The disruptive effects of mastitis on reproduction and fertility in dairy cows

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Abstract

Mastitis (intramammary infection) causes the deterioration of ovarian follicular responses in cows, resulting in low fertility. The short-term, acute clinical form of mastitis has a time-dependent disruptive effect on conception rate. It effectively lowers conception rate if events occur mainly 10 days before to 30 days after artificial insemination. Long-term subclinical mastitis is widely spread in commercial herds. Although it is less severe than clinical mastitis, its long-term nature causes a more pronounced decrease in conception rate. Even mild elevation of somatic cell count in subclinical cows significantly lowers conception rate. Disrupted follicular responses include depression of steroid production in the preovulatory follicle associated with low and delayed preovulatory luteinizing hormone surge, resulting in delayed ovulation in one-third of subclinical cows. Mastitis, clinical and subclinical, also impairs oocyte competence, reflected in low production of blastocysts. The corpus luteum seems to be insensitive to mastitis, possible due to the use of non-steroidal anti-inflammatory drugs when mastitis is first diagnosed.

Introduction

Mastitis is a widespread disease in dairy cattle. Intramammary infection (IMI) results in loss of milk production, increased use of veterinary drugs and higher culling rates (Halasa et al., 2007). The economic impact of mastitis on reproduction failure has only gained attention in the last decade. The IMI-induced reduction of fertility causes significant losses to dairy farms. This review deals with the impairment of reproduction by mastitis, with an emphasis on the ovarian response to IMI. This review does not discuss the mastitis-associated immune responses that are involved in impairment of reproduction. The short-term, acute immune responses associated with clinical mastitis and their effects on reproduction have been discussed previously (Bornstein et al., 2004; Sakamoto and Okuda, 2004; Malinowski and Gajewski, 2010). It does, however, address shortly the poorly documented immune responses associated with long-term, subclinical mastitis with respect to their involvement in disruption of reproduction.

Clinical mastitis is an acute short-term event caused mainly by Gram-negative (G-) bacteria, in particular Escherichia coli. It is characterized by systemic signs such as fever and others, mammary gland inflammation and a sharp rise in somatic cell count (SCC) in the milk. The IMI triggers a complex acute-phase response that includes increased secretion of inflammatory proteins, cytokines, prostaglandins and more, which can be detected in the milk and plasma (Shuster et al., 1991; Eckersall et al., 2001). Subclinical mastitis, caused mainly by Gram-positive (G+) bacteria (Staphylococcus aureus, coagulase-negative staphylococci, streptococci and more), is more common than clinical mastitis. It is widespread, found in 20% and more of cows in commercial herds worldwide. In subclinical mastitis elevates of SCC are moderate, with no detectable signs of local or systemic inflammation. Subclinical mastitis may persist for several months and years. Although the long-term effects of subclinical mastitis decrease fertility, its negative impact on reproduction has been poorly documented and is discussed in the current review.

Epidemiological studies

Studies performed in the last 15 years have indicated a negative effect of mastitis on cow fertility (Schrick et al., 2001; Santos et al., 2004). Its effect on conception rate is controversial, particularly with regard to the timing of the mastitis-occurring either before or after artificial insemination (AI), whether it is clinical or subclinical, and whether the IMI-causing bacteria are G- or G+. Clinical mastitis after AI is associated with low conception rate (Konig et al., 2006), regardless of whether the IMI-induced bacteria are G- or G+ (Schrick et al., 2001; Santos et al., 2004). The effect of IMI before AI on reproduction is controversial. Some studies show no effect on conception rates (Klaas et al., 2004). Others suggest that high SCC before AI has some effect on non-return rate (Miller et al., 2001). In another study, Schrick et al. (2001) suggested that cows with clinical or subclinical mastitis before first AI have a higher number of days open, and that reproductive performance does not differ between pathogen types. In agreement, Santos et al. (2004) showed that conception rates are lowered by clinical mastitis occurring before AI, caused by either G+ or G- bacteria. Konig et al. (2006) showed that elevated SCC during the entire lactation lowers pregnancy rate.

Our group performed a large epidemiological study on the effects of mastitis, determined by the pattern and level of the SCC around first AI, on probability of conception (Lavon et al., 2011a). Data from 287,000 AI were obtained from the Israeli Herd Book. Analyses examined the association of probability of conception with SCC elevation relative to timing of AI. A SCC cutoff of 150,000 cell/mL milk was set to distinguish between uninfected and mastitic cows. Accordingly, cows with high SCC before and after AI were designated chronic, likely subclinical, mastitic cows. Compared with uninfected cows, the subclinical subgroup showed reduced conception rate (39.4 and 31.5%, respectively). Among the chronic, subclinical group, the reduction in probability of conception was related to the degree of SCC elevation: probability of conception was lowered by 14.3% in the subgroups with mildly elevated SCC (between 150,000 and 450,000 cell/mL milk).
cells/mL milk) and moderately elevated SCC (between 450,000 and 10^6 cells/mL milk), and by 20.5% in cows exhibiting high SCC (above 10^6 cells/mL milk) elevation, relative to the uninfected group. Additional analysis in that study was related to clinical mastitis: it lowered the probability of conception by 24% when it occurred during the 10 days prior to AI, but not when it occurred earlier. When clinical mastitis occurred within a period of 30 days after AI, probability of conception was lowered by 23%. Similar findings were also reported by Hertl et al. (2010).

The corpus luteum

Mastitic induction of early regression of the corpus luteum (CL) post-AI can potentially lead to termination of pregnancy. However, evidence of reduced progesterone secretion following mastitis is controversial. Mastitis-induced increases in PGF_2α, and possibly TNFα, have been related to CL regression in several studies (Malinowski and Gajewski, 2010). However, evidence of impaired luteal function in mastitic cows is sketchy, mostly due to different approaches, to induction of IMI by G- or G+ toxin, and to a determination of the effects in clinical or subclinical mastitis.

Corpus luteum function in induced mastitis

Intravenous infusion of E. coli lipopolysaccharide (LPS) decreased progesterone concentration in pregnant cows (Giri et al., 1990). Another study showed that LPS causes a decline in CL size and a decrease in plasma progesterone level (Herzog et al., 2012). In contrast, intramammary administration of LPS at the onset of estrus did not cause early luteal regression and did not induce decline of progesterone concentrations (Lavon et al., 2008). The effect of G+ bacteria on luteal function is also debatable. Inoculation of the mammary gland with Streptococcus uberis did not change progesterone level (Hockett et al., 2005). In sheep, however, administration of G+ toxin of Streptococcus origin decreased progesterone concentration (Stewart et al., 2003; Dow et al., 2010). Collectively, these studies are inconclusive with respect to the effect of induced pathogenic stress on CL function.

Corpus luteum function in naturally occurring mastitis

Mastitic cows with G- isolates were more likely to have altered inter-estrus interval (Moore et al., 1991). A study in Hungary showed a shorter luteal phase due to a higher rate of premature luteolysis induced by G- isolates (Huszenicza et al., 2005). The latter contrast with a study conducted in Israel in which progesterone concentrations and CL volume in cows with clinical events occurring a few weeks earlier, or subclinical mastitis, did not differ from uninfected cows (Lavon et al., 2010). In agreement, a study conducted in England showed that lame cows exhibiting high SCC do not have altered progesterone concentrations (Morris et al., 2013).

Our group recently examined the effect of E. coli mastitis on inter-estrus interval (Shaani et al., 2012). We found that progesterone level following a clinical mastitic event in cyclic, inseminated or pregnant cows does not differ from that in uninfected controls, and none of the cows showed early progesterone decline. Thus, clinical mastitic events were not associated with early luteal regression. In a second experiment (Shaani et al., 2012), events of clinical E. coli mastitis were examined. Timing of the event and of oestrus around the time of detection of mastitis was determined by online electronic tag (Afikim, Israel) charts. We found that only 11 out of 201 cows (5.5%) exhibited estrus less than 8 days after the event, (serving as indication for a possible association between mastitis event and luteal regression). Results of the two experiments indicated a minimal association between E. coli mastitis and shortened inter-estrus interval. It should be noted that all mastitic cows received a non-steroidal anti-inflammatory treatment at the time of diagnosis. This might have attenuated or even blocked prostaglandin release, resulting in maintenance of the CL. The small, almost non-existent effect of clinical events on premature luteal regression suggests that the cause of the low fertility associated with clinical mastitis post-AI lies with the embryo and not the CL.

Follicular function: ovulation

As with the effect of IMI on the CL, distinguishing between clinical and subclinical mastitis is important. For instance, induction of clinical mastitis before AI during the follicular phase caused a significant reduction of pulsatile luteinizing hormone (LH) secretion, subsequently inducing low secretion of estradiol close to estrus, and delayed LH surge and ovulation (Hockett et al., 2005; Lavon et al., 2008). However, in those studies, an acute, short-term, experimental pathogenetic stress was induced by intramammary administration of endotoxin or inoculation with bacteria. Thus, the studies did not simulate cases of subclinical, long-term mastitis that last months or even years.

Recently, our group examined the effects of subclinical mastitis on timing of ovulation (Lavon et al., 2010). Naturally occurring subclinical mastitis was examined in lactating cows. It was found that 30% of subclinical mastitic cows exhibit an extended estrus to ovulation (E-O) interval of 56 h, compared with 28.5 h in healthy cows and the remaining 70% of mastitic cows. We also found that in mastitic cows with delayed ovulation, plasma estradiol concentration close to estrus was about 50% lower than that of controls. Importantly, the latter finding did not involve changes in pulsatile LH secretion, as had been documented for clinical events (Suzuiki et al., 2001).

Other differences between the responses of cows to clinical and subclinical mastitic were documented. Clinical mastitis was associated with activation of the glucocorticoid system, resulting in a sharp rise of systemic cortisol, known to be involved in depression of gonadotropin-releasing hormone (GnRH) and LH secretion. However, cortisol concentrations did not differ between cows with subclinical mastitis and their uninfected counterparts (Lavon et al., 2010), which might explain the lack of change in pulsatile LH in subclinical mastitic cows. However, unlike pulsatile LH secretion, the preovulatory LH surge in the 30% of subclinical mastitic cows with delayed ovulation was lower, delayed, or with no surge noted, compared to the normal LH surge exhibited in uninfected cows and the remaining 70% of IMI cows. Collectively, these results showed that about one-third of the cows with subclinical, long-term mastitis exhibit delayed ovulation that is caused by low secretion of estradiol and a low or delayed preovulatory LH surge. Low estradiol in the circulation close to estrus is associated with disruption of its positive effect on GnRH secretion, consequently leading to disruption of normal secretion of the preovulatory LH surge.

Follicular function: steroids

In a follow-up study, we examined the preovulatory follicles of a different group of cows with subclinical mastitis (Lavon et al., 2011b). Follicular fluid was collected and granulosa and theca cells from the preovulatory follicles were isolated. We found a clear diversity in responses among the subclinical mastitic cows: one-third of them exhibited low follicular...
estradiol and androstenedione concentrations, and the other two-thirds had normal steroid levels, similar to those of uninfected cows (P<0.01). It is important to note that the one-third vs. two-third proportions of sensitive vs. non-sensitive subclinical cows documented in that study was found in an independent set of cows, but was nevertheless similar to the 30:70 proportion of cows exhibiting delayed and normal ovulation, respectively, in our earlier study (Lavon et al., 2010). Reduced follicular concentrations of estradiol in the granulosa cells and of androstenedione (serving as a substrate for estradiol formation) in the theca cells were associated with reduced expression (P<0.05) of major steroidogenic enzymes and the LH receptor LHCR in theca and granulosa cells, CYP11A1 (P450-side-chain cleavage) and CYP17A1 (17α-hydroxylase) in theca cells, and CYP19A1 (aromatase) in granulosa cells; this may have contributed to the lower follicular steroid production in one-third of the IMI cows. To further validate these findings in naturally occurring IMI cows, exhibit subclinical infection, we induced subclinical mastitis experimentally and examined its effects on follicular responses (Furman et al., 2014). Cows were synchronized and received very small intramammary doses of G+ toxin or saline every 48 h for 20 days. During the 3 weeks of induced subclinical mastitis, no local or systemic inflammatory signs were detected, except for a rise in SSC. About 40% of the G+ treated cows exhibited reduced follicular steroid concentrations. This proportion was close to the earlier described proportion of one-third susceptible cows detected in naturally occurring subclinical mastitis (Lavon et al., 2010, 2011b). The main finding was a carry-over decrease, by about 40%, in follicular estradiol concentration in the G+ induced mastitic cows relative to controls (P<0.05). It was also found that induced subclinical mastitis causes depression of follicular growth, in agreement with a previous study performed by Rahman et al. (2012). Calculations based on the current experimental model indicated that subclinical mastitis induced by G+ toxin disrupts follicular functions of the ovarian pool of small, early antral follicles, subsequently reflected in low steroid production weeks later by the developing, large preovulatory follicle.

Diversity in responses

The above studies present a large diversity in follicular responses to the infection among individual cows, with proportions of about 30:70 sensitive to non-sensitive subclinical mastitic cows (Lavon et al., 2010, 2011b; Furman et al., 2014). Interestingly, other studies have shown similar diversity in responses among individual cows and sheep. Exposure to various stressors, such as bacterial inoculation, LPS administration, cortisol-induced stress and more have shown differences among individual responses (Adams et al., 1999; Battaglia et al., 2000; Hockett et al., 2005; Jacobsen et al., 2005; Bellingham et al., 2012). Unfortunately, none of those studies provided any explanation for the large differences in responses among individuals.

Follicular function: oocyte competence

Inflammatory agents such as PGF2α, added to the culture medium during oocyte maturation reduce the percentage of blastocyst formation (Soto et al., 2003b). Similarly, tumor necrosis factor (TNFα) added during maturation reduces the proportion of blastocysts formed (Soto et al., 2003a). Addition of TNFα after the oocyte was fertilized caused an increase in the number of blastomeres that underwent apoptosis (Hansen et al., 2004). Studies in mice found that addition of TNFα decreases cell number in the inner cell mass (Pampfer et al., 1994; Wuu et al., 1999). Another approach used follicular fluid from mastitic cows as oocyte maturation medium. Follicular fluid from G+ or G+ toxin-induced mastitic cows reduced the rates of cleavage and blastocyst formation (Asal et al., 2014).

In another study, oocytes from mastitic cows were collected for in vitro embryo production. Culled cows were diagnosed with mastitis based on SCC records and bacteriological analyses for the 3 months prior to oocyte collection after slaughter (Roth et al., 2013). Blastocyst rate was markedly lower in cows with medium and high SCC relative to the low-SCC (control) cows. That study provided first evidence of mastitis-induced impairment of oocyte competence in the pool of oocytes at the germinal vesicle stage (Roth et al., 2013). It indicates that follicles at earlier stages of development are susceptible to mastitis, for reasons unclear at present. Interestingly, others indicated impairment of function of small and less differentiated follicles that were associated with various inflammatory agents (Spicer and Alpizar, 1994; Shimizu et al., 2012; Bromfield and Sheldon, 2013). A summary of the effects of subclinical mastitis on alterations of reproductive processes in cows is presented in Table 1.

| Tissue/gland            | Biological action                     | Subclinical mastitis effect                                                                 |
|------------------------|--------------------------------------|-------------------------------------------------------------------------------------------|
| Pituitary              | Pulsatile LH                         | Unaltered                                                                                 |
| Adrenal                | Preovulatory LH surge                 | Low and delayed in 1/3 of cows                                                           |
| Follicles-preovulatory | Cortisol                             | Unaltered                                                                                 |
|                        | Ovulation                            | Delayed in 1/3 of cows                                                                    |
|                        | Follicular steroids                   | Low androstenedione and estradiol in 1/3 of cows                                          |
|                        | Gene expression                      | Low LHr and steroidogenic genes in thecal and granulosa cells                              |
| Follicular growth      | Medium size (6-9 mm)                 | Decreased in induced mastitis                                                             |
|                        |                                      | Increased in natural mastitis                                                             |
| Corpus luteum          | Progesterone and early luteal regression | Unaltered                                                                                 |
| Oocytes                | Matured with follicular fluid of mastitic cows before maturation | Low blastocyst formation, impaired maturation, increased apoptosis; low GDF9                |
|                        | Obtained from mastitic cows before maturation | Impaired competence of GV-stage oocytes, low blastocyst formation                          |

LH, luteinizing hormone; LHr, luteinizing hormone receptor; GV, germinal vesicle.
Conclusions

Mastitis in its subclinical form disrupts the functioning of the preovulatory follicle, resulting in low fertility. A major cause of this disruption is delayed ovulation associated with low follicular steroid production in about one-third of subclinical mastitic cows; the remaining two-thirds respond normally. The reason for the large response in mastitic cows is unclear. Mastitis also disrupts the developmental competence of the ovarian pool of oocytes at the germinal vesicle stage.

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