IMPACT OF MASTITIS ON REPRODUCTIVE PERFORMANCE

Pamela L. Ruegg, DVM, MPVM, Paul Fricke, PhD & Maria Jose Fuenzalida, University of WI, Dept. of Dairy Science, Madison WI USA

Introduction

On many dairy farms, reproductive failure and the occurrence of mastitis are two of the most common management problems. Risk factors for mastitis and reproductive disorders are similar and it can be difficult to determine the specific impact of mastitis on reproductive performance. Age of cow, heat stress, occurrence of metabolic diseases, immune suppression in early lactation and high milk yield are all associated with increased risk of mastitis and reduced fertility. However, the direct impact of mastitis on reducing reproductive performance was first noted more than 2 decades ago (Moore et al., 1991) and since that time researchers have consistently reported that mastitis can significantly reduce reproductive performance (Barker et al., 1998, Schrick et al., 2001; Santos et al., 2004a; Hertl et al., 2010). The occurrence of mastitis has been associated with reductions in the occurrence of natural estrus (Moore et al., 1991; Barker et al., 1981, Schrick et al., 20012, Santos et al., 2004a), the ability to conceive after breeding (Ahmadzadeh et al., 2009; Barker et al., 1981; Chebel et al., 2004; Santos et al., 2004a; Schrick et al., 20012) and increased pregnancy loss (Chebel et al., 2004; Hudson et al. 2012; Santos et al., 2004a).

The negative impact of mastitis on reproductive performance appears to be a consequence of the severity of the immune response to intramammary infection by bacteria (IMI). Subclinical mastitis (SM) is defined as an IMI that results in an influx of white blood cells to the udder without disruption of the appearance of milk and is usually detected based on recognition of an increased number of somatic cells in milk. Clinical mastitis (CM) is defined as an IMI that causes enough inflammation to disrupt the milk secretory process and results in the production of visually abnormal milk (with or without abnormalities in the mammary gland or systemic symptoms) (Pinzon-Sanchez and Ruegg, 2011, Ruegg et al., 2014). The occurrence of both SM (Schrick et al., 2001, Lavon et al., 2011a, Lavon et al., 2011b, Hudson et al., 2012) and CM (Moore et al., 1991, Santos et al., 2004) have been associated with reduced reproductive performance. The purpose of this paper is to review previous data about the impact of mastitis on reproductive performance and present new data that further defines the role of severity and etiology.

Clinical versus Subclinical Mastitis

As compared to SM, CM is defined based on a more easily recognizable inflammatory response. The severity of CM is a result of the degree of inflammation in response to specific characteristics of the pathogen, the magnitude of exposure to the pathogen and the ability of the cow to rapidly respond and eliminate the IMI (Burvenich et al., 1994). The occurrence of both SM and CM have been shown to be negatively associated with reproductive performance but it is difficult to differentiate between these conditions as detection methods and definitions of mastitis have varied among studies (Schrick et al., 2001; Pinedo et al., 2009). Researchers virtually always define SM based on monthly SCC values, but the thresholds have varied; most studies define SM as SCC > 200,000 cell/mL (Dohoo and Leslie, 1991) while others (Lavon et al. 2011b) have used a more sensitive threshold of >150,000 cells/mL.

As compared to SM, detection and classification of CM is problematic and varies enormously among studies. Some studies have provided no clear definition of CM (Barker et al., 1998; Schrick et al., 2001; Hudson et al., 2012) while others have defined CM based on the presence of abnormal milk or signs of inflammation in the udder (Chebel et al., 2004; Santos et al., 2004; Ahmadzadeh et al., 2009). Other

studies have defined CM based on visible changes in the udder, changes in milk consistency or based on electrical conductivity of milk (Hertl et al., 2010). Without a clear case definition and system of monitoring CM, it is likely that many mild cases are not detected and may be misclassified as SM or simply missed. To improve consistency of reporting, CM should be classified based on the severity of the symptoms: 1. Severity 1 is defined as mild and the only sign is abnormal milk, 2. Severity 2 is defined as moderate and the abnormal milk is accompanied by signs that are restricted to the udder (redness or swelling), and 3) severity 3 is defined as severe where the symptoms extend beyond the udder (decreased milk yield, off-feed, fever or other systemic symptoms) (Pinzón-Sánchez and Ruegg, 2011). The use of this simple classification system can aid in determining if mild cases are being detected as the normal distribution of cases is 50%, 35% and 15% severity 1, 2 and 3, respectively (Oliveira et al., 2013).

Schrick et al. (2001) found that cows that experienced either SM or CM before breeding had increased days to first service, days open and services per conception as compared to healthy cows. Progression of the symptoms from subclinical to a clinical state during the interval between first insemination and pregnancy examination had a profound impact on reproductive performance and resulted in reduced fertility (Schrick et al., 2001). The dynamics of SM relative to conception was further studied by Lavon et al. (2011b). In this study, SCC values were used to categorize SM occurring before and after breeding: 1) uninfected (SCC <150,000 cells/mL before and after AI); 2) cured (high SCC before AI and low SCC after AI); 3) newly infected (low SCC before AI and high SCC after AI); 4) or chronic (high SCC before and after AI). The authors found that as compared to the uninfected cows, the probability of conception decreased for all cows that experienced SM, however the impact was greatest for chronic cows.

Increased SCC before or after AI has been shown to have a strong association with decreased reproductive performance but the effect becomes greater as SCC increases (Lavon et al., 2011b; Hudson et al., 2012). To study the association of SCC with probability of conception Lavon et al., (2011b) classified the chronic cows into categories of mild, moderate, and high SCC. They concluded that any increased SCC occurring around the time of AI reduced the probability of conception but the effect was greater for cows with the greatest SCC. They also demonstrated that occurrence of a single SCC >1,000,000 cells/mL in the 10 day period before AI resulted in significantly decreased probability of conception (Lavon et al., 2011b).

For cows affected by SM, Hudson et al. (2012) observed that the probability of a AI leading to pregnancy decreased by 18% for cows with SCC between 200,000 and 399,000 cells/mL (from 1 to 30 d post AI), whereas the probability decreased by almost 26% when SCC exceeded 399,000 cells/mL. Thus it appears that the magnitude of inflammation is associated with greater reductions in fertility.

Time of Mastitis Relative to Breeding

The importance of the timing of mastitis relative to breeding has been evaluated in several studies (Hertl et al., 2010; Hudson et al., 2012; Barker et al., 1998; Schrick et al., 2001; Santos et al., 2004a). The impact of mastitis has been examined in the periods before AI, between AI and first pregnancy examination, and after first pregnancy examination (Table 1). Most studies indicate that the most critical period is immediately before and after breeding. Both clinical and subclinical mastitis occurring before AI have been associated with reduced conception (Schrick et al., 2001), increased days to first AI and increased days open as compared to cows without clinical mastitis or cows with a later case (Barker et al., 1998; Schrick et al., 2001; Santos et al., 2004a). The occurrence of clinical mastitis before AI

reduced conception at first AI, pregnancy rate at 320 DIM, increased the incidence of abortion and increased culling (Santos et al., 2004a) (Table 1).

The occurrence of mastitis in the period between AI and first pregnancy examination caused decreased conception, decreased pregnancy rate, increased services per conception, and has been associated with increased incidence of abortion, and increased days open (Barker et al., 1998; Schrick et al., 2001; Santos et al., 2004a) (Table 1). Santos et al. (2004a) concluded that occurrence of CM before or immediately after first AI resulted in a marked decrease in conception rates at first AI. The effect of mastitis on the first postpartum AI conception was exacerbated when CM occurred between AI and pregnancy diagnosis. Likewise, Hudson et al. (2012) indicated that occurrence of SM in the interval 1 to 30 days after AI was associated with the largest decrease in pregnancy rate.

While the impact of mastitis is less as the pregnancy advances, the occurrence of CM after pregnancy examination has been associated with increased pregnancy loss. In one study, cows with CM between AI and day 45 after AI were 2.8 times more likely to lose their pregnancy as compared to healthy cows (Chebel et al., 2004). Likewise, cows with CM during the first 45 d of gestation were 2.7 times more likely to abort within the following 90 d than cows without mastitis (Risco et al., 1999).

Barker et al. (1998) initially observed the importance of the temporal association between occurrence of CM and reproductive performance, and defined the greatest risk period as immediately before AI or between AI and first pregnancy diagnosis. Further studies have confirmed that this period is a high risk period and preventive strategies should be directed at reducing the incidence of both subclinical and clinical mastitis during this period.

Pathogen-specific Effects of Mastitis on Reproductive Performance

Gram-positive and negative bacteria have different cell wall components that activate the immune response after IMI. The lipopolysaccharide component of Gram-negative bacteria stimulates release of pro-inflammatory mediators that are thought to induce increased $PGF_{2\alpha}$, resulting in luteolysis and ultimately myometrial contraction (Giordano et al., 2012). This leads to reductions in fertilization and embryonic survival (Moore et al., 1991; Barker et al., 1998). Gram-positive bacteria contain peptidoglycan that may trigger a pyretic reaction similar to responses elicited by endotoxins (Barker et al., 1998). It is thus thought that inflammatory responses to both Gram-positive and Gran-negative bacteria can be associated with embryonic losses through inflammatory products and pyrexia.

Conflicting results have been reported for associations between CM caused by Gram-positive or negative pathogens and reproduction performance but most studies have likely been confounded by a failure to record severity of the symptoms. About 30% of Gram-negative cases of CM are severe while a much smaller proportion of Gram-positive cases result in severe symptoms and more Gram-positive IMI result in SM (Oliveira et al, 2014). Some studies have reported no differences in reproductive performance based on etiology (Barker et al., 1998; Schrick et al., 2001; Santos et al., 2004a). However, one study reported that the effect of CM on reproduction varied among cows that experienced mastitis caused by Gram-positive or Gram-negative bacteria (Hertl et al., 2010). At least one author has concluded that the magnitude of the inflammatory response, (rather than etiology) was associated with reduced oocyte competence Roth et al. (2013) and the combined effect of severity and pathogen type has just recently been investigated (Fuenzalida et al., in press).

Combined Effect of Severity and Pathogen

Fuenzalida et al., (in press) enrolled 3,144 dairy cows from 4 Wisconsin dairy herds in a prospective study with the objective to determine associations between occurrence and severity of clinical (CM) and subclinical mastitis (SM) during a defined breeding risk period (BRP, 3 d before to 32 d after artificial insemination) on pregnancies per artificial insemination at first service (P/AI1). Dairy cows were categorized based on occurrence of 1 or more CM or SM events during and before the BRP: 1) Healthy, 2) Mastitis before BRP, 3) SM during BRP, 4) Chronic SM 5) CM during BRP, or 6) Chronic CM. Clinical mastitis cases were categorized based on etiology (Gram-negative, Gram-positive, and no growth) and severity (mild, moderate, or severe). Compared to healthy cows, the odds of pregnancy were reduced by 40%, 30% and 20% for cows experiencing Chronic CM, CM or SM during the BRP, respectively (Table 2). The occurrence of chronic SM was not associated with reduced probability of P/AI1. Compared to healthy cows, the odds of pregnancy were 0.71 and 0.54 for cows experiencing mild or moderate-severe cases of CM during the BRP, respectively. The odds of pregnancy for cows experiencing CM caused by Gram-negative or Gram-positive bacteria during the BRP were 0.47 and 0.59, respectively. The occurrence of CM that resulted in no growth of bacteria in cultured milk samples was not associated with reductions in P/AI1. Regardless of etiology, microbiologically positive cases of CM with moderate or severe symptoms were associated with substantial reductions in P/AI1 (Table 2). Etiology, severity, and timing of CM were associated with decreases in the probability of pregnancy at first AI. Severity of the case was more important than etiology; however, regardless of etiology microbiologically negative cases were not associated with reduced probability of pregnancy. This study further reinforces the detrimental role of inflammation on reproductive performance. Reduced exposure to mastitis pathogens combined with management strategies that result in optimum immune function to clear IMI when they occur are critical for ensuring successful breeding programs.

Conclusions & Recommendations

Intramammary infections caused by different types of bacteria (Gram-positive and negative) stimulate the immune system of the cow and these immune responses can result in reduced reproductive performance. The interval beginning immediately before breeding and continuing until confirmation of pregnancy is a critical period and the occurrence of mastitis during this time can result in significantly reduced breeding performance. The timing of both SM and CM relative to breeding is critical and farmers should recognize that housing, feeding and milking programs should be designed to minimize the occurrence of mastitis during breeding periods.

References

Ahmadzadeh, A., F. Frago, B. Shafii, J. C. Dalton, W. J. Price, and M. A. McGuire. 2009. Effect of clinical mastitis and other diseases on reproductive performance of Holstein cows. Anim. Reprod. Sci. 11:273–282.

Barker, A. R., F. N. Schrick, M. J. Lewis, H. H. Dowlen, and S. P. Oliver. 1998. Influence of clinical mastitis during early lactation on reproductive performance of Jersey cows. Journal of dairy science 81:1285-1290.

Chebel, R. C., J. E. P. Santos, J. P. Reynolds, R. L. A. Cerri, S. O. Juchem, and M. Overton. 2004. Factors affecting conception rate after artificial insemination and pregnancy loss in lactating dairy cows. Anim. Reprod. Sci. 84:239–255.

Fuenzalida, M.J., P.M. Fricke, and P.L. Ruegg. 2015. The impact of clinical and subclinical mastitis on pregnancies per artificial insemination at first service of Holstein dairy cows. J. Dairy Sci. in press.

Giordano, J. O., M. C. Wiltbank, J. N. Guenther, M. S. Ares, G. Lopes Jr., M. M. Herlihy, and P. M. Fricke. 2012. Effect of presynchronization with human chorionic gonadotropin or gonadotropin-releasing hormone 7 days before resynchronization of ovulation on fertility in lactating dairy cows. J. Dairy Sci. 95:5612–5625.
Hertl, J. A., Y. T. Gröhn, J. D. G. Leach, D. Bar, G. J. Bennett, R. N. González, B. J. Rauch, F. L. Welcome, L. W. Tauer, and Y. H. Schukken. 2010. 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. J. Dairy Sci. 93:1551–1560.
Hudson, C. D., A. J. Bradley, J. E. Breen, and M. J. Green. 2012. Associations between udder health and reproductive performance in United Kingdom dairy cows. Journal of dairy science 95:3683-3697.
Lavon, Y., G. Leitner, E. Klipper, U. Moallem, R. Meidan, and D. Wolfenson. 2011a. Subclinical, chronic intramammary infection lowers steroid concentrations and gene expression in bovine preovulatory follicles. Domest Anim Endocrin 40:98-109.
Lavon, Y., G. Leitner, U. Moallem, E. Klipper, H. Voet, S. Jacoby, G. Glick, R. Meidan, and D. Wolfenson. 2011b. Immediate and carryover effects of Gram-negative and Gram-positive toxin-induced mastitis on follicular function in dairy cows. Theriogenology 76:942-953.
Moore, D. A., J. S. Cullor, R. H. Bondurant, and W. M. Sischo. 1991. Preliminary Field Evidence for the Association of Clinical Mastitis with Altered Interestrus Intervals in Dairy-Cattle. Theriogenology 36:257-265.
Oliveira, L., C. Hulland, and P. L. Ruegg. 2013. Characterization of clinical mastitis occurring in cows on 50 large dairy herds in Wisconsin. J Dairy Sci, 96:7538-7549.
Pinzon-Sanchez, C. and P. L. Ruegg. 2011. Risk factors associated with short-term post-treatment outcomes of clinical mastitis. Journal of dairy science 94:3397-3410.
Risco, C. A., G. A. Donovan, and J. Hernandez. 1998. Clinical mastitis associated with abortion in dairy cows. J. Dairy Sci. 82:1684–1689.
Roth, Z., A. Dvir, D. Kalo, Y. Lavon, O. Krifucks, D. Wolfenson, and G. Leitnert. 2013. Naturally occurring mastitis disrupts developmental competence of bovine oocytes. J. Dairy Sci. 96:1–7.
Ruegg, P. L., R. D. Erskine, and D. Morin. 2014. Mammary Gland Health. Pages 1015-1043 in Large Animal Veterinary INternal Medicine. 5th ed. B. P. Smith, ed. Elsevier, USA.
Santos, J. E. P., R. L. A. Cerri, M. A. Ballou, G. E. Higginbotham, and J. H. Kirk. 2004. Effect of timing of first clinical mastitis occurrence on lactational and reproductive performance of Holstein dairy cows. Animal reproduction science 80:31-45.
Schrick, F. N., M. E. Hockett, A. M. Saxton, M. J. Lewis, H. H. Dowlen, and S. P. Oliver. 2001. Influence of subclinical mastitis during early lactation on reproductive parameters. Journal of dairy science 84:1407-1412.

Table 1. Reproductive parameters associated with clinical mastitis (CM) events occurring before, between AI and pregnancy examination and after pregnancy examination

		Occurrence of CM Relative to Pregnancy Exam		
Parameter/Study	Control	BEFORE	BETWEEN	AFTER
Days to first AI				
Barker et al., 1998[1]	71.0±2.2[b]	93.6±5.6[a]	–	–
Schrick et al., 2001[2]	67.8±2.2[b]	77.3±2.7[a]	70.6±3.3[b]	–
Santos et al., 2004a[3]	64.0±1.4[ab]	68.0±1.9[b]	58.5±2.3[a]	62.3±2.6[ab]
Services per conception				
Barker et al., 1998[1]	1.7±0.1[b]	1.6±0.3[b]	2.9±0.3[a]	1.7±0.1[b]
Schrick et al., 2001[2]	1.6±0.2[b]	2.1±0.2[a]	3.0±0.2[c]	–
Santos et al., 2004a[3]	2.6±0.1[a]	2.6±0.1[a]	3.1±0.2[b]	2.5±0.2[a]
Ahmadzadeh et al., 2009[5]	1.6±0.1[a]	2.00±0.1[ab]	2.3±0.2[bc]	3.1±0.2[d]
Days open				
Barker et al., 1998[1]	92.1±4.6[b]	113.7±10.8[a]	136.6±13.3[a]	92.1±4.6[b]
Schrick et al., 2001[2]	85.4±5.8[b]	110.0±6.9[a]	143.6±8.5[c]	–
Santos et al., 2004a[3]	139.7±3.7[b]	165.0±5.7[c]	189.4±7.2[d]	118.4±6.4[a]
Ahmadzadeh et al., 2009[5]	88.0±2.0[a]	123.0±4.8[b]	141.0±5.5[c]	181.0±7.6[d]
Conception rate at first AI (%)				
Santos et al., 2004a[3]	28.7[a]	22.1[b]	10.2[c]	37.9[a]
Chebel et al., 2004[4]	24.0[a]	–	25.5[a]	–
Pregnancy rate at 320 DIM (%)				
Santos et al., 2004a[3]	85.4[a]	72.3[b]	58.5[c]	93.1[a]
Abortion incidence (%)				
Santos et al., 2004a[3]	5.8[a]	11.8[b]	11.6[b]	9.7[b]

[a-d] Means in a row with distinct letters differ ($P < 0.005$)

[1]Control (n=103), CM before AI (n=48), between AI and pregnancy examination (n=14), and after pregnancy examination (n=40). Study included 205 cows from 1 herd. For days to first AI, control, BET, and AFT categories were combined and compared to BEF.

[2] Control and AFT were grouped together. Control (n=326), CM before AI (n=374), and between AI and pregnancy examination (n=52). Study included 752 cows from 1 herd. For days to first AI d, services per conception and days open, control and AFT categories were combined to be compared to BEF and BET.

[3]Control (n=501), CM before AI (n=250), between AI and pregnancy examination (n=147), and after pregnancy examination (n=103). Study included 1001 cows from 2 herds.

[4]The impact of CM was assessed only between AI and pregnancy diagnosis. Study included 7,633 artificial inseminations from 3,161 cows in 2 herds.

[5]Control (n=572), CM before 56 d postpartum (n=91), from 56 to 105 d postpartum (n=64), and after 105 d postpartum (n=53). Study included 967 cows from 1 herd.

Table 2. Results of final models for associations of timing of subclinical (SM) or clinical mastitis (CM) during a defined breeding risk period (BRP; -3 to +32 days after 1st timed breeding) and etiology and severity of SM and CM on pregnancy per 1st AI (from Fuenzalida et al., in press)

	Number of Cows	Pregnancy per 1st AI	Odds of Pregnancy	P-value
Risk Group Relative to 1st Breeding				
Healthy	2,103	0.41	reference	reference
Mastitis before BRP	221	0.45	1.1	0.345
SM during BRP	271	0.34	0.8	0.031
Chronic SM	270	0.39	0.9	0.637
CM during BRP	207	0.32	0.7	0.012
Chronic CM	72	0.28	0.6	0.029
Association of etiology & severity				
Healthy	2,103	0.41	reference	reference
No Growth and mild	75	0.39	0.9	0.679
No Growth and moderate or severe	17	0.44	1.1	0.797
GP and GN and mild	59	0.33	0.7	0.245
GP and GN and moderate or severe	67	0.22	0.4	0.002