Effect of inflammatory response related to mastitis on dairy cattle reproduction

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Introduction

Mastitis is the inflammation of the mammary gland and is a common disease that affects dairy cattle health and wellbeing. Economic losses attributed to mastitis include lower milk production, increase in the amount of discarded milk, labor and medical costs, and premature culling (Fetrow, 2000). In addition to these costs, over the past two decades there has been mounting evidence that both clinical and subclinical mastitis reduce reproductive efficiency in dairy cattle (Kumar et al., 2017; Dahl et al., 2017). This paper discusses the effect of mastitis on dairy cattle reproduction from an inflammatory response basis of the mammary gland to infection.

Inflammatory response due to mastitis

Inflammation due to mastitis occurs when bacteria enter the mammary gland, multiply and produce toxins, enzymes, and cell-wall components, which stimulate production of various inflammatory mediators. The main impact of gram-negative bacteria is through lipopolysaccharides (LPS), a component of the cell wall (Sordillo and Babiuk, 1991). The phagocytizing activity of neutrophils and macrophages destroys the bacteria and releases LPS stimulating release of inflammatory mediators, particularly prostaglandin F2α (PGF2α). In addition, LPS and lipopolysaccharide binding protein binds to granulocytes and macrophages stimulating synthesis of pro-inflammatory cytokines, particularly tumor necrosis factor-α (TNF-α) and interleukins (IL-1α, IL-1β, IL-6, and IL-8); TNF-α and IL-1 are responsible for pyrogenic response to endotoxins (Danek and Zurek, 2014). Experimentally induced coliform mastitis through intramammary inoculation of *Klebsiella pneumonia* caused an increase of PGF2α in both milk and plasma (Cullor, 1990). Intravenous infusion of *E. coli* LPS increased PGF2α and decreased progesterone concentrations in plasma (Cullor, 1990). Intramammary infusion of *E. coli* LPS or *Streptococcus uberis*, elevated somatic cell count (SCC) in milk, and increased the activity of the inflammatory cytokines, TNF-α, IL-1β, and IL-8 in the milk, lymph, and circulation (Rainard and Paape, 1997; Hockett et al., 2000; Hoeben et al., 2000; Paape et al., 2002; Persson Waller et al., 2003; Rambeaud et al., 2003). Inflammatory response due to mastitis can also be related to elevated nitrous oxide (NO). Hansen et al. (2004) reported that intramammary infusion of LPS and *Streptococcus uberis* caused an increase in NO concentrations in blood or milk. Gram-positive bacteria can also produce toxins, extracellular enzymes, or enterotoxins that can cause a host inflammatory response including release of PGF2α and pro-inflammatory cytokines that result in pyrexia (Barker et al., 1998). Clinical mastitis induced via intramammary infusion of *Streptococcus uberis* suspension increased PGF2α concentration following oxytocin administration (Hockett et al., 2000).

Effect of mastitis on dairy cattle reproduction

Fertility

Poor fertility from the inflammatory and systemic response to clinical mastitis is related to an effect on the estrous cycle, impairment of oocyte maturation and development before ovulation, and delayed ovulation or anovulation. A study by Cullor (1990) indicated that mastitic cows have an altered interestrus interval (less than 18 days or more than 24 days), related to luteolysis as a function of PGF2α and/or pro-inflammatory cytokines. That is, PGF2α affects corpus luteum blood flow (Miyamoto and Shirasuna, 2009), and cytokines have extreme cytotoxic activity on the corpus luteum (Schams and Berisha, 2004).

Hansen et al. (2004) proposed a potential pathway by which infection of the mammary gland can reduce embryonic survival by modulating the hypothalamic–pituitary axis, ovary, oocyte and even the embryo. Endotoxins from gram-negative bacteria can disrupt luteinizing hormone (LH) secretion due to

induction of secretion of cytokines such as IFN-a as well as cortisol. The loss of LH support can potentially lead to anovulation, delayed ovulation or formation of follicular cysts, due to inhibition of the LH surge. Reduced LH secretion may also reduce progesterone secretion from the corpus luteum and thereby alter uterine function. In addition to apoptosis, TNF-a, disrupts the process of oocyte maturation. Cytokines can also cause increased secretion of other molecules that are disruptive to oocytes and embryos. In response to TNF- α and IL-1b, PGF2α is produced by several tissues including endometrium that can cause luteolysis and interfere with oocyte maturation. Elevated body temperature can also inhibit oocyte development before ovulation, processes in the oocyte occurring around the time of maturation (Hansen et al 2004).

Pregnancy survival

Early embryonic stages are sensitive to inflammatory responses. In rats, blastocysts have a receptor for TNF-α (Pampfer, 2001) which can cause apoptosis to embryo blastomeres leading to reduce inner cell mass number and affect embryo development (Hansen et al., 2004). Further, an in-vitro study reported that increased local concentrations of LPS, PGF2α, has a deleterious effect on oocyte function and NO on embryonic development (Soto et al., 2003).

Another possible pathway for pregnancy loss is that pregnancy survival is compromised by the effect of $PGF_2\alpha$ on the corpus luteum (CL) causing luteolysis and termination of pregnancy (Pate, 1994). This effect could be greater during late embryonic and early fetal development stages, as there is evidence that CL tissues from the luteal phase is different from that for the pregnant phase in the protein expression level (Chung et al., 2012). In a previous observational study, Risco el al. (1999) showed that cows affected with clinical mastitis during the first 45 d of gestation were 2.7 times as likely to experience pregnancy loss within the following 90d after diagnosis of mastitis, compared to cows not affected with clinical mastitis. Similarly, a recent matched case control study indicated that the odds of pregnancy loss in primiparous Holstein cows were 2.2 times higher in cows affected with clinical mastitis during early gestation, compared to cows without mastitis (Dahl et al., 2018, unpublished).

There is evidence that mastitis before or after breeding negatively impacts embryo survival. Circulating inflammatory mediators may reach the ovaries and affect follicular growth, and subsequently compromise oocyte and embryo development. Ribeiro et al. (2016) reported that the quality of embryos and the number of live embryos 1 to 5 or 6 d after breeding was reduced in cows exposed to clinical mastitis and other diseases before breeding. In addition, the uterine concentration of interferon tau (IFN-τ), was reduced in cows with clinical mastitis before breeding (Ribeiro et al., 2016). Interferon tau is involved in the pregnancy recognition process by stimulating gene expression in the endometritum necessary for embryo elongation and implantation (Brooks et al., 2014). In a recent matched case control study limited to multiparous Holstein cows with clinical mastitis within 42 days before breeding in parities four or later, were more likely to experience pregnancy loss, compared to cows without mastitis in parities two or three (OR = 3.25, or 2.69, respectively; Dahl et al., 2018 unpublished). Impact of mastitis and parity on embryo development and pregnancy may be exacerbated in older cows because of age-related changes in gene characteristic of bovine endometrial cells. Endometrial cells obtained from aged cows exhibited spontaneously higher levels of inflammatory signaling and dysfunction of cell division associated with chronic inflammation, that may lead to lower IFN-τ in older (> 150 months) cows compared to younger (28 to 68 months) cows (Tanikawa et al., 2017). In another study, (Hernandez et al., 2012), clinical mastitis during early lactation in combination with low body condition (BCS ≤ 2.75) at 70 DIM increased the risk of pregnancy loss in dairy cows. In that study, it was suggested that events of clinical mastitis during the first 10 to 50 days in milk can induce a prolonged negative energy balance in cows leading to losses of body weight and body condition days before or after insemination, and low body condition is a known predisposing factor for embryonic loss.

Reproductive performance

Adequate reproductive performance of the lactating herd is paramount to increase and sustain profitability in a dairy herd. Improved reproductive performance reduces the time from calving to

conception; thereby increasing the amount of milk produced per day of herd lifetime, reduces the number of cows culled for reproductive failure, and accelerates the rate of genetic progress, which collectively increase herd income (Risco et al, 1998). Observational studies indicate that clinical mastitis lowers reproductive performance from an increase in the number of days to first service, days non-pregnant, and services per conception (Moore et al., 1991; Barker et al., 1998). These changes can be related to the effect of clinical mastitis on the estrous cycle, CL function, oocyte maturation, delayed ovulation or anovulation, and embryonic survival.

Influence of pathogen causing mastitis

The type of pathogen causing mastitis has a similar effect on fertility in dairy cattle. Barker et al. (1998) reported that services per conception did not differ among cows with clinical mastitis caused by gram positive or negative pathogens. In agreement with this finding, other studies have shown that gram positive or negative bacteria had a similar effect on conception (Schrick et al., 2001; Santos et al., 2004). Similarly, Risco et al. (1999) reported no difference in abortion among bacteria types. The implication is that the inflammatory response elicited by gram-positive bacteria is similar to that of gram-negative bacteria. However, Huszenicza et al. (1998) reported significantly higher rate of premature luteolysis in mastitis induced by gram negative compared to gram positive bacteria. However, the occurrence of mastitis and type of pathogen in relation to time of breeding affects conception rate. Hertl et al. (2010) found that the occurrence of clinical mastitis between 14 days before artificial insemination (AI) or 7 days after AI, resulted in a significant effect on probability of conception and was affected by pathogen type. Mastitis due to gram-negative bacteria occurring between 8 and 14 days before AI was associated with a 32% reduction in conception rate, while mastitis caused by gram- positive and gram-negative bacteria from 1 to 7 days before AI was associated with a 50% reduction in probability of conception. Similarly, all types of clinical mastitis occurring from 0 to 7 days after AI were associated with reduced conception rates. Gram positive bacteria reduced the probability of conception by 47%, while gram negative reduced it up to 80%.

Effect of subclinical versus clinical mastitis

From the studies mentioned above, the systemic inflammatory response due to clinical mastitis is well known. In contrast, there has been a paucity of research on the systemic response from inflammation in cows with subclinical mastitis. This lack of information, may lead to the belief that the subclinical form of mastitis may not adversely affect fertility. However, various studies have indicated that subclinical mastitis does have an impact on fertility. Subclinical mastitis, defined as the presence of the same pathogen in at least two consecutive milk samples, with an elevated somatic cell count (SCC), resulted in lower reproductive performance of lactating cows, and was comparable to cows with clinical mastitis (Schrick et al, 2001). Furthermore, a negative effect of a linear SCC $\geq$ 4.5 within 30 days before AI on embryo survival was reported (Moore et al, 2005). Pinedo et al. (2009) evaluated the effect of subclinical mastitis (LNSCC $\geq$4.5) during early lactation on reproductive performance, and estimated their association with the risk of abortion in a population of central-southern Chilean dairy cattle. In that study, time to first breeding was 21.8 days longer in cows with at least one high LNSCC before the first breeding compared to controls. Cows with at least one high LNSCC before the fertile breeding had an increment in time to conception of 48.7 days and required on average 0.49 more services to conceive. The odds of conception at first service in cows with a high LNSCC within 30 d before or after breeding were 0.85 (0.81 to 0.89; 95% confidence interval) [0.82 (0.78 to 0.87; 95% confidence interval)] times the odds of conception for cows without a high LNSCC during that period. Cows registering a high LNSCC during the first 90 d of gestation had an increased risk of abortion. In another study, Lavon et al. (2010) reported that that subclinical, long-term mastitis and short-term clinical events induced delayed ovulation in about 30% of the cows. This finding was associated with low estradiol concentrations that subsequently induced low or delayed or no LH surge, without any change in pulsatile LH secretion, suggesting that mastitis directly affected follicular functioning.

Conclusion

Inflammation associated with clinical mastitis negatively affects reproductive performance in dairy cattle. In particular, LPS and PGF2α can have deleterious consequences on oocyte function, inflammatory cytokines like TNF-α and NO can disrupt embryonic development. Although, subclinical mastitis also impairs reproductive performance, its deleterious effect on fertility is yet to be determined. Consequently, veterinarians engaged in reproductive management of dairy cattle, should ensure that mastitis control practices are optimal in order to achieve a high level of reproductive performance.

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