Effects of mastitis on ovarian function and fertility in dairy cows

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Summary

Mastitis has a deleterious effect on reproductive responses and fertility of dairy cows, which depends on whether it occurs before or after artificial insemination (AI). Subclinical intramammary infection (IMI) before AI reduced steroid concentrations in the preovulatory follicle of approximately one third of lactating cows, and was associated with low expression of major steroidogenic genes. Consequently, IMI induced an attenuated LH surge and delayed ovulation in 30% of cows with subclinical IMI; the remaining 70% exhibited normal responses. The reason for the diversity in reproductive responses of individual cows to subclinical IMI is unclear. Mastitis induced by Gram-negative or Gram-positive bacteria disrupted the developmental competence of the pool of oocytes at the germinal vesicle stage, resulting in low blastocyst rates. The specific immune/inflammatory molecules involved in impairment of reproductive responses in subclinical mastitic cows are poorly documented. Exposure of small antral follicles to subclinical mastitis induced by Gram-positive bacterial toxins had a long-term effect by reducing estradiol concentrations of preovulatory follicles. Unlike chronic subclinical mastitis, the disruptive effect of short-term clinical IMI before AI is time-dependent and involves lowered conception when IMI occurs close to the time of insemination. The effect of clinical intramammary infection on corpus luteum function is equivocal. In a recent study, inter-estrus interval and progesterone concentration were unaltered in most (95%) E. coli-mastitic cows treated with anti-inflammatory drugs. Fertility studies showed that fertility of subclinical mastitic cows is improved by the Ovsynch program, probably because of synchronized timing of ovulation relative to AI in cows that could otherwise exhibit delayed ovulation.

Introduction

Bovine mastitis or intra-mammary infection (IMI) is highly prevalent in dairy cattle, with about 20–40% of the lactating cows infected with bacteria, mainly in a subclinical form. Traditionally, mastitis is related to reduced milk production and quality, increased veterinary costs, risk of culling and occurrence of other diseases (Halasa et al. 2007). However, in the last decade, attention has been drawn to the disruptive effect of mastitis on reproduction and fertility. Clinical mastitis is an acute short-term (on the order of days) event caused mainly by Gram-negative (G-) bacteria, in particular Escherichia coli. It is characterized by systemic and local signs of 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 immune responses such as increased secretion of inflammatory proteins, cytokines, prostaglandins and other elements, which are detected in milk and serum (Shuster et al. 1991, Eckersall et al. 2001). Subclinical mastitis, caused mainly by Gram-positive (G+) bacteria (Staphylococcus aureus, coagulase negative staphylococci and streptococci), is more common than clinical mastitis. It is a long-term chronic disease characterized by a moderate rise in SCC, with no apparent signs of local inflammation or systemic involvement. Infection may persist for several months or throughout lactation. Although long-term effects of subclinical mastitis decrease fertility, its negative impact on reproduction has been poorly documented and is discussed in detail herein.

The current review concentrates on the effect of clinical and subclinical, natural or induced mastitis on the reproduction of dairy cows, and describes ovarian responses and briefly discusses gonadotropin secretion and timing of ovulation. Immune or uterine responses to IMI and effects of lipopolysaccharide (LPS) or tumor necrosis factor alpha (TNFα) on follicular thecal and granulosa cells are beyond the scope of this review, and are discussed elsewhere (Terranova 1997, Bornstein et al. 2004, Sakumoto & Okuda 2004, Malinowski & Gajewski 2010).

The review comprises five sections: (1) epidemiological studies of the effect of mastitis on fertility; (2) effects of naturally occurring and induced mastitis, mainly subclinical, on follicular functions. This major section of the review includes IMI effects on timing of ovulation, follicular steroids and growth dynamics; (3) effects of mastitis on oocyte quality and early embryonic development; (4) impact of IMI on luteal function; (5) approaches to improving fertility of subclinical mastitic cows.

**Mastitis and fertility**

Although mastitis has a negative impact on reproductive performance, epidemiological studies are controversial, in particular with regard to the timing of the disease (before or after AI), involvement of clinical or subclinical IMI, and whether the responsible isolates are G+ or G- bacteria.

Clinical mastitis after AI is associated with low conception rate (CR) and more services per conception (Loeffler et al. 1999, Konig et al. 2006). Other studies show a negative effect of chronic SCC elevation (likely subclinical mastitis) after AI on conception (Konig et al. 2006, Pinedo et al. 2009). The effect of IMI before AI is controversial. A study by Klaas et al. (2004) claims that there is no effect on CR or days open. Others have reported that high SCC before AI has a minimal effect on the non-return rate (Miller et al. 2001). In contrast, Schrick et al. (2001) indicated that clinical and subclinical mastitis before AI increase days open and services per conception, irrespective of pathogen type. Supporting this, Santos et al. (2004) showed that CR is depressed by clinical mastitis caused by either G+ or G- bacteria occurring prior to AI. Reasons for the differences among studies could result from differences in cut-off values of SCC between healthy and infected cows, or timing of IMI relative to AI.

A recent study conducted in Israel based on 287,000 AIs examined the association of probability of conception with SCC elevation around AI (Lavon et al. 2011a). Cured and newly infected subgroups (likely clinical IMI cows) had lower CRs than uninfected cows, but the chronic (likely subclinical IMI) subgroup showed the largest reduction in CR (Fig. 1) and the magnitude of the reduction was related to SCC elevation. A single high elevation of SCC represented a clinical event of mastitis and a lower probability of conception if it occurred 30 days after AI or 10 days prior to AI, but not when it occurred more than 10 days before AI (Lavon et al. 2011a). The latter finding is supported by the physiological studies described
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Further on. A similar negative effect on probability of conception due to clinical mastitic events was documented in the US by Hertl et al. (2010). In the UK, Hudson et al. (2012) showed that clinical and subclinical IMI occurring before or after AI reduce reproductive performance. Another study in the US showed that the negative impact on fertility is exacerbated when cows experience both clinical mastitis and other diseases (Ahmadzadeh et al. 2009).

Follicular function

Timing of ovulation

Clinical mastitis. A variety of stressors can disrupt the timing of ovulation and lower the probability of successful fertilization. Stress induction during specific stages of the cycle determines specific response impairments. Intrauterine or i.v. administration of an acute dose of LPS during the follicular phase suppressed primarily pulsatile LH secretion, which was further associated with delayed or blocked preovulatory LH surge and ovulation in sheep and cows (Peter et al. 1989, Battaglia et al. 2000, Suzuki et al., 2001, Daniel et al. 2003). Intramammary challenge with Streptococcus uberis during the luteal phase (Hockett et al. 2005) caused acute clinical mastitis that was associated with inhibition of ovulation. Several in vitro studies have shown that transiently elevated LPS or TNFα during the acute phase of clinical mastitic events decreases follicular estradiol production by granulosa cells (Spicer 1998, Sakamoto et al. 2003, Herath et al. 2007, Bromfield & Sheldon 2011, Shimizu et al. 2012). Those studies indicated that acute mastitis occurring before estrus may directly suppress estradiol production, independent of any change in pulsatile LH, resulting in inhibition of estrus and ovulation. Interestingly, intramammary or i.v. administration of an acute dose of LPS at the onset of estrus did not alter pulsatile LH or estradiol concentrations, but lowered and delayed LH surge and ovulation (Battaglia et al. 2000; Lavon et al. 2008). Similarly, sheep studies showed the importance of the exact time of exposure to LPS prior to LH surge in obtaining delayed ovulation syndrome (Breen et al. 2004). Collectively, the above studies indicate that an acute clinical event may effectively alter the timing of ovulation or block it entirely, provided that it is induced close to estrus and potential ovulation. These studies and several others only mimicked acute clinical events associated with induction of short-term acute-phase mastitis.

Whereas LPS is released into circulation and can be detected in the follicular fluid in postpartum uterine infection (Herath et al. 2007), this does not occur in clinical mastitis. A study...
by Mehrzad et al. (2007) showed that LPS is not detected in the plasma of most cows exhibiting acute clinical mastitis, with the exception of one cow that had severe clinical per-acute IMI. In agreement, another study only detected LPS in the circulation during mostly severe gangrenous mastitis (Hakogi et al. 1989). As most pathogenic bacteria causing subclinical IMI are G+, LPS is not likely to be detected in the circulation of subclinical mastitic cows. Therefore, the site of LPS administration is important; intramammary infusion of endotoxin more closely mimics clinical mastitis than i.v. or intrauterine administration or adding LPS to culture systems.

Subclinical mastitis. Although fertility studies have revealed that subclinical IMI is as harmful to reproduction as clinical IMI, supportive studies are rare and the involved inflammatory responses are unclear. A UK study showed a synergistic effect of lameness and high SCC (>100,000 cell/mL) in reducing ovulation rate; however, cows with only a high SCC ovulated normally (Morris et al. 2009). In contrast, a study carried out in Israel (Lavon et al. 2010) showed that about 30% of subclinical mastitic cows (based on a SCC cutoff of >200,000 cell/mL and bacterial diagnosis) manifested an extended estrus-to-ovulation interval of 56 h vs. 28 h in uninfected cows (Fig. 2). Estradiol concentration was lower in subclinical mastitic cows, likely leading to low, delayed or no preovulatory LH surge in the cows exhibiting delayed ovulation. The decline in estradiol was not associated with any change in pulsatile LH concentration, in contrast to acute events that were associated with depressed pulsatile LH secretion (Suzuki et al. 2001) and high circulating cortisol concentrations (Hockett et al. 2005). However, activation of the hypothalamic–pituitary–adrenal axis reflected by cortisol elevation during the acute phase (Lavon et al. 2008) was not evident in subclinical or past clinical mastitic cows (Lavon et al. 2010). Collectively, this shows that low estradiol in the circulation of 30% of subclinical IMI cows is due to one or more as yet unidentified inflammatory mediators capable of disrupting follicular steroidogenesis. Consequently, it is suggested that the stimulatory effect of estradiol on GnRH induction of LH surge secretion was probably depressed, leading to low, delayed or no LH surge and resulting in delayed ovulation (Lavon et al. 2010).

![Fig. 2. Estrus-to-ovulation interval and percentage (%) of cows exhibiting normal and extended interval.](image)

Follicular steroid production

Naturally occurring subclinical mastitis. Only a few studies have examined the effects of subclinical mastitis on follicular responses. Lavon et al. (2011b) found a similar proportion
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of cows with delayed ovulation as in a previously described study (Lavon et al. 2010), with approximately one third of subclinical mastitic cows exhibiting low follicular fluid estradiol and androstenedione concentrations (Fig. 3B; 9/28 cows). These results corresponded with reduced expression of major genes associated with steroidogenesis: LHCGR in theca and granulosa cells, CYP11A1 and CYP17A1 in theca cells, and CYP19A1 in granulosa cells (Lavon et al. 2011b; Fig. 3A). Interestingly, expressions of STAR and HSD3B1 in theca cells and FSHR in granulosa cells were not affected by the subclinical mastitis (data not shown). However, the IMI events hardly affected follicular steroids when clinical mastitis occurred ca. 30 days prior to the study. The reason for the differential responses of the steroidogenic genes to IMI remains unclear.

Fig. 3. (A) Expression of mRNA for gonadotropin receptor and steroidogenic genes in theca and granulosa cells collected from preovulatory follicles. RNA was extracted from uninfected and subclinical mastitic cows exhibiting normal or low estradiol in the follicular fluid. LHCGR, LH receptor; CYP19A1, cytochrome P450 aromatase; CYP11A1, cytochrome P450 side-chain cleavage; CYP17A1, cytochrome P450 17-α-hydroxylase. (B) Concentrations of androstenedione in the follicular fluid of uninfected and subclinical mastitic cows exhibited normal or low estradiol concentrations in the follicular fluid.

Induced mastitis. The possible carryover effect of a clinical event on follicular responses (i.e., weeks after treatment) is of particular importance because clinical IMI before insemination lowers reproductive performance (Schrick et al. 2001, Santos et al. 2004, Pinedo et al. 2009). Intramammary administration of a single high dose of G- endotoxin (E. coli LPS) or G+ toxin (of Staphylococcus aureus origin) enabled us to examine the immediate and carryover effects of mastitis. As expected, G- LPS caused an immediate and short-term decrease in follicular steroid concentrations (Lavon et al. 2011c), emphasizing that fertility disruption by a clinical event depends strictly on its timing relative to the occurrence of specific reproductive processes (e.g. ovulation, as discussed earlier). However, the G+ toxin induced immediate and carryover (up
to 3 weeks after injection) decreases in preovulatory follicular steroids. This indicates a long-term effect of mastitis on reproduction (discussed in more detail later further on).

A single intramammary injection of *E. coli* LPS appears to be an acceptable model for clinical mastitis. An experimental model was designed for induction of subclinical mastitis over a period of 3 weeks to more closely study subclinical mastitis induced by G+ or G- toxin (Furman et al. 2012). Intramammary injections of small doses of G- or G+ toxin every 48 h for 20 days did not cause local signs of inflammation or systemic symptoms; body temperature, plasma cortisol and haptoglobin concentrations did not differ from those of non-treated controls. Similar to naturally occurring subclinical mastitis (Lavon et al. 2011b), diverse responses were recorded in a study in which cows had induced mastitis; about 40% of cows induced by G+ and 80% of cows induced by G- were defined as ‘affected’ by the toxin. Both toxins caused a 40 to 50% carryover decrease (without any immediate reduction in follicular estradiol concentrations) 16 days after mastitis induction ended (Furman et al. 2012). It was clearly shown that small antral follicles of cows with induced subclinical mastitis for 3 weeks were affected and carryover damage was evidenced by lower estradiol concentrations in the preovulatory follicle. Another interesting finding was the similarity in follicular reduction of estradiol concentration induced by the two toxins, even though the innate immune system recognizes G+ and G- bacteria mainly through different toll-like receptors (TLR2 and TLR4, respectively) with different intracellular signaling pathways (Takeuchi et al. 1999).

**Diversity in responses.** The above studies of natural and induced mastitis present a large diversity in the follicular responses to the disease, with proportions of about 30:70 sensitive to non-sensitive subclinical mastitic cows (Lavon et al. 2010, 2011b, Furman et al. 2012). Interestingly, other studies have shown similar occurrences in cows and sheep. Exposure to various stressors, such as bacterial inoculation, LPS administration, cortisol-induced stress and sewage chemicals have shown differences among individual responses (Adams et al. 1999, Battaglia et al. 2000, Breen & Karsch 2004, Hockett et al. 2005, Jacobsen et al. 2005, Bellingham et al. 2012). Variation also resides in the genetic differences among cows (Rupp & Boichard 2003). This notion is supported by a study showing that mammary epithelial cells presenting the Q allele on autosome 18 have a stronger and quicker reaction to *E. coli* or *S. aureus* exposure than cells with the q allele (Brand et al. 2011).

**Follicular growth**

The effect of subclinical mastitis on follicular growth is equivocal. It seems to depend on the severity of the IMI. Chronic mastitis in severely affected cows decreased the number of secondary preantral follicles, as well as the number of follicles that were >8 mm (Rahman et al. 2012). These findings were associated with a decreased density of blood vessels and decreased expression of growth differentiation factor 9 in the enclosed oocytes, known to be essential regulatory elements of follicular growth. These findings are in close agreement with our studies in which subclinical mastitis was induced by administering a single high dose of G+ toxin (Lavon et al. 2011c) or several small doses of G+ toxin over 3 weeks (Furman et al. 2012). Both toxin treatments caused a decrease in the number of medium-size follicles (6–9 mm, Fig. 4). Interestingly, naturally occurring subclinical mastitis caused an increase in the number of medium-size follicles that may be associated with depressed dominance (Lavon et al. 2010). The growth and size of the preovulatory follicle was not affected by either induced or naturally occurring mastitis (Lavon et al. 2010, 2011b, 2011c, Furman et al. 2012). It is worth noting that the latter finding is in contrast to the decline in size and growth of the dominant follicle recorded in cows exhibiting postpartum uterine disease (Sheldon et al. 2002). In summary, mastitis attenuates growth of
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preantral and medium-size follicles, but its association with reduced fertility of mastitic cows remains unclear.

**Oocyte competence**

Different approaches have been used to examine the effect of mastitis on oocyte competence. Initially, inflammatory agents (e.g. prostaglandin F$_{2\alpha}$, PGF$_{2\alpha}$) added to the culture medium during oocyte maturation reduced the proportion of blastocysts formed, while addition of the nitric-oxide generator sodium nitroprusside after fertilization prevented development to the blastocyst stage (Soto *et al.* 2003a). Additionally, TNFα added during maturation reduces the proportion of blastocysts formed, and when added after fertilization increases the percentage of blastomeres undergoing apoptosis (Soto *et al.* 2003b, Hansen *et al.* 2004). In mouse embryos, TNFα added during maturation decreased the number of cells in the inner cell mass, possibly associated with low embryonic survival (Pampfer *et al.* 1994, Wuu *et al.* 1999, Hansen *et al.* 2004).

Another approach that more closely mimics the effect of mastitis on oocyte competence is the use of follicular fluid from mastitic cows as oocyte maturation medium. Follicular fluid from G- or G+ toxin-induced mastitic cows used as maturation medium reduced cleavage rate, and proportion of blastocysts formed was lower in the G- group and numerically lower in the G+ group (Asaf *et al.* 2014). Although the inflammatory mediator(s) associated with reduced competence were not known, a main advantage of this study was the use of follicular fluids from mastitic cows containing inflammatory agents at physiological concentrations (Asaf *et al.* 2014).

Another method of examining the possible long-term effect of mastitis on oocyte competence involves oocyte collection for *in vitro* embryo production from mastitic cows. Oocytes were collected from culled Holstein cows that had been allotted to low-, medium- or high-SCC groups
based on the SCC records for the last 3 months before slaughter (Roth et al. 2013). Although cleavage rate did not differ among the groups, blastocyst rate was markedly lower in medium- and high-SCC groups than in the low-SCC (control) group (Fig. 5). Interestingly, blastocyst rates did not differ among G- or G+ bacterial types (Roth et al. 2013). This study provided evidence for disruption of the developmental competence of the ovarian pool of oocytes by naturally occurring mastitis at the germinal vesicle (GV) stage.

**Fig. 5.** Rate of blastocyst formation from oocytes aspirated from low- (controls), medium- and high-SCC groups recorded in culled cows for 3 months before collection of the ovaries at slaughter.

**Long-term disruptive effect of mastitis**

Several studies using different methodologies to explore long-term disruptive effects of mastitis on follicle function have revealed that small and less differentiated follicles are more susceptible to various inflammatory agents. Their early exposure to pathogenic stress may be expressed at a later stage as disrupted function in dominant follicles, resulting in low fertility. For instance, exposure of small antral follicles to intramammary administration of G+ or G- toxin induced a carryover decrease in follicular estradiol in preovulatory follicles and a decrease in the number of medium-size follicles (Lavon et al. 2011c, Furman et al. 2012). Another study not directly related to IMI showed that bovine granulosa cells obtained from only small (1–5 mm) antral follicles have reduced estradiol production in response to cytokines added to the culture medium. No effect was detected with other categories of follicles (Spicer & Alpizar 1994). It was also found in studies related to uterine infection that LPS inhibits the expression of TLR4, CD14, MD2 and NOD1 in granulosa cells from small (<5 mm in diameter) follicles (Shimizu et al. 2012) and reduces the number of bovine primordial follicles (Bromfield & Sheldon 2013). Similar disruptive effects of naturally occurring mastitis on subordinate follicles have been documented. For instance, chronic mastitis reduced the number of secondary preantral and medium-size (>8 mm in diameter) follicles (Rahman et al. 2012). Another study showed that exposure of the pool of oocytes at the GV stage for 3 months before oocyte collection to various forms of mastitis causes a low rate of blastocyst formation (Roth et al. 2013). Taken together, these studies indicate a long-term disruptive effect of IMI and other pathogenic stresses on follicular function.
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Corpus luteum

A possible cause of low fertility in mastitic cows could be early regression of the corpus luteum (CL) post-AI leading to pregnancy termination. Mastitis-induced increases in PGF$_{2\alpha}$ and possibly TNF$_{\alpha}$ have been related to CL regression in several studies (Sakumoto & Okuda 2004, Malinowski & Gajewski 2010). However, evidence for impaired luteal function in mastitic cows is controversial. Much of this is related to the various methodological approaches to stress induction by G- or G+ toxin, the route of toxin administration (intramammary, i.v. or intrauterine) and assessment of the effects in clinical or subclinical mastitis.

Induced stress

Intravenous infusion of *E. coli* LPS for 6 h into pregnant cows decreased progesterone concentration over a period of 120 h (Giri *et al.* 1990). Another study showed that i.v. injection of LPS causes a decline in CL size and a transient decline in plasma progesterone concentration, associated with increased caspase-3 and decreased STAR expression (Herzog *et al.* 2012). Infusion of live *E. coli* into the uterus tended to shorten cycle length (Gilbert *et al.* 1990). In contrast, intramammary or i.v. administration of LPS at the onset of estrus did not affect cycle length or progesterone concentrations in most treated cows (Lavon *et al.* 2008). Effects of G+ bacteria and their toxins on luteal function are also controversial. Mammary inoculation with *S. uberis* did not alter progesterone concentration for 7 days post-administration (Hockett *et al.* 2005); however, i.v. administration of G+ toxin (of *Streptococcus* origin) on day 5 after mating decreased progesterone concentrations for 16 to 19 days in sheep (Stewart *et al.* 2003, Dow *et al.* 2010). The above studies do not provide any conclusive evidence for the effect of induced stress on CL function.

Naturally occurring mastitis

Published outcomes from spontaneous cases of mastitis and their effect on CL lifespan have been unclear. Cows diagnosed as having mastitis with G- isolates were twice as likely to exhibit altered inter-estrus interval than healthy cows (Moore *et al.* 1991) and had a shorter luteal phase due to a higher rate of premature luteolysis (Huszenicza *et al.* 2005). In contrast, mid-luteal progesterone concentrations and CL volume in cows with past clinical events or subclinical mastitis did not differ from uninfected cows (Lavon *et al.* 2010). Another study showed that lameness combined with high SCC does not alter progesterone concentrations in cows (Morris *et al.* 2013). Similarly, progesterone profiles over 10 days following naturally occurring clinical mastitis (of *E. coli* origin) were not affected by IMI (Shaani *et al.* 2012). It is important to note that all mastitic cows in this study were given a commercial medication with a non-steroidal anti-inflammatory treatment at the time of diagnosis. This most likely attenuated or blocked prostaglandin release, resulting in CL maintenance. The same study showed that only a small portion of the cows (5.5%; n = 201) manifested estrus less than 8 days after *E. coli* mastitis, suggesting a negligible association between *E. coli* mastitis and short inter-estrus interval (Shaani *et al.* 2012). The above suggests that the cause of low fertility induced by *E. coli* mastitis after fertilization is directed at the embryo rather than the CL.
Approaches to improving fertility

Among all of the reproductive functions disrupted by subclinical mastitis, the delayed ovulation found in one third of mastitic cows (Lavon et al. 2010) can be corrected. Two approaches were tested with the objective of improving fertility of subclinical mastitic cows; one was a management method and the other a hormonal treatment protocol.

The management approach to correcting the timing of AI in mastitic cows with delayed ovulation was based on the use of two inseminations: one was performed routinely after estrus, the second 24 h later (D Wolfenson 2010, unpublished observations). The findings of a study with 219 lactating cows showed that a single vs. two AIs does not change CR of either healthy or subclinical mastitic cows. It is possible that the low LH surge seen in about 30% of the mastitic cows is involved in delayed ovulation and possibly altered oocyte maturation, preventing fertility improvement by double AI.

The Ovsynch protocol may improve timing of ovulation relative to that of estrus because the second GnRH injection induces an LH surge regardless of low steroidogenic capacity of the preovulatory follicle. In a recent study (D Wolfenson 2011, unpublished observations), 1,553 Holstein cows were subjected to either the Ovsynch 56 program (consisting of i.m. injection of GnRH followed 7 days later by PGF$_{2\alpha}$, 56 h later by a second injection of GnRH and 16 h later by timed AI) or AI following natural estrus. A SCC threshold of 150,000 cell/mL milk was set for subclinical cows, and cows with postpartum uterine disease were recorded. Ovsynch significantly improved CR in subclinical mastitic cows relative to that in their control counterparts (Fig. 6). In contrast, Ovsynch did not improve CR in cows exhibiting postpartum uterine disease. Thus, the two major diseases affecting the dairy industry responded oppositely to the Ovsynch protocol, suggesting that different mechanisms underlie the disrupted fertility. A possible difference between the two diseases could be that subclinical mastitis induces delayed ovulation (Lavon et al. 2010), whereas postpartum uterine disease decreases the percentage of ovulating cows (Williams et al. 2008). This suggests that Ovsynch is capable of ‘correcting’ ovulation timing in mastitic but not endometritic cows.

![Graph showing conception rates](image)

**Fig. 6.** Effect of Ovsynch 56 program on first-AI conception rate (CR) of subclinical mastitic and uninfected cows. Controls were artificially inseminated following natural estrus.
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Conclusions

1. Reduced fertility of subclinical mastitic cows before AI is associated with both follicle-induced delayed ovulation and impaired oocyte competence.
2. The potential of subclinical mastitis to disrupt follicular responses is higher than that of clinical mastitis, particularly before AI due to its long-term chronic nature.
3. Mastitis induces long-term disruption of ovarian follicular functions. A carryover effect (occurring weeks later) of subclinical mastitis induced by G+ or G- toxin is reflected in depressed follicular growth and low steroid production in the preovulatory follicle. Naturally occurring clinical or subclinical mastitis affects the pool of GV-stage oocytes and this is reflected after maturation in low blastocyst formation rates. The inflammatory agent(s) associated with subclinical mastitis that disrupts follicular functions is unknown.
4. There is large diversity in individual cows’ reproductive responses to subclinical mastitis. The reason for the susceptibility of a particular subpopulation of cows to mastitic stress is unclear.
5. Several similarities are noted in the impairment of reproductive responses by G+ and G- toxins despite their large differences in induction of immune responses and in their molecular/cellular pathways.
6. The effect of clinical IMI on possible disruption of CL function post-AI is equivocal; however, it seems to play a minor role in cows with clinical mastitis treated with anti-inflammatory drugs.
7. Conception rate of subclinical IMI cows can be improved by the Ovsynch-timed AI program, likely due to its synchronization of time of ovulation in cows that might otherwise exhibit delayed ovulation relative to AI.

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