day was higher in the lactation preceding the birth of twins in comparison with those from other lactations of the same cow. Results from these retrospective studies may be affected by censoring bias introduced through normal abortions and culling.

Prospective studies using early pregnancy diagnosis data may reduce censoring bias and accurately assess associations between twin gestations and cow characteristics. The objective of this prospective cohort study was to identify the presence of twin pregnancies (TP) early in gestation and examine several characteristics that might be associated with the diagnosis and calving of twins. Characteristics examined included parity, days open, days in gestation at pregnancy diagnosis, and milk production in the lactation preceding the birth of twins. Results should contribute to a better understanding of the risks that predispose animals to the conception of twins and aid in increasing the precision of diagnosis of TP.

## Materials and Methods

Four cooperating veterinarians, who routinely diagnose pregnancies as early as 30 day postbreeding and routinely diagnose twins, contributed information on 14 large, well-managed, Holstein dairy herds (>500 milking cows) in California's central valley. Good management was defined as use of regular (at least monthly) veterinary service for reproduction, diagnosis of pregnancy beginning at 30 to 35 days, the use of a DHIA or similar outside service to monitor milk production, rolling herd average >8182 kg, and the use of

<sup>a</sup>Current address and address for reprint: Dairy Health Services/MPS, 2235 S Lincoln #6, Jerome, ID 83338.

records to monitor cow production and reproduction.

Lactating cows (n=5493) were enrolled in the study at the initial diagnosis of pregnancy (30 to 70 day postbreeding) as determined by rectal palpation by the cooperating veterinarian. Pregnancies were categorized as a single pregnancy (SP) or TP. Twins were diagnosed by palpation of two amniotic vesicles and were further classed as bicornual (BP; one vesicle in each horn) or unicornual (UP; both vesicles in one horn). Calving outcome was categorized as single birth (SB) or TB. Enrollment of cows on the dairies began with genital tract examinations conducted in June through August 1989 and continued for 6 to 8 months. Cows were included in the study if diagnosed pregnant during the enrollment period by one of the participating veterinarians. Data for cows that were diagnosed pregnant >150 day postpartum were excluded from analyses except for determination of diagnosis rate by days open.

### Statistical Analysis

Chi-square tests of independence<sup>1</sup> were used to evaluate whether the type of pregnancy diagnosed and the incidence of TB were associated with days open, parity, or days in gestation at palpation in the lactation SP or TP were diagnosed. Means and their 95% confidence intervals (CI) were used to evaluate peak milk production (highest milk test of first four tests) and ME in the lactation diagnosed SP or TP by outcome of pregnancy diagnoses and calving.

An Analysis of Variance (ANOVA) for a randomized complete blocks design<sup>1</sup> was used to evaluate milk production further by pregnancy diagnosis and calving outcome. In the ANOVA for pregnancy diagnosis, SP or TP cows constituted the treatment and each dairy represented a block. The BP and UP cows were combined into the TP category for all randomized complete blocks evaluations because a preliminary analysis indicated no significant difference between these two groups for milk production. In the ANOVA for calving outcome, each dairy represented a block and the outcome constituted the treatment.

### Results

A total of 5493 cows entered into the study. Of those, 5223 (95.08%) were SP and 270 (4.92%) were TP. Of those twins, 196 (3.57%) were BP and 74 (1.35%) were UP. A large number of twin gestations were not detected at pregnancy diagnosis.<sup>5</sup> Of the 4401 cows that calved, 4099 (93.14%) had SB and 302 (6.86%) had TB (Table 1). Of the remaining 1092 cows, 676 aborted, and 416 cows that had not aborted were culled or lost to follow up.

**Table 1.** Incidence of twins based on diagnosis by rectal palpation and at calving.

Cows	(n)	(%)
Entered study	5493	
Singles diagnosed	5223	95.08%
All twin diagnosed	270	4.92%
Bicornual twin diagnosed	196	3.57%
Unicornual twin diagnosed	74	1.35%
Calving	4491	
Single calves	4099	93.14%
Twin calves	302	6.86%

Comparison of diagnoses conducted at <41 days, 41 to 50 days, 51 to 60 days, and 61 to 70 days of pregnancy showed that diagnosis was dependent on days in gestation at palpation ( $X^2=13.16$ ;  $P<.05$ ); the greatest number of twins was diagnosed in the interval from 51 to 60 days. However, the risk of calving with twins was independent of days in gestation at palpation ( $X^2=1.93$ ;  $P>.10$ ).

Diagnosis of SP, BP, and UP was independent of days open (Table 2) for days open intervals of <61 days, 61 to 90 days, 91 to 120 days, 121 to 150 days, and >150 day in milk ( $X^2=10.32$ ). Lack of correlation between days open and twinning was further substantiated in that the number of cows calving with singles and twins was also independent of days open ( $X^2=5.58$ ; Table 2).

**Table 2.** Chi-square tests of independence for diagnosis of singles and twins by rectal palpation and at calving with days open, days in gestation at palpation, and parity.

	Diagnosis <sup>1</sup>			Diagnosis <sup>2</sup>			Calving		
	X <sup>2</sup>	df	P	X <sup>2</sup>	df	P	X <sup>2</sup>	df	P
Days Open	10.32	8	>.10	6.51	4	>.10	5.58	3	>.10
Days @ Palp <sup>3</sup>	13.16	6	<.05	10.58	3	<.025	1.93	3	>.10
Parity	13.62	4	<.01	3.90	1	<.05	4.29	1	<.05

<sup>1</sup>Comparison of diagnosis between SP, BP, and UP

<sup>2</sup>Comparison of diagnosis between SP and TP

<sup>3</sup>Days pregnant at initial pregnancy diagnosis

The diagnosis rates of BP and UP twins were 3.3 and .9%, 3.3 and 1.1%, and 4.1 and 2.1% for 1, 2, and >2 parities, respectively. Twins constituted 6.2% of the second lactation calvings and 7.8% of the calvings among cows in third and greater lactations. Classification of pregnancy diagnosis as SP, BP, and UP or SP and TP was dependent of parity ( $X_2=13.62$ ,  $P<.01$ ; and  $X_2=3.899$ ,  $P<.05$ , respectively), as was the incidence of twins at calving ( $X_2=4.29$ ,  $P<.05$ ).

Overall peak milk production (Table 3) was increased in UP and TP cows during the lactation of that pregnancy ( $P<.01$ ). However, when peak milk production was examined by parity, only peak milk production for TP cows at the highest parity was higher ( $P<.05$ ). A tendency toward twins in higher producing cows ( $r=.25$ ;  $P=.056$ ), when peak milk production was compared among dairies in a two-way ANOVA. However, the correlation coefficient for peak milk production by dairy was .89 ( $P<.005$ ).

Peak milk production was also higher when data for prior milk production were examined for cows calving with twins (Table 3;  $P<.01$ ). However, when stratified by parity, TP cows calving for at least the third time had higher peak milk production in the lactation of that pregnancy than cows calving with singles ( $P<.01$ ). When cows were stratified by individual dairy, the correlation of peak milk with that dairy ( $r=.87$ ;  $P=.014$ ) was strong, but no correlation existed for peak milk production by calving outcome.

**Table 3.** Comparison of peak milk production of cows by parity to pregnancy diagnosis classification and calving result.

Peak milk						
Parity	Pregnancy Diagnosis				Calving	
	SP <sup>1</sup>		TP <sup>2</sup>		TB <sup>4</sup>	
	(kg)	(sd) <sup>5</sup>	(kg)	(sd)	(kg)	(sd)
ALL	39.5 <sup>a</sup>	8.4	41.3 <sup>b</sup>	8.9	39.5 <sup>a</sup>	8.4
1	32.3	4.8	32.3	5.7	32.1	5.3
2	42.7	6.0	43.9	7.1	43.6	6.3
>2	45.5 <sup>c</sup>	6.8	46.6 <sup>d</sup>	6.0	47.3 <sup>b</sup>	6.2

<sup>1</sup>Single pregnancy

<sup>2</sup>All twin pregnancies

<sup>3</sup>Single births

<sup>4</sup>Twin births

<sup>5</sup>Standard deviation

<sup>a,b</sup>Columns with different superscripts differ  $P<.001$

<sup>c,d</sup>Columns with different superscripts differ  $P<.010$

The ME did not differ between SP and TP cows (Table 4). When ME was examined by parity, first lactation TP cows had a reduced ME in comparison with SP cows ( $P<.05$ ). The ME did not differ between the older cows diagnosed with singles or twins. When ME was compared by individual dairy farms and pregnancy diagnosis, ME was not correlated with diagnosis, but was correlated with dairy ( $r=.98$ ;  $P<.005$ ). A non significant tendency existed for an increase in ME (Table 4) in the preceding lactation in cows in lactations >2 that were calving with twins compared with cows calving with singles ( $P<.10$ ). When ME was compared between dairy and calving outcome, no correlation of ME with calving outcome occurred, but ME correlation with dairy was strong ( $r=.95$ ;  $P<.005$ ).

**Table 4.** Comparison of 305 day milk production of cows by parity and pregnancy diagnosis classification and calving result.

Parity	305 day Mature Equivalent							
	Pregnancy Diagnosis				Calving			
	SP <sup>1</sup>		TP <sup>2</sup>		SB <sup>3</sup>		TB <sup>4</sup>	
	(kg)	(sd) <sup>5</sup>	(kg)	(sd)	(kg)	(sd)	(kg)	(sd)
ALL	10,475	1,702	10,384	1,798	10,490	1,676	10,511	1,811
1	10,325 <sup>a</sup>	1,586	9,998 <sup>b</sup>	1,635	10,307 <sup>c</sup>	1,567	10,086 <sup>d</sup>	1,668
2	10,805	1,771	10,689	2,223	10,858	1,755	10,823	1,989
>2	10,499	1,741	10,499	1,571	10,420 <sup>e</sup>	1,698	10,653 <sup>d</sup>	1,731

<sup>1</sup>Single pregnancies

<sup>2</sup>All twin pregnancies

<sup>3</sup>Single births

<sup>4</sup>Twin births

<sup>5</sup>Standard deviation

<sup>a,b</sup>Columns with different superscripts differ  $P<.05$

<sup>c,d</sup>Columns with different superscripts differ  $P<.10$

## Discussion

The 6.86% of TB was higher than the 4.54% reported in 1959,<sup>7</sup> 4.75% reported in 1978,<sup>2</sup> and 5.8% reported in 1989.<sup>13</sup> The increased prevalence of twins in this study may be due to enrollment of cows from herds with high milk production. The increased plane of nutrition necessary for higher producing cows may increase the number of multiple ovulations.<sup>2</sup> Amounts of bST and somatomedin in genetically superior cows may also have an effect on ovarian activity. Direct effects of growth hormone on ovarian tissue of the rat,<sup>4</sup> porcine,<sup>8</sup> and human<sup>12</sup> have been reported. Furthermore, Jia *et al.*<sup>9</sup> found that growth hormone enhanced the effect of FSH on differentiation of cultured rat granulosa cells. However, De La Sota *et al.*<sup>6</sup> reported that treatment with bST stimulated class 2 and 3 follicles in lactating dairy cows but did not increase the incidence of multiple ovulations.

Our observation of increased twinning rate as parity increased is in general agreement with Markusfeld<sup>11</sup> who reported an increasing relative risk for twinning as parity increased from first to second lactation, and with Cady and Van Vleck,<sup>2</sup> who reported rates similar to those reported herein.

Cady and Van Vleck<sup>2</sup> suggested that twinning may increase with parity because of a higher rate of double ovulations in older cows or an increased abortion rate of twin pregnancies by younger cow, perhaps because of metabolic demand or size constriction of the uterus. Unless abortions occurred earlier than 35 days, additional data from our study<sup>5</sup> does not support the latter theory because abortions were independent of parity and days in gestation at abortion. Furthermore, occurrence of abortions from increased metabolic demand or uterine size restriction does not play an active role at this

early stage of embryonic development.

Peak milk production increased in cows diagnosed with twins and in cows preceding the birth of twins, especially in cows in the third and greater parities. However, dairy plays an important role, as shown by examination of peak milk production by calving outcome and diagnosis by dairy. The full extent of the association of peak milk production and the diagnosis of twins may be masked by the variability between dairies and the decrease of power from small sample sizes when cows are classified by different classes of twins, parity, and dairy.

Difference in ME in the lactation preceding the birth of twins or singles was not found. The ME was decreased in TP cows in their first lactation. However, when ME was examined among the dairies, no correlation existed between milk production and twin diagnosis or calving, and the majority of the variation in ME could be explained by the dairy effect. Nielen *et al.*<sup>13</sup> also found that cows calving with twins had only an early increase in milk production in comparison to cows calving with singles. Others<sup>3,10</sup> have found an increased ME in cows preceding twin calving. Both studies<sup>3,10</sup> utilized only records from cows that had at least three lactations and compared within-cow production in different lactations, not with cohorts in the same lactation and year. The data presented herein and the data of Nielen *et al.*<sup>13</sup> suggest that higher producing cows early in lactation are at a greater risk of TP. However, this production advantage is lost during the lactation, possibly as a result of differing energy status in gestation or because of the lack of sensitivity of 270- and 305-day milk production as an evaluative measure.

Although anecdotal reports indicate that cows that conceive later have a greater number of twins, in our study, the number of embryos detected at pregnancy diagnosis and the number of twin calves born were independent of days open. Research or management programs requiring establishment of twin pregnancy diagnosis by rectal palpation should consider that palpation was best able to distinguish twin pregnancies between 51 and 60 days postbreeding.

**Incidence of twins in this study is higher than previously reported and was associated with increased parity and possibly with increased milk production. The TP were most likely to be diagnosed when cows were examined at 51 to 60 days pregnant. Days open were not associated with twinning rate.**

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