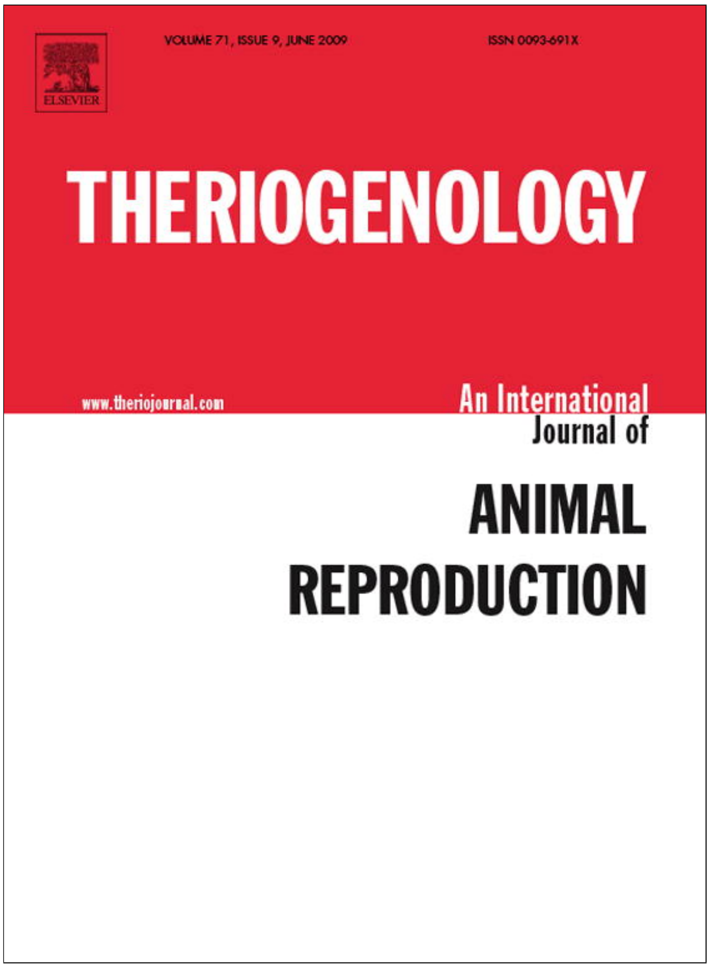
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Factors affecting pregnancy loss for single and twin pregnancies in a high-producing dairy herd

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Abstract

Our objective was to determine the magnitude of, and factors affecting, pregnancy loss for lactating Holstein cows on a commercial dairy farm when diagnosed with twin ($n = 98$) or single ($n = 518$) pregnancies using transrectal ultrasonography. Pregnancy losses were assessed with records of non-viable embryos at first pregnancy examination and embryo losses between the first (25–40 d after AI) and second (48 and 82 d after AI) post-breeding pregnancy examinations. Among cows diagnosed with single pregnancies, 3.7% were diagnosed with a non-viable embryo at first pregnancy examination, and 4.6% of those diagnosed with a viable embryo underwent pregnancy loss by the second examination. A total of 11.2% of cows diagnosed with twins experienced a single embryo reduction, whereas 13.3% lost both embryos. Overall, the total proportion of cows experiencing pregnancy loss or experiencing embryo reduction was greater for cows diagnosed with twin than single pregnancies (odds ratio; OR = 3.6), resulting in an embryo survival rate of 91.9% for cows diagnosed with single compared to 75.5% for cows diagnosed with twin pregnancies. Season of breeding and milk production were associated with pregnancy loss for single pregnancies, whereas CL number was associated negatively with embryo reduction and pregnancy loss for twin pregnancies. The risk of twinning and double ovulation among pregnant cows increased with days in milk (DIM), and the risk of double ovulation was greater for cows diagnosed with ovarian cysts and lacking a CL at initiation of an Ovsynch protocol. We concluded that in this herd, embryo reduction and pregnancy loss during early gestation was greater for lactating Holstein cows diagnosed with twin compared to single pregnancies. In addition, cows diagnosed with ovarian cysts and lacking a CL had an increased risk for double ovulation.

Keywords: Dairy cattle; Pregnancy loss; Double ovulation; Twinning

1. Introduction

In dairy cattle production systems, the incidence of twin calvings ranges from 2.2 to 6.9%, and has generally increased over time [1,2]. Many factors are associated with the increased risk of dizygous (DZ) twinning, including milk production, parity, genetics, breed, and season [3]. However, Kinsel et al. [1] singled out the concurrent increase in milk production over time as the most important risk factor for twinning. Therefore, as feeding management practices and genetic selection continue to increase milk production, the trend towards increased twinning rate in the dairy cattle population is likely to continue [3]. Twinning has detrimental effects on calves born as twins, as well as cows calving twins, incurring losses estimated to be $125 per twin calving event [4,5]. If embryonic and

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fetal losses were included in the economic assessment, the negative economic impact of twinning would be even greater [5–7].

For single pregnancies, most pregnancy loss occurs during early stages of development, with approximately 30% occurring by Day 16 of gestation [8]. Because of the technical difficulties associated with assessment of early embryonic loss, the rate at which cows with double ovulations produce a twin pregnancy is not known. Defining the conceptus as an embryo from conception to Day 45 of gestation, and as a fetus from Day 45 of gestation to birth [9], the incidence of late embryonic and early fetal losses averaged 12.8% in a summary of published studies [8]. However, studies comparing pregnancy loss for twin versus single pregnancies, late embryonic and early fetal losses were greater for cows diagnosed with twins (OR = 2.0–3.7; [6,10–13]). Interestingly, pregnancy loss assessed between 36 and 90 d after insemination occurred on average 52 d after insemination for single pregnancies and 75 d after insemination for twin pregnancies [11]. Thus, risk factors for pregnancy loss might differ with pregnancy type. Additionally, other factors such as dietary components, metabolic status, heat stress, disease status, and abnormal ovarian patterns have been associated with pregnancy loss [8,11]. More information is needed regarding the incidence of pregnancy loss for high-producing dairy cows carrying twins, as well as on the maternal and environmental factors affecting embryonic and fetal survival.

The primary objective of this study was to characterize pregnancy loss and its potential risk factors for lactating Holstein cows on a commercial dairy farm diagnosed (transrectal ultrasongraphy) with single versus twin fetuses. A secondary objective was to investigate, among pregnant cows, factors associated with double ovulation and twinning.

2. Materials and methods

2.1. Animals and reproductive management

This study was conducted on a commercial dairy farm located in north-central Wisconsin, USA milking 1100 lactating Holstein cows housed in free-stall barns. Cows were milked thrice daily, and the annual rolling herd average was 13,500 kg throughout the duration of the study. Herd personnel examined fresh cows weekly to identify cows with postpartum reproductive disorders such as retained placenta, metritis, and pyometra. Cows became eligible for insemination after exceeding the voluntary waiting period of 50 d in milk (DIM).

Detection of estrus was initiated at the end of the voluntary waiting period, and AI was conducted based on visual observation of estrous behavior (Estrus). Cows not detected in estrus by 90 DIM and cows diagnosed not pregnant at the weekly herd visit by the veterinarian were submitted for timed AI (Ovsynch) using i.m. injections of 100 μg of GnRH (Cystorelin; Merial, Ltd., Duluth, GA, USA) and 25 mg of $PGF_{2\alpha}$ (5 mL of Lutalyse; Pfizer Animal Health, New York, NY, USA) as follows: GnRH (Day 0), $PGF_{2\alpha}$ (Day 7), GnRH (54 h after $PGF_{2\alpha}$) [14]. Unless detected in estrus during the protocol, cows received a timed AI immediately after the second GnRH injection of the Ovsynch protocol. Cows were classified as cystic by the herd veterinarian at a nonpregnancy diagnosis, based on ultrasonographic morphology when a fluid-filled cystic ovarian structure(s) $\geq$25 mm in diameter was identified in the absence of a detectable corpus luteum (CL). No attempt was made to further classify cystic structures as follicular, luteal or benign, based on ultrasonography. Cows classified as cystic were submitted to an Ovsynch protocol (C-Ovsynch), as described previously.

2.2. Data collection

Data from reproductive examinations were collected by the herd veterinarian during weekly herd health visits for cows inseminated from December 2004 to December 2005 and submitted for pregnancy diagnosis from January 2005 to February 2006. All pregnancy examinations were performed by the same veterinarian throughout the study period. Transrectal ultrasonography was conducted using a portable scanner (Easi-scan, BCF Technology Ltd., Livingston, Scotland, UK) equipped with a 5 MHz linear-array transducer and a monocular video display headset.

During the first pregnancy examination, the ovaries and uterine horns were ultrasonically examined, and the number and location of CL and embryo(s) were recorded. Embryonic viability was confirmed based on a fetal heart beat when fetal size, fluid character, and/or placental separation appeared to be abnormal. Based on the first pregnancy examination, cows were classified as (1) not pregnant, (2) pregnancy recheck, when pregnancy diagnosis was unconfirmed, (3) pregnant with one embryo, (4) pregnant with two embryos, or (5) loss of an embryo (absence of a fetal heart beat and/or signs of fetal degeneration). Cows identified as "not pregnant" were submitted to an Ovsynch protocol for synchronization of ovulation and timed AI. After the first pregnancy examination, cows classified as "pregnancy recheck", "pregnant with one embryo", or ''pregnant with two embryos'' were submitted for a second examination using transrectal ultrasonography. During the second pregnancy examination, data on fetal viability, and fetal number were recorded.

2.3. Data set description

The initial data set included 2048 observations, 1389 from a first pregnancy examination and 659 from a second pregnancy examination. A total of 730 observations did not include a second pregnancy examination; 673 of those cows were diagnosed not pregnant, 22 cows were diagnosed as undergoing loss of an embryo, 33 cows were diagnosed pregnant with a single fetus, and 2 cows were diagnosed pregnant with twin fetuses. Cows with complete information at the first and second pregnancy examinations included 511 cows diagnosed pregnant with singles, 102 cows diagnosed pregnant with twins, and 46 cows diagnosed as ''pregnancy recheck'' at the first pregnancy examination. Thirty-nine cows classified as ''pregnancy recheck'' at the first pregnancy examination were confirmed pregnant at the second pregnancy examination.

For cows to be included in the final data set, their first pregnancy examination had to be conducted between 25 and 40 d after AI (mean ± S.D.; 33.7 ± 10.5 d after AI), followed by a second pregnancy examination between 48 and 82 d after AI (mean ± S.D.; 66.5 ± 13.0 d after AI). The final data set included records from cows diagnosed as undergoing loss of an embryo ($n = 19$) and from cows diagnosed with one ($n = 499$) or two ($n = 98$) viable embryos at the first pregnancy examination that included a second pregnancy examination. The data set included information on CL number (1 CL, 2 CL), number of embryos/fetuses and its viability (early embryonic loss; pregnancy loss; pregnant with singles; pregnant with twins), date of pregnancy examination, date of AI, DIM at AI, breeding method (Estrus, Ovsynch, or C-Ovsynch), and milk production from the Dairy Herd Improvement Association (DHIA) test nearest to the insemination date.

2.4. Statistical analyses

To analyze factors contributing to the probability of pregnancy loss for singles (early embryo loss and pregnancy loss), embryo reduction for twins (single embryo reduction and pregnancy loss), twinning and double ovulation, a multivariable logistic regression model was developed using the maximum likelihood method of the LOGISTIC procedure of SAS [15]. To construct the final model, a preliminary scrutiny of the data was conducted for the following factors of interest: season (January–March, S1; April–June, S2; July–September, S3; October–December, S4), breeding method (Estrus, Ovsynch, and C-Ovsynch), DIM, and milk production. For each variable, a univariable model was fitted, and those variables with P-values <0.25 were included in the final model. All selected variables and their interactions were included in the final model using the backward selection option set at $\alpha = 0.25$. Orthogonal contrasts were performed for the variables season and breeding method. Proportional differences in pregnancy loss, with pregnancy type and location of CL, were determined based on Chi-square tests using the FREQ procedure of SAS. Statistical significance was declared at $P \leq 0.05$ and a tendency towards significance at $0.05 < P \leq 0.10$. Data are presented as odds ratios (ORs), based on the analysis using the LOGISTIC procedure of SAS.

3. Results

3.1. Pregnancy loss and embryo reduction for cows diagnosed with twins versus singles

Descriptive statistics for milk production near the time of AI and DIM at AI for pregnancy loss and embryo reduction, pregnancy type, CL number and breeding method are shown (Table 1). Embryo viability, pregnancy loss, and twin pregnancies experiencing a single embryo reduction were classified by the number of corpora lutea (CL) detected at first pregnancy examination (Table 2). At first pregnancy examination, 19 cows were diagnosed as having a single non-viable embryo ($n = 4$ cows with 2 CL; $n = 15$ cows with 1 CL). Overall, 23 of the cows diagnosed with viable single embryos at the first pregnancy examination lost their pregnancy by the second examination ($n = 13$ CL in the right ovary, $n = 9$ CL in the left ovary, and $n = 1$ bilateral CL; Table 2). Information on CL and embryo location was missing for 27 and 54 single pregnancies, respectively. Cows with one CL and pregnant with singles had a CL located more frequently on the right (61.6%; 245/398) compared to the left ovary (38.4%; 153/398; $P < 0.01$; Table 3). After a double ovulation, a similar proportion of single and twin pregnancies were observed, independent of CL location (Table 3).

At the first pregnancy examination, 85 cows were identified with twins ($n = 8$ cows with 1 CL; $n = 77$ cows with 2 CL). In addition, 13 cows ($n = 7$, cows with 1 CL; $n = 6$, cows with 2 CL) were diagnosed with single pregnancies at the first pregnancy examination, but were confirmed pregnant with twins at the second

Table 1
Descriptive statistics for daily milk production near the time of AI and days in milk (DIM) at AI for lactating Holstein cows diagnosed with single versus twin pregnancies.

	No. of cows	Milk (kg/d) near AI		DIM at AI	
		Mean ± S.D.	Range	Mean ± S.D.	Range
Single pregnancies					
Pregnancy maintained	476	49.7 ± 10.5	12–85	122 ± 51	49–321
Pregnancy loss[a]	42	54.4 ± 15.5	16–73	122 ± 56	59–284
Twin pregnancies					
Pregnancy maintained	74	47.6 ± 12.0	16–85	142 ± 58	69–336
Pregnancy loss/Embryo reduction[b]	24	50.3 ± 8.2	39–74	183 ± 78	78–383
CL number					
1 CL	459	49.5 ± 10.9	12–85	123 ± 53	49–336
2 CL	156	50.5 ± 10.9	16–85	140 ± 60	66–383
Pregnancy type					
Single	518	50.0 ± 10.9	12–85	122 ± 51	49–321
Twin	98	48.3 ± 10.8	16–81	152 ± 66	69–383
Breeding method[c]					
Ovsynch	250	49.5 ± 11.4	24–85	140 ± 54	61–383
Estrus	293	50.4 ± 10.0	12–85	108 ± 48	49–336
C-Ovsynch	73	48.1 ± 11.9	16–76	160 ± 56	86–321

[a] Cows diagnosed with a non-viable embryo and cows diagnosed with a viable embryo at the first pregnancy examination (25–40 d after AI) experiencing pregnancy loss by the second pregnancy examination (48–82 d after AI).
[b] Cows diagnosed with two viable embryos at first pregnancy examination and with one or no viable embryos by the second pregnancy examination.
[c] Cows were inseminated based on visual detection of estrous behavior and/or rubbed tail chalk (Estrus), after synchronization of ovulation and timed artificial insemination (Ovsynch), or submitted to an Ovsynch protocol after diagnosis as cystic (ovarian structure ≥25 mm) and lacking a CL (C-Ovsynch).

pregnancy examination. For these 13 cows, information on the location of the embryo within the uterus was collected for only one of the embryos; therefore, embryo location was classified as ‘‘unknown’’ and the first pregnancy examination outcome, was reclassified as ‘‘pregnant with two embryos’’. At the second pregnancy examination, 11.2% of the cows diagnosed pregnant with twins were classified as undergoing a single embryo reduction (1 CL on the right ovary (R; $n = 3$), 1 CL on the left ovary (L; $n = 2$), bilateral CL (RL; $n = 4$), 2 CL on the left ovary (LL; $n = 2$), whereas both embryos were lost in 13.3% of the cows diagnosed with twin pregnancies (2 CL on the right ovary (RR; $n = 1$), 7LL, 4RL and 1R; Table 2). Cows diagnosed with twin pregnancies and with complete information on CL and embryo location had a similar frequency of unilateral and bilateral twin pregnancies, with 30.1% ($n = 25$) located in the right uterine horn, 22.9% ($n = 19$) located in the left uterine horn, and 47.0% ($n = 39$) bilateral pregnancies (Table 3). Overall, the total proportion of cows experiencing embryo loss and pregnancy loss was less for cows diagnosed with single compared to twin pregnancies (OR = 3.6), resulting in an embryo survival rate of 91.9% for single compared to 75.5% for twin pregnancies ($P < 0.01$; Table 2).

3.2. Risk factors for embryo reduction and pregnancy loss

Milk production near the time of AI and season of AI were associated with pregnancy loss for cows with single pregnancies; however, number of CL, DIM at AI, and breeding method did not affect pregnancy loss (Table 4). The association between milk production and pregnancy loss was defined as a quadratic effect, with greater losses among those cows with the greatest and the least milk production. Cows inseminated from January to March experienced more pregnancy loss than those inseminated from October to December (OR = 11.4; $P < 0.05$; Table 4). Nevertheless, no differences in pregnancy loss were observed for cows inseminated during cold (January–March and October–December) or warm (April–June and July–September) seasons. There were no statistical differences in pregnancy loss for cows pregnant with singles and having 1 CL (8.3%; 37/444) versus 2 CL (6.8%; 5/74).

Table 2
Embryo viability, pregnancy loss (PL), and single embryo reduction (ER) classified based on the number of corpora lutea (CL) identified at pregnancy examinations using transrectal ultrasonography in lactating Holstein cows.

Item	Pregnancy type	
	Single	Twin
Cows with embryos (viable[a] + non-viable[b]) at FPE[c], *n*	518	98
Cows with non-viable embryos at FPE, % (*n*)	3.7 (19)	–
1 CL	15	–
2 CL	4	–
Cows with viable embryos at FPE, *n*	499	98
Cows with viable embryos at FPE experiencing PL by SPE[d], % (*n*)	4.6 (23)	13.3 (13)
1 CL	22	1
2 CL	1	12
Cows with twins at FPE undergoing single ER by SPE, % (*n*)	–	11.2 (11)
1 CL	–	5
2 CL	–	6
Cows maintaining preganancy by the SPE, % (*n*)	91.9 (476)	75.5 (74)
1 CL	407	9
2 CL	69	65

[a] Viable embryos were classified based on visualization of an organized embryo and presence of a heart beat using transrectal ultrasonography.
[b] Non-viable embryos were classified based on visualization of a degenerate embryo and/or lack of an embryonic heart beat using transrectal ultrasonography.
[c] First pregnancy examination (FPE) was conducted between 25 and 40 d after AI using transrectal ultrasonography.
[d] Second pregnancy examination (SPE) was conducted between 48 and 82 d after AI using transrectal ultrasonography.

Milk production near the time of AI, DIM at AI, and season of AI did not affect embryo losses for cows diagnosed with twins. However, there were differences in embryo losses for twin pregnant cows based on whether they had 1 CL (40%; 6/15) or 2 CL (21.6%; 18/83; Table 2). There was a tendency for an interaction ($P = 0.092$) between CL number and DIM for twin pregnancies undergoing embryo reduction and pregnancy loss (Table 4). Cows maintaining twin pregnancies tended to conceive earlier in lactation when having 2 CL than when having 1 CL.

3.3. Double ovulation and twining for cows identified pregnant at first pregnancy examination

Overall, the risk of double ovulation increased with DIM at AI (OR = 1.004; Table 5). The odds of double ovulation were 3.3 times greater for cows classified with ovarian cysts and lacking a CL that were submitted to timed AI after Ovsynch (C-Ovsynch) than for cows inseminated at Estrus or after Ovsynch.

The likelihood of twinning increased with each additional DIM at AI (OR = 1.007; $P < 0.001$, Table 6) and decreased as milk production increased (OR = 0.974; $P = 0.043$; Table 6). After double ovulation, the odds of twinning were 33.3 times greater ($P < 0.001$) than after a single ovulation.

4. Discussion

In this study, we investigated the incidence and risk factors associated with pregnancy loss for high-producing cows on a large commercial dairy. In this study, the risk of embryo loss from the first pregnancy examination to the second pregnancy examination was greater for cows carrying a twin pregnancy (13.3% lost both embryos and 11.2% lost a single embryo), than for cows carrying a single pregnancy (8.1% pregnancy loss). However, overall pregnancy loss for cows carrying a twin pregnancy might have been greater, considering that twin pregnancy losses occurred on average 75 d after insemination, whereas in the present study the second pregnancy examination was performed on average 67 d after insemination [11]. Taking into account the diagnostic error of 13 twin pregnancies initially classified as singles, even more embryo losses might have occurred for twin pregnancies but were never detected. Moreover, the 11 cows undergoing embryo reduction by the second pregnancy examination were at a greater risk to experience pregnancy loss later in gestation, based on a previous study in which 37.1% of cows diagnosed with twins experiencing single embryo reduction ended in pregnancy loss by 90 d of gestation [16]. It is important to characterize early embryonic death and pregnancy loss from a management and economic standpoint, especially considering the high incidence of early twin pregnancies in our study herd. Prompt identification of embryo losses provided the opportunity to rapidly resubmit cows to a subsequent AI service. The cost associated with a pregnancy loss has been estimated at \$640 [17] and at \$600–\$800 [18]; therefore, it is important to identify potential risk factors for pregnancy loss, particularly for cows pregnant with twins.

For this commercial dairy farm, there was an association between milk production and the risk of pregnancy loss for cows carrying a single but not a twin pregnancy. Cows with single pregnancies producing the lowest and the highest level of milk near the time of AI

Table 3
Embryonic location within the uterus and location of corpora lutea (CL) for lactating Holstein cows diagnosed with single versus twin pregnancies that had one or two CL at the first pregnancy diagnosis conducted between 25 and 40 d after AI.

Location of CL	Pregnancy type	*n*	Embryonic location within the uterine horns			
			Right	Left	Both	Unknown
Cows with 2 CL						
Unilateral right ovary	Single	18	17	0	0	1
	Twin	25	25	0	0	0
Unilateral left ovary	Single	11	0	7	0	4
	Twin	19	0	19	0	0
Unilateral total	Single	29	17	7	0	5
	Twin	44	25	19	0	0
Bilateral ovaries	Single	41	26	12	0	3
	Twin	39	0	0	33	6
Unknown	Single	4	0	0	0	4
	Twin	0	0	0	0	0
Overall	Single	74	43	19	0	12
	Twin	83	20	25	33	6
Cows with 1 CL						
Right ovary	Single	264[a]	241	4	–	19
	Twin	7	7	0	–	0
Left ovary	Single	157[a]	0	153	–	4
	Twin	8[b]	0	8	–	0
Unknown	Single	23	1	3	–	19
	Twin	0	0	0	–	0
Overall	Single	444	242	160	–	42
	Twin	15	7	8	–	0

[a,b]Cows diagnosed with one CL and pregnant with singles had their CL located more frequently on the right ovary ($P < 0.05$).

Table 4
Variables selected by the backward stepwise selection process of the logistical regression procedure affecting pregnancy loss (PL) for Holstein cows diagnosed with singletons, and reduction of one embryo or pregnancy loss (ER-PL) for cows diagnosed with twins.

Variable	*n*	%	Odds ratio	95% confidence interval	*P*-value
PL for singles[a]					
Season of AI[b]					
S1	22/167	13.2	11.44	1.489–87.960	0.003
S2	10/142	7.0	5.45	0.676–44.043	0.487
S3	9/137	6.6	5.06	0.619–41.342	0.619
S4	1/72	1.4	–	–	–
Milk	–	–	0.83	0.727–0.970	0.018
Milk × milk	–	–	1.002	1.001–1.003	0.006
ER-PL for twins[c]					
CL no.					
1	9/15	60.0	–	–	–
2	18/83	21.7	4.87	1.325–21.006	0.033
DIM at AI	–	–	1.005	0.996–1.013	0.232
CL no. × DIM[d]	–	–	0.993	0.985–1.001	0.092

[a] Likelihood ratio test; 22.45, 5 d.f., $P < 0.001$. Hosmer and Lemeshow goodness-of-fit test; 2.145, 8 d.f., $P = 0.976$.
[b] S1 = January–March; S2 = April–June; S3 = July–September; S4 = October–December.
[c] Likelyhood ratio test; 11.04, 3 d.f., $P = 0.011$.
[d] Cows maintaining twin pregnancy tended to conceive earlier in lactation when diagnosed with 2 CL compared to 1 CL.

Table 5
Variables selected by the backward stepwise selection process of the logistic regression procedure affecting the incidence of double ovulation for lactating Holstein cows.

Variable[a]	*n*	%	Odds ratio	95% confidence interval	*P*-value
AI method[b]			–	–	<0.01
Ovsynch	58/250	23.2	0.298	0.169–0.523	0.003
Estrus	62/293	21.2	0.307	0.173–0.545	0.007
C-Ovsynch	36/73	49.3	–	–	–
DIM at AI	–	–	1.004	1.000–1.007	0.029

[a] Likelihood ratio test; 29.45, 3 d.f., $P < 0.001$. Hosmer and Lemeshow goodness-of-fit test; 8.872, 8 d.f., $P = 0.353$.
[b] Cows were inseminated based on visual detection of estrous behavior and/or rubbed tail chalk (Estrus), after synchronization of ovulation and timed artificial insemination (Ovsynch), or submitted to an Ovsynch protocol after diagnosis as cystic (ovarian structure ≥25 mm) and lacking a CL (C-Ovsynch).

Table 6
Variables selected by the backward stepwise selection process of the logistic regression procedure affecting the risk for twinning in lactating Holstein cows.

Variable[a]	*n*	%	Odds ratio	95 % confidence interval	*P*-value
Milk near AI	–	–	0.974	0.949–0.999	0.043
DIM at AI	–	–	1.007	1.003–1.012	0.003
CL					
1 CL	15/459		–	–	–
2 CL	83/157		33.32	17.92–61.96	<0.001

[a] Likelihood ratio test; 203.04, 3 d.f., $P < 0.001$. Hosmer and Lemeshow goodness-of-fit test; 2.9187, 8 d.f., $P = 0.939$.

experienced greater pregnancy loss than average producers within the herd. In this analysis, there were 18 cows producing <30 kg of milk and 82 cows producing >60 kg of milk. Because of the limited number of cows included in the lowest and highest milk production groups, these results should be interpreted with caution. In our study herd, lower milk production may have been associated with compromised health status and increased incidence of disease. In previous studies, cows suffering from clinical and subclinical mastitis [19–21], pyometra and retained placenta [22] experience greater pregnancy loss. By contrast, the highest producers in the herd were likely to be healthy animals, but physiologic and metabolic challenges associated with high milk production may have compromised maintenance of pregnancy. Increased milk production has been negatively associated with conception rate [23–25], but no effect of milk production was reported for pregnancy loss [10,26–28]. The discrepancy with previous studies on the association of high milk production and the risk of pregnancy loss could be explained by the greater level of milk yield observed for the highest producing cows in our study herd, or by the use of cumulative milk production rather than actual milk production during early pregnancy.

In agreement with previous studies, we found no differences in late embryonic pregnancy loss between cows inseminated at detected estrus or at fixed-time after Ovsynch or Heatsynch protocols [20,26,29]. There were no differences in pregnancy loss among seasons; however, for no obvious reasons, pregnancy losses from January to March were greater than from October to December. The effects of season on pregnancy loss are still unclear, whereas some authors [8,11,19,30] have reported important pregnancy loss associated with season or heat stress, others [26,31] failed to observe the same effects. These equivocal results may be explained by the age of the embryo when suffering the heat stress insult. García-Ispierto et al. [13] reported a relationship between heat stress during the peri-implantation period (between 21 and 30 d of gestation) and subsequent pregnancy loss, but not from 0 to 21 d and 31 to 40 d after insemination.

In the current study, there was no evidence that CL number affected the risk of pregnancy loss for cows carrying singles. By contrast, other authors reported that cows pregnant with singles had 8 times the odds of pregnancy loss when bearing 1 CL versus 2 CL [10,11]. Furthermore, Fricke and Wiltbank [32] reported greater conception rates at 28 d after AI for cows that double ovulated compared to those that single ovulated, implicating either an increase in fertility or a reduction in early pregnancy loss for cows bearing 2 CL. Results of pregnancy loss for cows carrying singles after a single ovulation were comparable to that reported previously [10,11]; however, pregnancy loss for cows pregnant with singles after a double ovulation were 6.8% in our study and 1.1% in the former cited studies. One explanation for this discrepancy across studies may be due to differences in progesterone concentration, but none of the previous cited studies reported progesterone data. Interestingly, in a recent study [33], cows with two or more CL during the early fetal period had three times the odds of having high progesterone concentrations than cows with a single CL; however, having an additional CL did not affect pregnancy loss. By contrast, Starbuck et al. [31] reported no effects of CL number on progesterone concentrations, but numerically more pregnancy losses were observed for cows with 2 CL. Nevertheless, the number of fetuses was not determined in this study [31] and more twin pregnancies may have confounded the results, favoring more pregnancy loss for cows with 2 CL.

Embryo losses for cows pregnant with twins were not associated with embryo location within the uterus. By contrast, previous studies reported greater pregnancy loss when two embryos implanted in the same uterine horn for primiparous cows [34], and for primiparous and multiparous cows [16]. Overall, twin embryo losses were greater for cows with 1 CL (monozygous twins; MZ) than for cows with 2 CL (dizygous twins; DZ). These results were expected based upon twinning data in women, which revealed a greater likelihood of pregnancy loss for MZ than DZ twins [36]. This high rate of mortality agrees with the low incidence of MZ twins observed at birth [37].

Cows pregnant with singles had more ovulations on the right than on the left ovary. This observation was consistent with the greater functional activity of the right ovary in cows [32,38,39] and other mammalian species, including rats and women [40,41]. Furthermore, an extensive study demonstrated that bilateral asymmetry of the reproductive system did not affect the pregnancy rate [42] or embryo survival [43] in cows. After a double ovulation, a similar proportion of single and twin pregnancies were observed, independent of whether CL were located unilaterally or bilaterally. After a double ovulation, a single pregnancy may occur when a single egg is fertilized, or when one of the twin embryos die, but the other embryo survives [44]. However, modeling survival rates of embryos transferred to induced twins, McMillan [35] found no evidence that the survival of one embryo was independent of that of its co-twin. Similarly, Lopez-Gatius and Hunter [16] reported that pregnancy losses were greater for unilateral than bilateral twin pregnancies and the survival of an embryo after its co-twin die was more likely to occur in bilateral than unilateral twin pregnancies [16].

In the present study, the odds of double ovulation were 3.3 times greater for cows identified with ovarian cysts and lacking a CL that were submitted to an Ovsynch protocol (C-Ovsynch) than for cows inseminated after visual observation of estrous behavior (Estrus) or non-cystic cows submitted to an Ovsynch protocol (Ovsynch). In agreement with this observation, an association between cystic ovarian condition and double ovulation has been reported [38,45]. Cows with co-dominant follicles had lower serum progesterone concentrations from 48 h before and after expected deviation than cows with a single dominant follicle [46]. Accordingly, postpartum double ovulation was greater for cows with anovulatory condition (46.3%) [47] or anovular with ovarian cysts (100%) [48]. Thus, rather than the cystic condition itself, a high proportion of cows in the C-Ovsynch group were likely anovular based on their lack of a CL and, therefore, experienced a greater double ovulation rate in response to the Ovsynch protocol than cycling cows. Although C-Ovsynch cows had numerically more twins (28.8%) than Osynch and Estrus cows (14.0%), the difference did not attain statistical significance. Based on a study population of nearly 9000 cows, Bendixen et al. [49] reported a greater incidence of twinning for cows diagnosed with cysts either during the first 40 d postpartum or after the estrus before AI. The limited number of twin pregnancies included in the present study likely prevented us from detecting significant differences.

In our study, double ovulation and twinning rate increased with DIM. However, as milk production near the time of AI increased, the likelihood of twinning decreased ($P = 0.043$), but ovulation rate was not affected by milk production. The present results may be explained to some extent by DIM and milk production for cows inseminated after being identified as having a cystic condition compare to those inseminated at Estrus or after Ovsynch (Table 1). López-Gatius et al. [38] did not detect any association between milk production and double ovulation. Conversely, prior epidemiological studies identified both seasonal changes and milk production as risk factors for twinning [1,2,7,32,47]. Nevertheless, considering the increase in double ovulation through genetic selection [50] and the high incidence of double ovulation among non-lactating dairy cows (28.3%) [51], factors such as milk production or season of breeding might not play a major role in double ovulation.

In summary, pregnancy losses after an initial pregnancy examination (using transrectal ultrasonography) were of sufficient magnitude to impact the overall reproductive efficiency on a dairy, particularly for cows diagnosed with twins. Conception of MZ twins may be greater than expected, but a greater incidence of pregnancy loss reduces their incidence at birth. Twinning and double ovulation were associated with an increase in DIM. Finally, cows diagnosed with ovarian cysts and lacking a CL have an increased risk for double ovulation which was likely associated with their cyclicity status rather than the cystic condition itself.

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References

[1] Kinsel ML, Marsh WE, Ruegg PL, Etherington WG. Risk factors for twinning in dairy cows. J Dairy Sci 1998;81:989–93.
[2] Silva-del-Río N, Stewart S, Rapnicki P, Chang YM, Fricke PM. An observational analysis of twin births, calf sex ratio, and calf mortality in Holstein dairy cattle. J Dairy Sci 2007;90:1255–64.
[3] Fricke PM. Review: twinning in dairy cattle. Prof Anim Sci 2001;61–7.
[4] Beerepoot GMM, Dykhuizen AA, Nielen Y, Schukken YH. The economics of naturally occurring twinning in dairy cattle. J Dairy Sci 1992;75:1044–51.
[5] Eddy R, Davies O, David C. An economic assessment of twin births in British dairy herds. Vet Rec 1991;129:526–9.
[6] Day JD, Weaver LD, Franti CE. Twin pregnancy diagnosis in Holstein cows: discriminatory powers and accuracy of diagnosis by transrectal palpation and outcome of twin pregnancies. Can Vet J 1995;36:93–7.
[7] Nielen M, Schukken YH, Scholl DT, Wilbrink HJ, Brand A. Twinning in dairy cattle: a study of risk factors and effects. Theriogenology 1989;32:845–62.
[8] Santos JE, Thatcher WW, Chebel RC, Cerri RL, Galvao KN. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. Anim Reprod Sci 2004;82/83:513–35.
[9] Committee on Bovine Reproductive Nomenclature. Recommendations for standardizing bovine reproductive terms. Cornell Vet 1972;216–37.
[10] López-Gatius F, Santolaria P, Yaniz J, Rutllant J, Lopez-Bejar M. Factors affecting pregnancy loss from gestation Day 38 to 90 in lactating dairy cows from a single herd. Theriogenology 2002;57:1251–61.
[11] López-Gatius F, Santolaria P, Yaniz JL, Garbayo JM, Hunter RH. Timing of early foetal loss for single and twin pregnancies in dairy cattle. Reprod Domest Anim 2004;39:429–33.
[12] Romano JE, Thompson JA, Kraemer DC, Westhusin ME, Forrest DW, Tomaszweski MA. Early pregnancy diagnosis by palpation per rectum: influence on embryo/fetal viability in dairy cattle. Theriogenology 2007;67:486–93.
[13] García-Ispierto I, López-Gatius F, Santolaria P, Yáñiz JL, Nogareda C, López-Béjar M, De Rensis F. Relationship between heat stress during the peri-implantation period and early fetal loss in dairy cattle. Theriogenology 2006;65:799–807.
[14] Pursley JR, Mee MO, Wiltbank MC. Synchronization of ovulation in dairy cows using PGF and GnRH. Theriogenology 1995;44:915–23.
[15] Institute S. SAS/STAT 9.1. User's Guide; 2004.
[16] López-Gatius F, Hunter RH. Spontaneous reduction of advanced twin embryos: its occurrence and clinical relevance in dairy cattle. Theriogenology 2005;63:118–25.
[17] Thurmond MC, Picanso JP. A surveillance system for bovine abortion. Prev Vet Med 1990;9:41–53.
[18] Eicker S, Fetrow J. New tools for deciding when to replace used dairy cows. In: Proceedings of the Kentucky Dairy Conference Cave City, KY. Lexington: University of Kentucky; 2003. p. 33–46.
[19] Schrick FN, Hockett ME, Saxton AM, Lewis MJ, Dowlen HH, Oliver SP. Influence of subclinical mastitis during early lactation on reproductive parameters. J Dairy Sci 2001;84:1407–12.
[20] Santos JEP, Cerri RLA, Ballou MA, Higginbotham GE, Kirk JH. Effect of timing of first clinical mastitis occurrence on lactational and reproductive performance of Holstein dairy cows. Anim Reprod Sci 2004;80:31–45.
[21] Chebel RC, Santos JE, Reynolds JP, Cerri RL, Juchem SO, Overton M. Factors affecting conception rate after artificial insemination and pregnancy loss in lactating dairy cows. Animal Reprod Sci 2004;84:239–55.
[22] López-Gatius F, Labérnica J, Santolaria P, López-Bejar M, Rutllant J. Effect of reproductive disorders previous to conception on pregnancy attrition in dairy cows. Theriogenology 1996;46:643–8.
[23] Dematawewa CMB, Berger PJ. Genetic and phenotypic parameters for 305-Day yield, fertility, and survival in Holsteins. J Dairy Sci 1998;81:2700–9.
[24] Lucy MC. Reproductive loss in high-producing dairy cattle: where will it end? J Dairy Sci 2001;84:1277–93.
[25] Royal MD, Darwash AO, Flint APF, Webb R, Woolliams JA, Lamming GE. Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. Anim Sci 2000;70:487–501.
[26] Chebel RC, Santos JE, Reynolds JP, Cerri RL, Juchem SO, Overton M. Factors affecting conception rate after artificial insemination and pregnancy loss in lactating dairy cows. Anim Reprod Sci 2004;84:239–55.
[27] Silke V, Diskin MG, Kenny DA, Boland MP, Dillon P, Mee JF, Sreenan JM. Extent, pattern and factors associated with late embryonic loss in dairy cows. Anim Reprod Sci 2002;71:1–12.
[28] Gábor G, Tóth F, Ozsvári L, Abonyi-Tóth Z, Sasser R. Factors influencing pregnancy rate and late embryonic loss in dairy cattle. Reprod Domest Anim 2008;43:53–8.
[29] Cartmill JA, El-Zarkouny SZ, Hensley BA, Rozell TG, Smith JF, Stevenson JS. An alternative AI breeding protocol for dairy cows exposed to elevated ambient temperatures before or after calving or both. J Dairy Sci 2001;84:799–806.

[30] Grimard B, Freret S, Chevallier A, Pinto A, Ponsart C, Humblot P. Genetic and environmental factors influencing first service conception rate and late embryonic/fœtal mortality in low fertility dairy herds. Anim Reprod Sci 2006;91:31–44.
[31] Starbuck MJ, Dailey RA, Inskeep EK. Factors affecting retention of early pregnancy in dairy cattle. Anim Reprod Sci 2004;84:27–39.
[32] Fricke PM, Wiltbank MC. Effect of milk production on the incidence of double ovulation in dairy cows. Theriogenology 1999;52:1133–43.
[33] Bech-Sábat G, López-Gatius F, Yániz JL, García-Ispierto I, Santolaria P, Serrano B, Sulon J, de Sousa NM, Beckers JF. Factors affecting plasma progesterone in the early fetal period in high producing dairy cows. Theriogenology 2008;69:426–32.
[34] Echternkamp SE, Thallman RM, Cushman RA, Allan MF, Gregory KE. Increased calf production in cattle selected for twin ovulations. J Anim Sci 2007;85:3239–48.
[35] McMillan WH. Statistical models predicting embryo survival to term in cattle after embryo transfer. Theriogenology 1998;50: 1053–70.
[36] Steinman G, Valderrama E. Mechanisms of twinning. III. Placentation, calcium reduction and modified compaction. J Reprod Med 2001;46:995–1002.
[37] Silva-del-Río N, Kirkpatrick BW, Fricke PM. Observed frequency of monozygotic twinning in Holstein dairy cattle. Theriogenology 2006;66:1292–9.
[38] López-Gatius F, Lopez-Bejar M, Fenech M, Hunter RH. Ovulation failure and double ovulation in dairy cattle: risk factors and effects. Theriogenology 2005;63:1298–307.
[39] Sellars CB, Dalton JC, Manzo R, Day J, Ahmadzadeh A. Time and incidence of ovulation and conception rates after incorporating estradiol cypionate into a timed artificial insemination protocol. J Dairy Sci 2006;89:620–6.
[40] Chavez R, Cruz ME, Dominguez R. Differences in the ovulation rate of the right or left ovary in unilaterally ovariectomized rats: effect of ipsi- and contralateral vagus nerves on the remaining ovary. J Endocrinol 1987;113:397–401.
[41] Fukuda M, Fukuda K, Andersen CY, Byskov AG. Right-sided ovulation favors pregnancy more than left-sided ovulation. Hum Reprod 2000;15:1921–6.
[42] López-Gatius F. Transuterine sperm transport is not affected by bilateral asymmetry of the reproductive system in dairy cows. Theriogenology 1997;47:1319–25.
[43] Echternkamp SE, Gregory KE. Reproductive, growth, feedlot, and carcass traits of twin vs. single births in cattle. J Anim Sci 2002;80:1–10.
[44] Erb RE, Morrison RA. Effects of twinning on reproductive efficiency in a Holstein–Friesian herd. J Dairy Sci 1958;42: 512–9.
[45] Labhsetwar AP, Tyler WJ, Casida LE. Analysis of variation in some factors affecting multiple ovulations in Holstein cattle. J Dairy Sci 1963;46:840–2.
[46] López H, Sartori R, Wiltbank MC. Reproductive hormones and follicular growth during development of one or multiple dominant follicles in cattle. Biol Reprod 2005;72:788–95.
[47] López H, Caraviello DZ, Satter LD, Fricke PM, Wiltbank MC. Relationship between level of milk production and multiple ovulations in lactating dairy cows. J Dairy Sci 2005;88:2783–93.
[48] Savio JD, Boland MP, Hynes N, Roche JF. Resumption of follicular activity in the early post-partum period of dairy cows. J Reprod Fertil 1990;88:569–79.
[49] Bendixen PH, Oltenacu PA, Andersson L. Case-referent study of cystic ovaries as a risk indicator for twin calvings in dairy cows. Theriogenology 1989;31:1059–66.
[50] Echternkamp SE, Cushman RA, Allan MF, Thallman RM, Gregory KE. Effects of ovulation rate and fetal number on fertility in twin-producing cattle. J Anim Sci 2007;85:3228–38.
[51] Mann GE, Robinson RS, Hunter MG. Corpus luteum size and function following single and double ovulations in non-lactating dairy cows. Theriogenology 2007;67:1256–61.