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Effects of clinical mastitis caused by gram-positive and gram-negative bacteria and other organisms on the probability of conception in New York State Holstein dairy cows

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ABSTRACT

The objective of this study was to estimate the effects of different types of clinical mastitis (CM) on the probability of conception in New York State Holstein cows. Data were available on 55,372 artificial inseminations (AI) in 23,695 lactations from 14,148 cows in 7 herds. We used generalized linear mixed models to model whether or not a cow conceived after a particular AI. Independent variables included AI number (first, second, third, fourth), parity, season when AI occurred, farm, type of CM (due to gram-positive bacteria, gram-negative bacteria, or other organisms) in the 6 wk before and after an AI, and occurrence of other diseases. Older cows were less likely to conceive. Inseminations occurring in the summer were least likely to be successful. Retained placenta decreased the probability of conception. Conception was also less likely with each successive AI. The probability of conception associated with the first AI was 0.29. The probability of conception decreased to 0.26, 0.25, and 0.24 for the second, third, and fourth AI, respectively. Clinical mastitis occurring any time between 14 d before until 35 d after an AI was associated with a lower probability of conception; the greatest effect was an 80% reduction associated with gram-negative CM occurring in the week after AI. In general, CM due to gram-negative bacteria had a more detrimental effect on probability of conception than did CM caused by gram-positive bacteria or other organisms. Furthermore, CM had more effect on probability of conception immediately around the time of AI. Additional information about CM (i.e., its timing with respect to AI, and whether the causative agent is gram-positive or gram-negative bacteria, or other organisms) is valuable to dairy personnel in determining why some cows are unable to conceive in a timely manner. These findings are also beneficial for the management of mastitic cows (especially those with gram-negative CM) when mastitis occurs close to AI.
Key words: dairy cow, gram-positive mastitis, gram-negative mastitis, conception

INTRODUCTION

Fertility plays a crucial role in successful management of dairy cows to optimize the profitability of an enterprise. If a cow does not conceive, her productive life will end soon after her milk production drops to unprofitable levels. A cow may have trouble conceiving for various reasons. Factors related to poor fertility include missed estrus and poor heat expression. To circumvent these problems, many farms in the United States, including the farms in this study, use ovulation synchronization and planned breeding programs. Despite the use of such programs, some cows still do not conceive in a timely manner. Weather (heat stress; Chebel et al., 2004; Huang et al., 2008), high milk production (Leroy et al., 2008), and diseases, including clinical mastitis (**CM**) (Santos et al., 2004), the focus of this study, may be partly responsible for reproduction problems.

Fourichon et al. (2000), in a review of diseases and reproduction in dairy cows, reported that although mastitic cows were less likely to be inseminated, mastitis had little effect on other reproductive parameters. In contrast, many studies, including those discussed below, have found mastitis to play an important role in dairy cow reproduction. For example, Moore et al. (1991) found that mastitic cows were more likely to have abnormal estrous cycle lengths. Also, cows that contracted CM in the first 45 d of gestation were al-

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most 3 times more likely to abort during the next 90 d than were cows without CM (Risco et al., 1999).

Several studies have looked at the timing of CM; for example, CM occurring before first AI, between first AI and pregnancy diagnosis, or after pregnancy diagnosis (Barker et al., 1998; Schrick et al., 2001; Santos et al., 2004; Gunay and Gunay, 2008). When CM occurred before first AI, the first AI was reported to be delayed (Barker et al., 1998; Gunay and Gunay, 2008). If CM occurred after first AI and before pregnancy diagnosis, days open were longer (Barker et al., 1998; Santos et al., 2004; Gunay and Gunay, 2008), and more services per conception were needed (Barker et al., 1998; Gunay and Gunay, 2008). Schrick et al. (2001) also considered subclinical mastitis: cows having CM following subclinical mastitis had poor reproductive performance. Ahmadzadeh et al. (2009) found that the timing of the first CM case in lactation affected the number of services per conception. If CM first occurred after 56 DIM, the number of services per conception was greater than if it had occurred earlier in lactation or not at all. They also reported that a greater proportion of mastitic cows failed to conceive, compared with nonmastitic cows. Cows with an intramammary infection before AI were twice as likely to lose the embryo by 35 to 41 d (Moore et al., 2005).

Different types of CM (i.e., caused by different pathogens) may have varying effects on reproduction (Wilson et al., 2008). Clinical mastitis caused by *Escherichia coli* and *Streptococcus* spp. was associated with a 50% lesser probability of conception (Wilson et al., 2008).

One method of classifying CM is by Gram staining, which we have used in this study. The majority of the mastitis-causing pathogens in dairy cows can be reliably classified as gram-positive or gram-negative by using an on-farm culture system, which seems more convenient to guide treatment decisions and is often faster than sending a milk sample to an outside laboratory. Clinical signs, severity, and treatment protocols differ for gram-positive and gram-negative CM. We therefore think it would be valuable to compare the effect of CM on reproduction by categorizing CM-causing pathogens by Gram status.

Although several of the studies discussed above considered CM occurrence before AI, between AI and pregnancy diagnosis, and after pregnancy diagnosis, no study, to our knowledge, has looked at the effects of different types of CM occurring in weekly intervals before and after AI on conception. The objective of this study, therefore, was to estimate the effects of different types of CM (gram-positive, gram-negative, other) occurring in different weekly intervals before or after AI (from 42 d before until 42 d after AI) on the probability of conception in Holstein dairy cows.

MATERIALS AND METHODS

Study Herds

Data were available on 55,372 AI in 23,695 lactations in 14,148 New York State Holstein dairy cows in 7 herds. Data were collected for 3 to 5 yr: from January 2003 (1 farm), February 2004 (2 farms), June 2004 (2 farms), July 2004 (1 farm), and October 2004 (1 farm), until March 2008. The 305-d rolling herd average milk production was 11,320 kg/cow per year; monthly mean SCC was 225,000 cells/mL. Cows were housed in freestalls in covered barns and managed in groups according to lactation, production, and reproductive status. They were fed a balanced TMR and were milked 3 times per day. All farms recorded milk production, milk conductivity, and other information, including medical data and drying-off, calving, and culling dates.

All cows in these 7 herds were enrolled in ovulation synchronization and planned breeding programs. Herds were specifically selected for this management characteristic as these synchronization and planned breeding programs eliminate many of the potential biases that may occur in herds where the herd managers make decisions on estrus detection and breeding.

Case Definition

All lactating cows that had been inseminated at least once between 40 and 90 DIM were eligible for inclusion in the study. Cases of CM were characterized by a warm, swollen udder or changes in milk consistency and were mostly detected by milkers. Herdspersons examining cows whose milk electrical conductivity was elevated and had concurrent milk loss found the additional cases. Sick cows were treated according to well-defined protocols that were similar on all 7 farms and throughout the study. Farm personnel sent milk samples from quarters with CM signs to the Quality Milk Production Services laboratories for microbiological diagnosis.

Recommended procedures for diagnosis of bovine intramammary infections were followed (see also Gröhn et al., 2004). Briefly, milk samples were plated by streaking 0.01 mL on trypticase soy agar II with 5% sheep blood and 0.1% esculin (BBL, Becton Dickinson Microbiology Systems, Cockeysville, MD). Plates were incubated at 37°C for 48 h. After observation of colony morphology and hemolytic patterns on blood agar, isolates were examined further by means of 3% KOH, Gram staining of organisms, catalase and oxidase testing, and additional biochemical and metabolic evaluations as needed. Colony morphology on MacConkey agar, and the BBL Crystal ID System (Becton Dickinson), identified gram-negative organisms.

Table 1. Clinical mastitis (CM)-causing organisms present in 7 New York State Holstein herds, 2003–2008

Group/species	n[1]
Gram-positive	
Streptococcus spp.	1,911
Staphylococcus aureus	546
Staphylococcus spp.	535
Gram-negative	
Escherichia coli	2,189
Klebsiella spp.	1,073
Citrobacter spp.	81
Enterobacter spp.	55
Other	
Arcanobacterium pyogenes	193
Mycoplasma spp.	98
Corynebacterium bovis	40
Pseudomonas spp.	4
Yeast	220
Others	1,026
Contamination	224
Unknown	685
No growth	1,928

[1]Total number of CM cases (comprising first, second, third, and fourth occurrences) in which the organism was identified. A cow may have a mixture of organisms in any one episode. For purposes of classifying an episode as gram-positive, gram-negative, or other, if more than one organism was present in an isolate and they were of different types, a "major" pathogen was chosen as the leading cause over a "minor" pathogen (Hassan et al., 2009); for example, an isolate containing both *Escherichia coli* and *Staphylococcus* spp. was classified as gram-negative.

In this study, gram-positive organisms included *Streptococcus* spp., *Staphylococcus aureus*, and other *Staphylococcus* species, whereas gram-negative organisms included *E. coli*, *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp. Other organisms, such as *Arcanobacterium pyogenes*, *Mycoplasma* spp., *Pseudomonas* spp., and yeast, that did not fall into these 2 categories, were classified as "other" (Table 1).

If a second clinical episode of CM occurred in the same quarter 5 or fewer days after the first one (with either the same or a different etiologic agent isolated), or occurred within 14 d with the same etiologic agent isolated from both episodes, it was considered to be the same case of CM. Any episode occurring more than 14 d after the previous episode was considered a new case (Barkema et al., 1998).

Other Diseases

Our main interest was the effect of gram-positive, gram-negative, and other CM on the probability of conception. Other common disorders [milk fever, retained placenta, metritis, ketosis, and displaced abomasum (**DA**)] may also play a role. These were defined as follows (Gröhn et al., 2004): 1) milk fever: a cow was unable to rise or had cool extremities and sluggish rumen motility near the time of calving, but was successfully treated with calcium; 2) retained placenta: retention of fetal membranes for at least 24 h after calving; 3) metritis: a cow was febrile and had a purulent or fetid vaginal discharge, or an enlarged uterus detected by veterinary palpation; 4) ketosis: presence of ketones in milk or urine, and response to treatment; and 5) DA: an abomasum enlarged with fluid, gas, or both, and which was mechanically trapped in either the left or right side of the abdominal cavity. Disease definitions and diagnostic criteria were consistent across farms. Written disease definitions were given to participating dairy producers and veterinarians before starting the study.

Statistical Analysis

We modeled the effects of cases of CM and other factors [herd, parity, season of AI, milk yield level (corrected for parity group: primipara and multipara), and other diseases] on the probability of conception associated with a particular AI (first, second, third, fourth).

We used SAS PROC GLIMMIX (SAS Institute, 2006) to fit the generalized linear mixed model:

$$g(\mathbf{Y}) = \mathbf{X}\boldsymbol{\beta} + \varepsilon, \qquad [1]$$

where g is a link function (here, the natural log of the odds of a cow conceiving after an AI), $\mathbf{Y}$ is the vector of observations, $\boldsymbol{\beta}$ is an unknown vector of fixed-effect parameters with known design matrix $\mathbf{X}$, and ε is an unknown random error vector.

We modeled the probability P of an event (conception) occurring, due to various factors, using the following specification:

$$P = [\exp(\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k)] / [1 + \exp(\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k)], \qquad [2]$$

where P is the probability of conception associated with a particular AI, β_0 is the regression parameter for the intercept, and $\beta_1, \beta_2, \ldots, \beta_k$ are the regression parameters for the effects $x_1, x_2, \ldots, x_k$, respectively, in the model.

Potential correlation of repeated AI (1, 2, 3, or 4) within a cow's lactation was accounted for by specifying the *_residual_* effect in the RANDOM statement in PROC GLIMMIX (SAS Institute, 2006). The "RANDOM _residual_" statement fits a model with overdispersion; that is, the observed variance is greater than the predicted variance. The *subject* effect was a particular lactation of a specific cow in a specific herd; that is, events (AI) were correlated within each unique cow-lactation, but were assumed independent between subjects after conditioning on the fixed effects in the model.

Only CM cases occurring between 6 wk before and 6 wk after an AI were included. Based on preliminary analyses from our group (unpublished data), we had concluded that cases occurring outside this interval had no effect on the probability of conception associated with that particular AI.

Dummy variables for each type of CM in relation to when CM occurred with respect to an AI were created. Separate variables were created for the following time periods: 36 to 42 d, 29 to 35 d, 22 to 28 d, 15 to 21 d, 8 to 14 d, 1 to 7 d before, and 0 to 7 d, 8 to 14 d, 15 to 21 d, 22 to 28 d, 29 to 35 d, and 36 to 42 d after an AI. Insemination events that had more than one CM case in this 12-wk period were not used, so that only the effect of a CM case occurring in that week in relation to AI was taken into account.

Various models were fitted. Possible candidates for the final model were compared, based on the residuals from the maximum likelihood estimation ($-2 \times$ residual log likelihood), to assess the goodness of fit, and also the statistical (at least one level of a covariate had to be significant at $\alpha = 0.05$) and biological importance of the covariates. The first model included terms for CM occurring from 42 d before until 42 d after AI, plus other variables (but not milk yield). The second model was the same as the first but contained an additional variable for cumulative milk yield in the first 35 DIM. The third model included terms for CM occurring from 14 d before until 35 d after AI, plus other variables (but not milk yield). The fourth and final model was the same as the third, but also contained a variable for cumulative milk yield in the first 35 DIM. The third model had the best goodness of fit as judged by the value of $-2 \times$ residual log likelihood. All results discussed in this paper are based on this third model.

The voluntary waiting period in all 7 study herds was approximately 60 d; that is, farm practice was to wait until a cow was 60 DIM before inseminating her. Nevertheless, a few cows were detected in estrus early (before 40 DIM) and inseminated without synchronization treatment. At the other extreme, cows whose first AI occurred after 90 DIM were not considered to have been synchronized and inseminated normally. Therefore, the data set contained only cows who had received their first AI between 40 and 90 DIM.

RESULTS

Descriptive Findings

Table 1 shows the distribution of CM-causing organisms in the study herds. *Escherichia coli* was the most commonly isolated organism from CM cases, followed by *Streptococcus* spp. and *Klebsiella* spp. Although we identified specific agents, the number of observations was insufficient to allow estimation by agent. In the data set, 73% of the lactations did not have CM. The remaining lactations had approximately equal numbers of cases of gram-positive and gram-negative CM, and more "other" CM cases.

At the first AI, 23,504 cows were inseminated, and at the second, third, and fourth AI, 15,228 cows, 10,144 cows, and 6,496 cows were inseminated, respectively. Insemination events were not used if more than one CM case occurred in the 12-wk period around the AI event (as explained in the Statistical Analysis section). The corresponding number of cows confirmed pregnant, associated with each AI, was 6,747, 3,930, 2,537, and 1,552, respectively. Among cows that had CM, the number that conceived or did not conceive after the first, second, third, or fourth AI is shown in Table 2.

Effects of Factors (Other than CM) on Probability of Conception

Parameter estimates and standard errors for the variables used in the model are given in Table 3. Parity was divided into 4 groups: 1, 2, 3, and 4+. Probability of conception decreased with age. Parity 2 cows had a 17% lesser $[1 - \exp(-0.18)]$ probability of conception ($P < 0.0001$) than did parity 1 cows. Parity 3 and 4+ cows had a 28% lesser probability of conception ($P < 0.0001$) than did parity 1 cows.

The distribution of AI (n = 55,372) was as follows: 42.5% were the first AI in the studied lactation, 27.5% were the second, 18.3% were the third, and 11.7% were the fourth. Probability of conception was less with each successive AI. The study farms varied in the success of their breeding programs. Conception probability associated with the first AI varied from 0.29 to 0.38 between individual herds.

Inseminations were less successful in warmer months. Probability of conception in the summer was 8% less $[1 - \exp(-0.08); P = 0.0028]$ than in the winter. Moderate weather favored conception: cows inseminated in the spring or autumn had a 12% greater probability of conception ($P < 0.0005$) than did cows inseminated in the winter.

Cows with retained placenta had a 25% $[1 - \exp(-0.28); P < 0.0001]$ lesser probability of conception than did cows without retained placenta. No diseases (other than CM) were associated with probability of conception.

When cumulative milk yield of the first 35 DIM (corrected by parity group: primiparous and multiparous) was added to the model, estimates for CM and the

Table 2. Among cows with clinical mastitis (CM), the number that did or did not conceive after a first, second, third, or fourth AI, by timing and type in 7 New York State Holstein herds, 2003–2008

		First AI		Second AI		Third AI		Fourth AI	
		Conceived		Conceived		Conceived		Conceived	
Timing of CM	CM type	Yes	No	Yes	No	Yes	No	Yes	No
36–42 d before AI	Gram-positive	6	20	9	27	9	17	8	12
	Gram-negative	12	36	11	55	6	22	4	14
	Other	7	38	10	18	10	22	4	12
29–35 d before AI	Gram-positive	4	19	11	21	8	18	5	13
	Gram-negative	17	40	13	41	12	23	5	14
	Other	18	24	11	25	7	17	7	18
22–28 d before AI	Gram-positive	9	23	1	20	4	19	5	11
	Gram-negative	14	53	10	44	9	25	5	15
	Other	12	32	7	30	4	21	8	10
15–21 d before AI	Gram-positive	9	30	5	18	9	13	1	9
	Gram-negative	19	51	9	37	9	22	4	12
	Other	10	27	9	23	6	26	4	12
8–14 d before AI	Gram-positive	7	32	8	21	5	27	4	14
	Gram-negative	7	64	9	32	10	22	6	17
	Other	18	30	6	27	5	12	2	11
1–7 d before AI	Gram-positive	3	29	8	30	4	15	0	13
	Gram-negative	10	37	1	30	4	15	1	13
	Other	13	26	4	23	1	17	2	12
0–7 d after AI	Gram-positive	7	40	6	17	1	22	3	13
	Gram-negative	8	86	4	52	1	28	0	16
	Other	9	38	2	32	2	23	6	16
8–14 d after AI	Gram-positive	12	30	11	35	0	22	3	18
	Gram-negative	9	59	6	42	2	29	4	16
	Other	9	44	11	22	2	22	1	17
15–21 d after AI	Gram-positive	13	37	7	19	6	12	3	11
	Gram-negative	16	71	2	55	2	26	4	9
	Other	9	40	5	27	6	10	0	15
22–28 d after AI	Gram-positive	5	33	7	30	6	20	5	9
	Gram-negative	13	78	7	36	4	19	5	9
	Other	8	37	5	24	2	19	4	11
29–35 d after AI	Gram-positive	5	36	7	40	5	17	0	9
	Gram-negative	16	63	9	22	3	20	4	19
	Other	13	35	7	28	10	24	5	12
36–42 d after AI	Gram-positive	10	56	7	23	9	14	1	13
	Gram-negative	24	46	9	35	4	25	6	16
	Other	13	26	7	29	1	15	2	10

other variables did not change meaningfully. The greatest overall probability of conception (30%) was associated with average-yielding cows. The smallest overall probability of conception (29%) was associated with the lowest yielding cows. Although this 1% difference was statistically significant ($P = 0.0003$), it is not practically important.

Effect of CM on Probability of Conception

Clinical mastitis, regardless of type, occurring 15 or more days before or 36 or more days after an AI was not associated with the probability of conception. Within the interval from 14 d before until 35 d after an AI, however, the effect of CM on probability of conception varied with both type and timing of CM. Generally, the CM effect was more pronounced if it occurred around the time of AI and when the causative agent was gram negative (Table 3).

Gram-negative CM occurring from 8 to 14 d before an AI was associated with a 32% lower $[1 - \exp(-0.39)]$ probability of conception ($P = 0.0498$), compared with no CM occurring in that period. Gram-positive or gram-negative CM occurring from 1 to 7 d before an AI was associated with a 50% reduction in probability of conception ($P < 0.02$; Table 3).

All types of CM occurring 0 to 7 d after an AI were associated with a reduction in probability of conception (Table 3). Gram-positive and "other" CM were associated with reductions in probability of conception of 47% ($P = 0.0183$) and 49% ($P = 0.0073$), respectively. Gram-negative CM occurring in this period was associated with a much larger reduction of 80% ($P < 0.0001$). A case of "other" CM occurring 8 to 14 d or 15 to 21 d after an AI was associated with a reduction in probability of conception of 39% ($P < 0.05$). A case of gram-negative CM occurring in this period was associated with a reduction in probability of conception of 59% ($P < 0.0003$). A case of "other" CM or gram-negative CM occurring 22 to 28 d after an AI was associated with a 42% lesser probability of conception ($P < 0.04$), compared with no CM in this period. A case of gram-positive CM occurring 29 to 35 d after an AI was associated with a 53% ($P = 0.0048$) lesser probability of conception (Table 3).

Figure 1 shows the probability of conception in a parity 2 cow being bred for the first time in the spring,

Table 3. Parameter estimates and standard errors for the generalized mixed model used to estimate the effect of different types of clinical mastitis (CM) and other factors on probability of conception in 23,695 lactations in 14,148 Holstein cows in 7 New York State herds[1]

Parameter	Level	Estimate	SE
Intercept		−0.90***	0.04
Parity	1	Ref.[2]	—
	2	−0.18***	0.02
	3	−0.32***	0.03
	4+	−0.33***	0.03
Insemination attempt (AI)	First	Ref.	—
	Second	−0.13***	0.02
	Third	−0.17***	0.03
	Fourth	−0.22***	0.03
Season of AI	Winter	Ref.	—
	Spring	0.10***	0.03
	Summer	−0.08**	0.03
	Autumn	0.13***	0.03
Retained placenta	Absent	Ref.	—
	Present	−0.28***	0.03
Farm	1	0.41***	0.03
	2	0.02	0.05
	3	0.11*	0.04
	4	0.04	0.04
	5	0.06	0.04
	6	0.16***	0.04
	7	Ref.	—
CM occurring 8–14 d before AI	Gram-positive	−0.35	0.23
	Gram-negative	−0.39*	0.20
	Other	0.09	0.21
	None	Ref.	—
CM occurring 1–7 d before AI	Gram-positive	−0.70*	0.28
	Gram-negative	−0.68*	0.27
	Other	−0.28	0.25
	None	Ref.	—
CM occurring 0–7 d after AI	Gram-positive	−0.63*	0.27
	Gram-negative	−1.63***	0.29
	Other	−0.67**	0.25
	None	Ref.	—
CM occurring 8–14 d after AI	Gram-positive	−0.34	0.22
	Gram-negative	−0.88***	0.23
	Other	−0.50*	0.23
	None	Ref.	—
CM occurring 15–21 d after AI	Gram-positive	−0.00	0.22
	Gram-negative	−0.87***	0.22
	Other	−0.49*	0.25
	None	Ref.	—
CM occurring 22–28 d after AI	Gram-positive	−0.34	0.23
	Gram-negative	−0.55**	0.21
	Other	−0.53*	0.25
	None	Ref.	—
CM occurring 29–35 d after AI	Gram-positive	−0.75**	0.26
	Gram-negative	−0.30	0.20
	Other	−0.01	0.20
	None	Ref.	—

[1]Values have been rounded to 2 decimal places, so results presented in the text may differ slightly due to rounding.

[2]Reference level of the factor.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

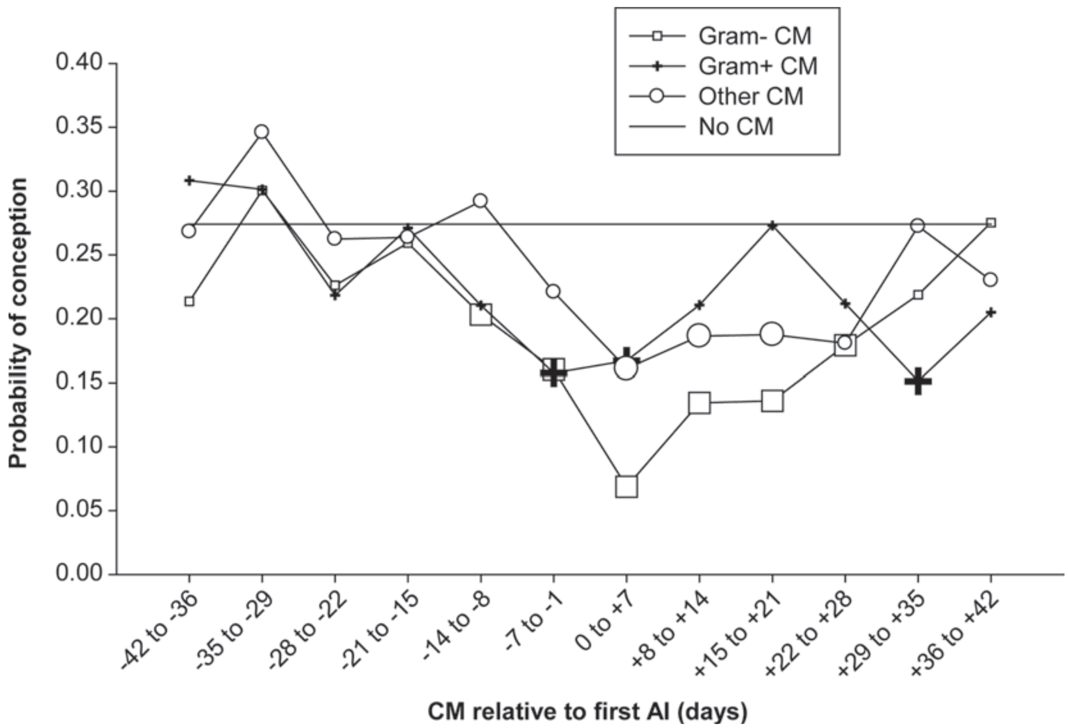

Figure 1. Probability of conception on first AI, for a parity 2 cow bred in spring, without mastitis (CM; —) or with mastitis caused by gram-positive (+) or gram-negative bacteria (□) or other organisms (○). Larger symbols indicate probabilities that were significantly different ($P < 0.05$) compared with "No CM" in the interval.

with either no CM or 1 of 3 types of CM occurring in the period around AI. In the week immediately before and the week immediately after AI, as well as the period 29 to 35 d after AI, a cow with gram-positive CM had a lower probability of conception than did a cow with no CM at that time. Gram-negative CM was associated with the most detrimental effect on probability of conception: in all weekly intervals from 14 d before until 28 d after AI, a cow with gram-negative CM had a lower probability of conception than did a cow without CM in those intervals. The effect was largest when gram-negative CM occurred in the week following AI. "Other" CM was not associated with probability of conception more than 8 d before or 29 d after AI. Overall, the lowest probability of conception was associated with occurrence of CM immediately after AI.

DISCUSSION

This study demonstrated that CM occurring around the time of insemination, whether before or after AI, was associated with a reduction in probability of conception. In general, the interval from 14 d before until 35 d after an AI was most affected; CM occurring 0 to 7 d after AI was associated with the greatest reduction in probability of conception. Of the 3 types of CM considered in this study, gram-negative CM was generally associated with larger decreases in probability of conception than was either gram-positive or "other" CM. This was in contrast to some previous studies, which found no difference between gram-positive and gram-negative CM in their effects on reproduction (Barker et al., 1998; Schrick et al., 2001; Santos et al., 2004). One reason may be related to the statistical power of our study, which included many more lactations, allowing us to statistically isolate the effect of Gram status.

A major strength in our study design was the equal opportunity for AI (and conception) in all cows in the study because we only included herds in which all cows were enrolled in a strict estrus synchronization protocol. All cows, irrespective of disease history, milk production, genetic merit, or previous reproductive performance, were subjected to AI within very tight time limits. For this reason, we expect that very little producer bias affected the probability of conception. In many other data sets, the producer's preconceived notions of lower or higher fertility in cows with certain characteristics may bias the actual observed biology of conception.

In our data, probability of conception decreased with each successive AI through the fourth AI. In our study, probability of conception was only 5% after the fourth

AI if a cow had experienced gram-negative CM in the week after insemination, a substantial decrease from the probability of conception of 0.29 after the first AI in a healthy cow. Other researchers have reported similar findings. Cows that do not conceive display difficulties in conceiving in subsequent AI attempts. In 4 high-producing Spanish Holstein-Friesian herds, cows bred 4 or more times were less likely to conceive than those needing fewer inseminations (García-Ispierto et al., 2007). Rizzo et al. (2007) found that repeat breeders (cows with normal estrus cycles and no pathological findings but that did not conceive after 3 or more AI) had higher levels of β-endorphins and free radicals (stress factors), which may inhibit establishment of a pregnancy, and lower levels of progesterone, than did cows needing fewer AI to conceive. Repeat breeding was reported to be associated with several risk factors, including but not limited to difficulty conceiving in the previous lactation, season of the first insemination, milk yield, and diseases, including CM (Gustafsson and Emanuelson, 2002).

Substantive research has focused on the mechanisms of action of pathogen-associated molecules, such as endotoxin from gram-negative bacteria, and their effects on conception. Endotoxin effects have been reported to be present in various tissues involved in reproduction, including the hypothalamus (Schrick et al., 2001; Nugent et al., 2002), ovary (Herath et al., 2007), and endometrium (Herath et al., 2009). Herath et al. (2007) reported that in the ovary, follicle growth might be interrupted because of bacterial infection. Similarly, in the endometrium, *E. coli* LPS were associated with production of prostaglandin E_2 rather than prostaglandin $F_{2\alpha}$, resulting in a prolonged luteal phase (Herath et al., 2009) and potentially lower probability of ovulation and conception. Williams et al. (2008) observed that fewer animals ovulated following intrauterine infusion with LPS compared with control cows; it was concluded that LPS suppressed ovarian cell function. The key cell wall component of gram-positive bacteria that is involved in induction of inflammation is lipoteichoic acid (**LTA**). Although LTA has been shown to affect fertility in the same manner as LPS in mice, the effective dose of LTA needed to show a similar effect was much larger than that of LPS (Kajikawa et al., 1998). This may explain our observed differences in the effect of gram-negative and gram-positive mastitis on the probability of conception.

Intramammary infections often result in pathogen-specific release profiles of pro-inflammatory cytokines such as tumor necrosis factor-α and several interleukin molecules (Bannerman, 2009). These cytokines have been linked to embryo loss (Hansen et al., 2004), by inducing hyperthermia (fever), which inhibits oocyte function and embryo growth, or through increased prostaglandin production. Alternatively, these cytokines might be directly involved in functional luteolysis (Neuvians et al., 2004). The cytokines may originate in the mammary gland, or be produced elsewhere in response to signals from the mammary gland (Hansen et al., 2004).

Perrin et al. (2007) evaluated whether CM was affecting fertilization of oocytes or early embryonic death. In that study, they also categorized the occurrence of CM with respect to the timing of AI: CM occurring 3 to 6 wk before, 0 to 3 wk before, 0 to 3 wk after, or 3 to 6 wk after AI. When CM occurred 0 to 3 wk before the first AI, twice as many nonmastitic cows conceived as mastitic ones. A decreased conception risk was not observed in any of the other periods relative to AI, suggesting that CM primarily affects ovulatory factors and oocytes, rather than products of conception (embryos; Perrin et al., 2007).

Our finding that CM (especially gram-negative CM) occurring immediately after an AI was associated with a very low probability of conception suggests that CM may also be interfering with oocyte fertilization or embryonic development. Soto et al. (2003) studied oocytes before and after fertilization and cocultured these with LPS. Their results indicated that increased local concentrations of LPS had deleterious consequences on oocyte function and embryonic development. This observation was in agreement with a study by Hockett et al. (2000), where cows were challenge infected in one quarter with gram-positive bacteria. This challenge infection resulted in an increased uterine sensitivity to prostaglandin $F_{2\alpha}$, making cows more susceptible to embryo losses (Hockett et al., 2000).

From the above arguments linking bacterial infections with impaired reproductive performance, we conclude that it is likely that multiple interrelated mechanisms are responsible for the reduced fertility associated with CM. We therefore suggest that our data combined with the known pathophysiological mechanisms indicate that a causal relationship exists between CM and reduced fertility.

In our study, the only disorder besides CM that was associated with probability of conception was retained placenta. Furthermore, in a model in which all of the diseases (milk fever, retained placenta, metritis, DA, ketosis) were included (results not shown), the parameter estimates for the mastitis terms were virtually identical to those in Table 3, indicating that confounding was not a problem (Kleinbaum et al., 1982). Other studies have reported various periparturient disorders to be associated with impaired fertility. In a literature review, Fourichon et al. (2000) reported that clinical ketosis, dystocia, and retained placenta were associated with longer days to first service and lower conception rate after first service, resulting in 6 to 12 d longer, on average, until conception. Metritis was associated with an average of 19 additional days open, cystic ovaries with 20 to 30 additional days open, anestrus with 41 additional days open, and abortion with 70 to 80 additional days open (Fourichon et al., 2000). Chebel et al. (2004) found that milk fever and retained placenta reduced conception rate.

A practical advantage of this study is that the probability of conception for any individual cow with a given set of factors can be easily calculated, based on the parameter estimates in Table 3 obtained from a generalized mixed model. For example, a parity 3 cow bred in winter after her second AI has a probability of conception of $[\exp(-0.90 - 0.32 - 0.0 - 0.13)]/[1 + \exp(-0.90 - 0.32 - 0.0 - 0.13)] = 0.21$.

If this cow had gram-positive CM 1 wk before AI, her probability of conception would be $[\exp(-0.90 - 0.32 - 0.0 - 0.13 - 0.70)]/[1 + \exp(-0.90 - 0.32 - 0.0 - 0.13 - 0.70)] = 0.11$. If this cow had, instead, gram-negative CM in the week after AI, her probability of conception would be $[\exp(-0.90 - 0.32 - 0.0 - 0.13 - 1.63)]/[1 + \exp(-0.90 - 0.32 - 0.0 - 0.13 - 1.63)] = 0.05$.

Such calculations may assist farmers in determining whether it is worthwhile to inseminate a particular cow; for example, one with CM (especially if caused by gram-negative bacteria) and that has already undergone several AI. The costs of the resources (e.g., semen, technician time, treatment of the CM) may outweigh the benefits. A more likely scenario would be the selection of cheaper versus more expensive semen in cows with a very low probability of conception. Not only is semen from high-genetic-merit bulls more expensive, but sexed semen is also substantially more expensive than conventional semen. Depriving cows with a known low probability of conception from these expensive resources makes economic sense.

Our study differs from previous studies in that both type and precise timing of CM, as well as the first through fourth AI, were accounted for in our model estimates. Some previous studies have examined gram-positive versus gram-negative CM and reproduction (Barker et al., 1998; Schrick et al., 2001; Santos et al., 2004) and some have examined CM occurring in the intervals before AI, AI to pregnancy diagnosis, and after pregnancy diagnosis (Barker et al., 1998; Santos et al., 2004) or other intervals (Perrin et al., 2007; Ahmadzadeh et al., 2009). Our study differs in that we accounted both for type of CM and timing of CM in 6 weekly intervals before AI and 6 weekly intervals after AI. Our findings suggest that additional information about CM (e.g., its timing with respect to AI and whether the causative agent is gram-positive, gram-negative, or other) is beneficial to farmers in determining why some cows have trouble conceiving. Such knowledge is useful in the management of CM cows before and after AI, and could therefore be instrumental in improving the profitability of a dairy enterprise.

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