

Mastitis and Fertility in Cattle – Possible Involvement of Inflammation or Immune Activation in Embryonic Mortality*

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Causes for pre-implantation embryo loss, which can be as high as 50% or more of fertilized embryos, are multifactorial and largely undescribed. Studies in cattle using mastitis as a model indicate that one cause of early embryonic loss is infectious disease or activation of immune responses at sites outside the reproductive tract. Infection of the mammary gland in dairy cattle is associated with a reduction in pregnancy rate (proportion of inseminated cows that become pregnant) and an increase in the number of inseminations required to establish pregnancy. Also, intravenous challenge with bacterial peptidoglycan and polysaccharide at ~days 3–5 after breeding reduced subsequent pregnancy rate in sheep that had been previously immunized against the same material. The mechanism by which extrauterine activation of immune and inflammatory responses leads to embryonic loss is not clear although cytokines probably play a crucial role. Effects could be exerted at the level of the hypothalamic–pituitary axis, ovary, reproductive tract or embryo. Interferon (IFN)- α , for example, which can reduce pregnancy rate in cattle when injected around 13–19 days after breeding, increases body temperature, inhibits secretion of luteinizing hormone, and reduces circulating concentrations of progesterone. Other cytokines or products of cytokine activation could cause embryonic loss by causing hyperthermia (as elevated temperature blocks oocyte function and embryonic development), exerting toxic effects on the corpus luteum [for example, IFN- γ , tumor necrosis factor- α (TNF- α) and prostaglandin F_{2 α}], stimulating endometrial prostaglandin synthesis [TNF- α and interleukin(IL)-1 β], reducing endometrial cell proliferation (IL-1 β), and interfering with oocyte maturation and embryonic development (TNF- α , nitric oxide, and prostaglandin F_{2 α}). Although largely neglected by reproductive immunologists, study of the involvement of the immune system in pre-implantation embryonic loss is likely to lead to new methods for enhancing fertility.

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INTRODUCTION

The fate of the newly formed embryo is an uncertain one. It is estimated that about 50% of human embryos are lost before implantation.¹ In dairy cattle, pregnancy rate (the proportion of cows that are inseminated that are diagnosed pregnant) has been decreasing over the last 30 years. Pregnancy rates of 50–60% in

the 1970s have declined to values of 35–45% today.^{2,3} The mechanisms mediating embryo survival and death are incompletely understood. Recently, studies in cattle have led to the emergence of the idea that infectious disease outside the reproductive tract can lead to reduced pregnancy rate. Such observations are suggestive that inflammatory or immune responses associated with infectious disease can cause anovulation,

fertilization failure, and embryonic mortality. The purpose of this paper is to review the still-incomplete evidence that mastitis compromises reproductive function and discuss some of the possible mechanisms by which mastitis interferes with embryonic survival.

MASTITIS AND REPRODUCTIVE FUNCTION

Mastitis is an infection of the mammary gland caused in cattle primarily by bacteria. Clinical symptoms include decreased milk production, increased numbers of leukocytes in milk, altered milk composition and appearance, increased body temperature, and red, warm, and swollen mammary quarters. Often cows have mastitis without obvious clinical symptoms: these subclinical infections also result in decreased milk production and elevated leukocyte counts in milk.

The first study to suggest a relationship between mastitis and fertility involved comparison of reproductive records of 102 lactating Jersey cows diagnosed with clinical mastitis with records of 103 lactating Jersey cows without mastitis.⁴ Cows were inseminated at all estrus periods after ~ 60 days postpartum. Pregnancy was confirmed by rectal palpation 50–65 days after insemination. Cows with clinical mastitis were further subdivided into three groups based on the time of diagnosis of mastitis: before first insemination, between first insemination and confirmed pregnancy, and after confirmed pregnancy. Among the associations between mastitis and impaired reproductive function was an increase in services per conception (a term to describe the number of inseminations required to achieve a confirmed pregnancy) for cows diagnosed with clinical mastitis between first insemination and confirmed pregnancy when compared with cows that were diagnosed with mastitis at other times or to cows without clinical mastitis. Effects of mastitis on reproduction were independent of bacterial type (gram-positive or gram-negative).

Similar findings were obtained using a larger data set of 758 lactating Jersey cows in which cows were classified as being free of mastitis, having subclinical mastitis (based on detection of bacteria in milk), or having clinical mastitis.⁵ Cows were separated by the type of mastitis and by the timing of mastitis diagnosis. Services per conception were greater for cows with mastitis first diagnosed between first insemination and confirmed pregnancy than for other groups (Fig. 1A). There was also a slight increase in services per conception for cows diagnosed with mastitis before first insemination than for cows that were either uninfected or that developed mastitis after pregnancy

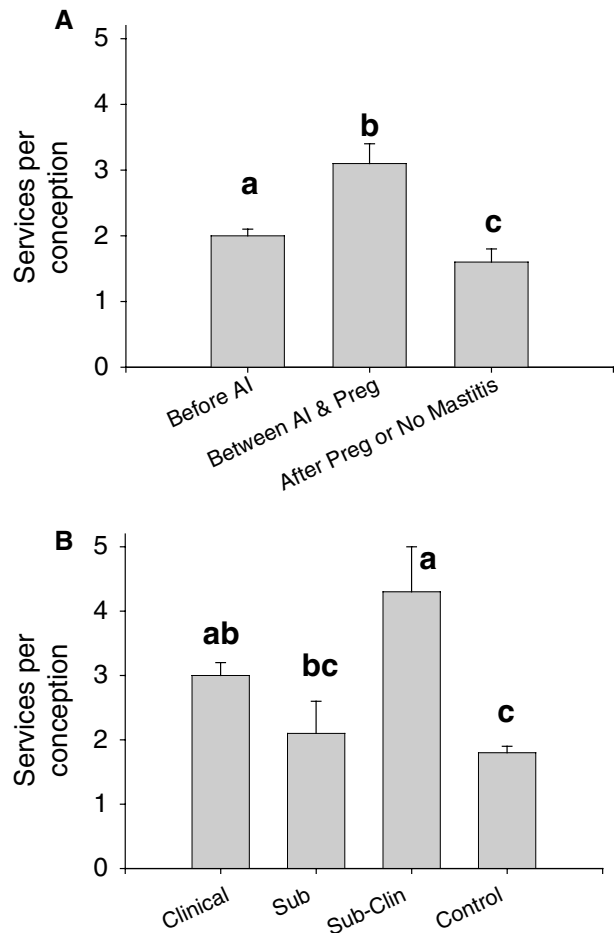


Fig. 1. Association between mastitis and services per conception in lactating dairy cattle. (A) Cows were grouped into three groups based on the timing of mastitis diagnosis: before first insemination (Before AI), between insemination and confirmed pregnancy (pregnancy diagnosis conducted 50–65 days after insemination) (Between AI & Preg), or a combined group of cows that were either not diagnosed with mastitis or were diagnosed after confirmed pregnancy (After Preg or No Mastitis). (B) Cows were classified as having clinical mastitis (Clin), subclinical mastitis (Sub), subclinical mastitis converting to clinical mastitis (Sub-Clin), or either being free of mastitis or diagnosed with mastitis after pregnancy confirmation (Control). Bars with different superscripts differ significantly from each other. Data are redrawn from Schrick et al.⁵

diagnosis (Fig. 1A). Effects of mastitis were similar regardless of whether the pathogen affecting the mammary was a gram-negative or gram-positive organism. The greatest increase in services per conception was observed when cows experienced subclinical mastitis that progressed to clinical mastitis (Fig. 1B).

The increase in services per conception caused by mastitis in these studies suggests that mastitis is associated with either anovulation at estrus, fertilization failure, or embryonic mortality. Some caution in

interpretation is warranted, however. One cannot rule out the possibility that the association between mastitis and services per conception occurs because cows that are pre-disposed to developing mastitis (for example, because they are experiencing some form of stress) are also predisposed to reproductive problems. Mastitis also affected interval from calving to first service.^{4,5} Such a result could indicate additional actions of mastitis on reproductive processes or could reflect the fact that cows prone to develop mastitis are more debilitated physiologically. It should also be kept in mind that changes in services per conception, while suggesting alterations in embryonic survival, could in part be due to changes in estrus detection, anovulation, oocyte quality, or fertilization rate.

Recently, experimental evidence has been developed in sheep to indicate that immune activation associated with immunization to bacteria involved in mastitis causes reduced pregnancy rate.⁶ Ewes were assigned to one of four groups: unimmunized controls, unimmunized controls challenged at day 47, ewes immunized twice, at a 22-day interval beginning at day 0, with heat-killed streptococcal cells, and ewes immunized according to the same schedule with an extract of streptococcal peptidoglycan and polysaccharide (PG-PS). Estrous cycles of all ewes were synchronized using progesterone, prostaglandin, and gonadotropin, and rams were introduced. At day 47 after initial immunization, a time approximately equal to 3–5 days after breeding, all groups expect controls were challenged with injection of PG-PS. Pregnancy rate was reduced from 73% for unimmunized controls and 65% for ewes receiving challenge only to 56% for ewes immunized with killed cells and 47% for ewes immunized with PG-PS.

HYPERTHERMIA AS A CAUSE OF REDUCED PREGNANCY RATE

There are an abundance of possible mechanisms by which mastitis could negatively affect fertility. One possibility is that the elevation in body temperature coincident with mastitis compromises reproductive processes. It has long been known from studies on effects of heat stress that elevated body temperature compromises fertility. In cattle, for example, heat stress during the period of oocyte maturation⁷ or early embryonic development^{8,9} leads to reduced embryonic survival. At least some of the effect of heat stress on embryonic survival is because of the direct effects of elevated temperature on oocyte and embryo function as culture of maturing oocytes¹⁰ or pre-implantation embryos^{10,11} reduced development to the blastocyst

stage. It also appears that oocyte competence can be compromised by heat stress during follicular development. Heat stress 10 days before estrus reduced pregnancy rate in sheep.¹² Bovine oocytes collected during warm weather have reduced ability to yield blastocysts when subjected to *in vitro* fertilization.^{13,14}

MEDIATORS OF INFLAMMATION AS DISRUPTORS OF OOCYTE MATURATION AND EMBRYONIC DEVELOPMENT

Mastitis leads to production of a variety of bioactive molecules that can potentially disrupt reproductive tract tissues. Milk-derived cells from infected mammary glands contained increased amounts of mRNA for interleukin(IL)-1 α , IL-1 β , tumor necrosis factor- α (TNF- α), IL-10 and IL-12¹⁵ as well as increased amounts of TNF- α protein.¹⁶ Mammary infusion of lipopolysaccharide (LPS) from *Escherichia coli* increased milk concentrations of IL-1 β , IL-8, and TNF- α but not interferon (IFN)- γ .^{17–19} For TNF- α , IL-1, IL-6 and probably other cytokines, circulating concentrations in blood can be elevated during mastitis.^{17,18,20,21} Experimental treatment with LPS increased peripheral blood concentrations of TNF- α .^{17,18,22,23} Mastitis and LPS treatment is also associated with increased production of molecules whose synthesis can be activated by specific cytokines. In particular, mastitis and endotoxin treatment increased concentrations of nitric oxide (NO) in milk^{17,24} and intramammary infusion of *E. coli* endotoxin resulted in increased milk concentrations of prostaglandin F_{2 α} (PGF_{2 α}).²⁵ It has also been reported²⁶ that cows with mastitis had a higher peak concentration of 13,14-dihydro-15-keto PGF_{2 α} (the major PGF_{2 α} metabolite, PGFM) in blood following oxytocin challenge compared with control cows.

Of these molecules, TNF- α , NO, and PGF_{2 α} can affect embryonic development by acting either on the oocyte or on the developing embryo. As illustrated in Fig. 2, addition of TNF- α to bovine oocytes matured *in vitro* did not alter subsequent cleavage when oocytes were fertilized but the proportion of oocytes that became blastocysts was reduced.²⁷ Addition of TNF- α to bovine embryos after fertilization did not affect the proportion that became blastocysts but TNF- α did increase the proportion of blastomeres that were apoptotic when added to embryos at day 5 after fertilization.²⁷ Such an apoptosis-inducing effect of TNF- α could contribute to the decreased inner cell mass number and reduced post-transfer survival of mouse embryos treated with TNF- α .^{28,29} Overexpression of TNF- α leading to embryonic apoptosis has also

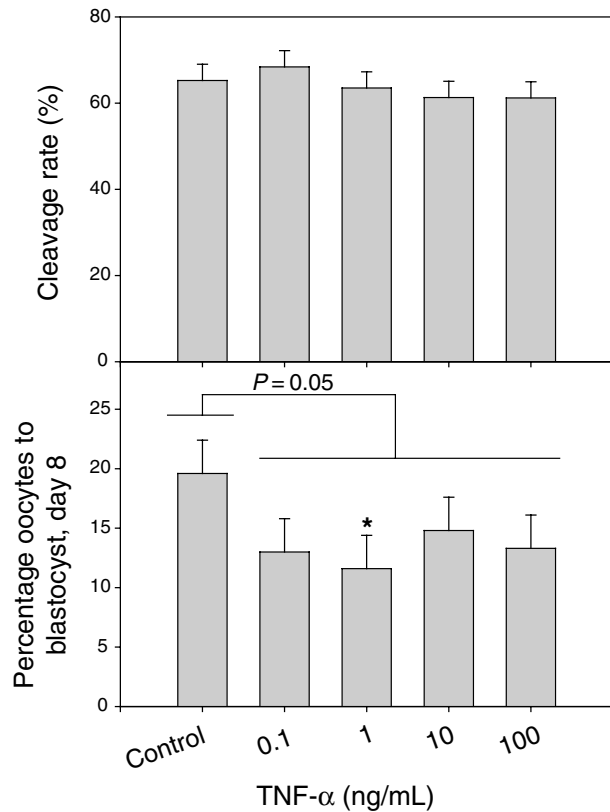


Fig. 2. Effects of tumor necrosis factor- α during oocyte maturation on the percentage of oocytes that cleaved (top panel) and developed into blastocyst on day 8 after insemination (bottom panel). Results are least-squares mean \pm SEM. Mean values that differ ($P < 0.05$) from control oocytes (0 ng/mL) are indicated by asterisks. Significant orthogonal contrasts are indicated by horizontal lines over the bars. Data are reproduced from Soto et al.²⁷

been associated with embryonic death in diabetic rats.^{30,31}

Prostaglandin $F_{2\alpha}$ has been reported to have a negative effect on embryo development in cattle: administration of $PGF_{2\alpha}$ to cows receiving supplemental progesterone compromised embryonic development and decreased pregnancy rate.^{32,33} Removal of the corpus luteum reduced the embryotoxic effects of $PGF_{2\alpha}$,³² suggesting that $PGF_{2\alpha}$ induces secretion of an embryotoxic molecule of luteal origin. It is also possible that $PGF_{2\alpha}$ acts directly on the embryo to block development. Although no effect of addition of $PGF_{2\alpha}$ after fertilization was observed on development of cultured bovine embryos,³⁴ another report³⁵ indicated that addition of $PGF_{2\alpha}$ to frozen-thawed bovine embryos at the morula stage decreased development to the expanded and hatched blastocyst stage. Oocyte maturation can also be compromised by $PGF_{2\alpha}$.³⁴

Elevated concentrations of NO have also been associated with early embryonic death. Culture with

sodium nitroprusside dihydrate, a NO donor, inhibited development to the blastocyst stage of bovine^{34,36} and mouse embryos.^{37,38} The toxic effects of NO may occur through interaction between NO and O_2^- to form the oxidant peroxynitrite.³⁹

It is not known whether mastitis is associated with increased synthesis of $PGF_{2\alpha}$ and NO in the reproductive tract but this is possible because synthesis of both molecules is under cytokine regulation. For example, TNF- α , which can be elevated in the blood during mastitis,^{17,18,20,21} can increase endometrial synthesis of $PGF_{2\alpha}$ (Starzynski et al., 2001).⁴⁰ IL-1 α can also induce endometrial secretion of $PGF_{2\alpha}$.⁴¹ Among the molecules inducing NO synthase are IFN- γ , TNF- α , LPS, and $PGF_{2\alpha}$.^{42,43}

Addition of *E. coli* LPS to cumulus-oocyte complexes during *in vitro* oocyte maturation reduced the proportion of oocytes that developed into blastocysts after subsequent *in vitro* fertilization³⁴ but the concentrations of LPS required to interfere with oocyte function during maturation were too high (1 ng/mL or higher) to be relevant to the situation in mastitis. There was no effect of up to 1000 ng/mL LPS on blastocyst development when added to embryo culture after fertilization.³⁴ Thus, the major reproduction-disrupting role that LPS plays during mastitis is to trigger release of cytokines and other molecules that interfere with reproduction and not to directly interfere with oocyte and embryo function.

CHANGES IN UTERINE FUNCTION MEDIATED BY CYTOKINES

As mentioned previously, endometrial synthesis of prostaglandins is under the control of several cytokines including TNF- α , which can increase $PGF_{2\alpha}$ secretion from cultured bovine stromal endometrial cells,⁴⁰ and IL-1 β , which can increase secretion of $PGF_{2\alpha}$ and PGE_2 from endometrial stromal and epithelial cells.⁴¹ Thus, it is possible that the release of cytokines into the bloodstream during mastitis could lead to induction of endometrial $PGF_{2\alpha}$ release and pre-mature luteolysis. Endotoxin caused an increase in serum concentrations of PGFM when administered i.v. (although not when administered in the mammary).⁴⁴ Moreover, cows in which mastitis was induced by *Streptococcus uberis* infusion experienced a greater rise in circulating PGFM in response to oxytocin treatment than control cows.²⁶ Intrauterine infusion of live *E. coli* into heifers on days 7–9 of the estrous cycle caused luteal regression and shortened estrous cycles.⁴⁵

Cytokines could also exert other effects on endometrial or oviductal tissue that impede embryonic

development. IL-1 β , for example, reduced proliferation of endometrial stromal cells⁴¹ and IFN- α reduced proliferation of oviductal epithelial cells.⁴⁶

DISRUPTION OF THE HYPOTHALAMIC-PITUITARY-OVARIAN AXIS

One possible reason for increased number of services per conception in cows with mastitis is inhibition of gonadotropin secretion leading to reduced gonadotropin support for ovulation, oocyte maturation, folliculogenesis and luteal function. Certain cytokines can decrease LH release.⁴⁷ In cattle, for example, IFN- α has been shown to have such an action.⁴⁸ Secretion of LH can also be blocked by cortisol,^{49–51} a hormone whose secretion can be elevated during mastitis²⁶ or after endotoxin treatment.^{52–54} Treatment of heifers with endotoxin near estrus can lead to inhibition of the LH surge, anovulation or delayed ovulation and formation of follicular cysts.^{52,54} The effect of endotoxin on LH secretion involves activation of the opioid system as naloxone treatment counteracted inhibitory effects of endotoxin on LH secretion in heifers.⁵³

Cytokines released during mastitis can also have direct effects on the ovary. IL-6 blocks follicle stimulating hormone-induced estradiol secretion from bovine granulosa cells, especially from cells isolated from small follicles.⁵⁵ Both TNF- α and IFN- γ are cytotoxic to bovine luteal cells.^{56,57}

CONCLUSIONS

While definitive experiments are still necessary, observations from cows experiencing mastitis suggest that activation of inflammatory or immune responses external to the reproductive tract can lead to embryonic mortality. This embryonic loss appears to follow activation of multiple pathways that disrupt the reproductive axis at several points including the hypothalamic-pituitary axis, ovary, oocyte and the embryo.

A model illustrating these pathways and the central role proposed for cytokines in orchestration of these events is shown in Fig. 3. According to this model, invasion of the mammary gland leads to the release of LPS, proteoglycans and other molecules of bacterial origin that activate inflammatory and immune responses. As a result, there is an increase in cytokine synthesis from the mammary gland, lymph nodes draining the mammary, and, perhaps, at other sites including the reproductive tract. Certain cytokines are directly inhibitory to oocyte and embryonic function.

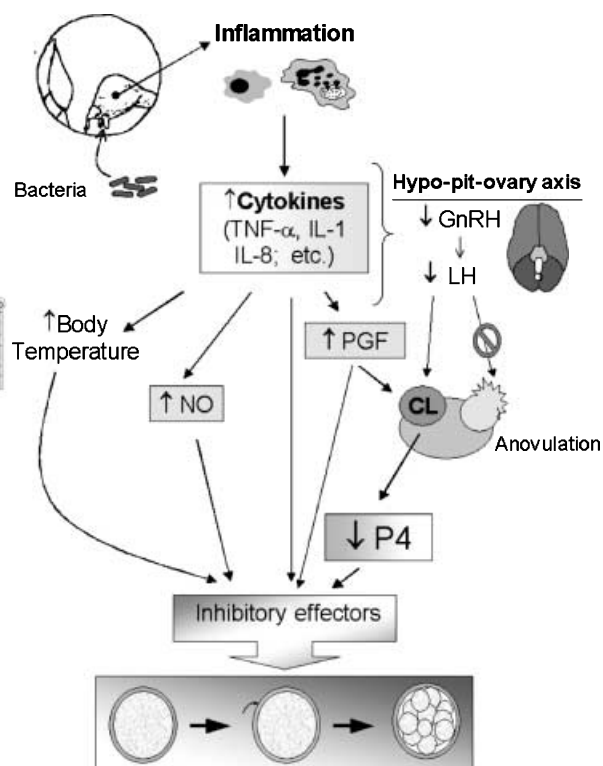


Fig. 3. Potential pathways by which infection in the mammary gland can reduce embryonic survival. According to this model, the central phenomenon leading to embryonic mortality associated with mastitis is increased secretion of cytokines (either produced in the mammary and draining lymph nodes and secreted or produced in other tissues in response to mammary-derived signals) that in turn modulate reproductive function at several levels (see text for details).

TNF- α , for example, disrupts the process of oocyte maturation and can induce apoptosis in embryos.²⁷ Cytokines can also cause increased secretion of other molecules that are disruptive to oocytes and embryos. One example is PGF_{2 α} , which is produced in response to TNF- α and IL-1 β by several tissues including endometrium^{40,41} and which can cause luteolysis and interfere with oocyte maturation³⁴ and embryonic development.³⁵ Another such molecule is NO, whose synthesis is increased by LPS, IFN- γ , TNF- α , and PGF_{2 α} ^{42,43} and which inhibits post-fertilization embryonic development.^{34,36} Hyperthermia associated with pyrogenic actions of various cytokines could be a major cause of embryonic mortality as elevated body temperature can inhibit oocyte development before ovulation,¹² processes in the oocyte occurring around the time of maturation,^{7,10} and cleave-stage embryonic development.^{10,11}

Also illustrated in Fig. 3 is the idea that cytokines can also affect embryonic survival by disrupting function of the hypothalamus, pituitary, ovary, and uterus. For example, endotoxins can disrupt LH

secretion^{52,54} because of induction of secretion of cytokines such as IFN- α ⁴⁸ as well as cortisol.^{49–51} The resultant loss of LH support can potentially lead to inhibition of the LH surge, anovulation or delayed ovulation and formation of follicular cysts.^{52,54} Reduced LH secretion may also reduce progesterone secretion from the corpus luteum and thereby alter uterine function. Cytokines can also act to interfere with follicular function (IL-6),⁵⁵ luteal maintenance (TNF- α and IFN- γ),^{56,57} and proliferation of endometrial (IL-1 β)⁴¹ and oviductal cells (IFN- α).⁴⁶

Appropriate temporal–spatial secretion of cytokines in the reproductive tract is essential for pregnancy – several cytokines have been shown to be important for establishment of maintenance of pregnancy including leukemia inhibitory factor, IL-1, macrophage colony-stimulating factor and others.^{58–60} Nonetheless, aberrant cytokine secretion can be harmful for the survival of the embryo. The potential for a cytokine that is ordinarily essential for pregnancy to disrupt reproduction when secreted inappropriately is highlighted by the example of type 1 IFN in cattle. Maintenance of the corpus luteum in this species is dependent upon embryonic secretion of large quantities of IFN- τ around days 15–25 of pregnancy to prevent uterine PGF_{2 α} synthesis.⁶¹ The related molecule, IFN- α , can also block luteolysis.⁶² Despite the essential role for IFN- τ in pregnancy maintenance, injection of IFN- α into heifers at 13–19 days after breeding reduces pregnancy rate,⁶³ probably because IFN- α increases body temperature, inhibits secretion of luteinizing hormone, and reduces circulating concentrations of progesterone.^{48,64}

The link between mastitis and embryonic mortality can be viewed as one example of how the interface between the immune and reproductive systems can impinge on reproductive success. Moreover, it is likely that mastitis is not the only type of infectious disease that can compromise female fertility but that other infections outside the reproductive tract may interfere with establishment of pregnancy. Indeed, vaccination may be inimical to optimal fertility.⁶ Thus, research to further define the connection between immune activation and infertility and the mechanisms involved may expand our understanding of the nexus between the immune and reproductive systems and lead to new treatments to improve fertility in domestic animals and women.

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