

Minireview

Defining Postpartum Uterine Disease and the Mechanisms of Infection and Immunity in the Female Reproductive Tract in Cattle¹

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ABSTRACT

Uterine microbial disease affects half of all dairy cattle after parturition, causing infertility by disrupting uterine and ovarian function. Infection with *Escherichia coli*, *Arcanobacterium pyogenes*, and bovine herpesvirus 4 causes endometrial tissue damage. Toll-like receptors on endometrial cells detect pathogen-associated molecules such as bacterial DNA, lipids, and lipopolysaccharide (LPS), leading to secretion of cytokines, chemokines, and antimicrobial peptides. Chemokines attract neutrophils and macrophages to eliminate the bacteria, although persistence of neutrophils is associated with subclinical endometritis and infertility. Cows with uterine infections are less likely to ovulate because they have slower growth of the postpartum dominant follicle in the ovary, lower peripheral plasma estradiol concentrations, and perturbation of hypothalamic and pituitary function. The follicular fluid of animals with endometritis contains LPS, which is detected by the TLR4/CD14/LY96 (MD2) receptor complex on granulosa cells, leading to lower aromatase expression and reduced estradiol secretion. If cows with uterine disease ovulate, the peripheral plasma concentrations of progesterone are lower than those in normal animals. However, luteal phases are often extended in animals with uterine disease, probably because infection switches the endometrial epithelial secretion of prostaglandins from the F series to the E series by a phospholipase A2-mediated mechanism, which would disrupt luteolysis. The regulation of endometrial immunity depends on steroid hormones, somatotrophins, and local regulatory proteins. Advances in knowledge about infection and immunity in the female genital tract should be exploited to develop new therapeutics for uterine disease.

bovine, female reproductive tract, immunity, immunology, infection, inflammation, ovary, prostaglandins, toll-like receptors, uterus

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INTRODUCTION

Microbial disease of the female genital tract is most common and of greatest economic importance in humans and cattle among the mammals [1, 2]. Microbial infections of the genital tract cause infertility by disrupting uterine and ovarian function. Many of the mechanisms underlying the recognition of microbial pathogens by the innate immune system in vertebrates have been identified during the past 10 years [3–5]. These mechanisms of innate immunity not only are important for classic immune cells such as neutrophils and macrophages but also are evident in the endometrial and ovarian cells of mammals [6–8]. As well as causing an immune and inflammatory response, microbes or pathogen-associated molecules disrupt endocrine function in the female reproductive tract of rodents and cattle [6, 7, 9, 10]. Herein, we outline advances in scientific knowledge about how infection and innate immunity affect the female reproductive tract to cause infertility in cattle.

DEFINING POSTPARTUM REPRODUCTIVE TRACT DISEASES

Uterine disease within a week of parturition (metritis) is present in up to 40% of dairy cows (Fig. 1). Metritis incidence depends on the definition of disease (see herein), but maximal herd rates for obvious clinical disease of 36%–50% have been reported in large surveys [16, 17], and 18.5%–21% of animals have metritis with signs of systemic illness such as pyrexia [18, 19]. Subsequently, 15%–20% of cattle have clinical disease that persists beyond 3 wk postpartum (endometritis), and about 30% have chronic inflammation of the uterus without clinical signs of uterine disease (subclinical endometritis) [2, 15, 20, 21].

Metritis occurs within 21 days and is most common within 10 days of parturition. Metritis is characterized by an enlarged uterus and a watery red-brown fluid to viscous off-white purulent uterine discharge, which often has a fetid odor [2]. The severity of disease is categorized by the signs of health. We propose that cows are classified as having grade 1 metritis if they have an abnormally enlarged uterus and a purulent uterine discharge without any systemic signs of ill health. Animals with additional signs of systemic illness such as decreased milk yield, dullness, and fever >39.5°C are classified as having grade 2 clinical metritis. Animals with signs of toxemia such as inappetence, cold extremities,

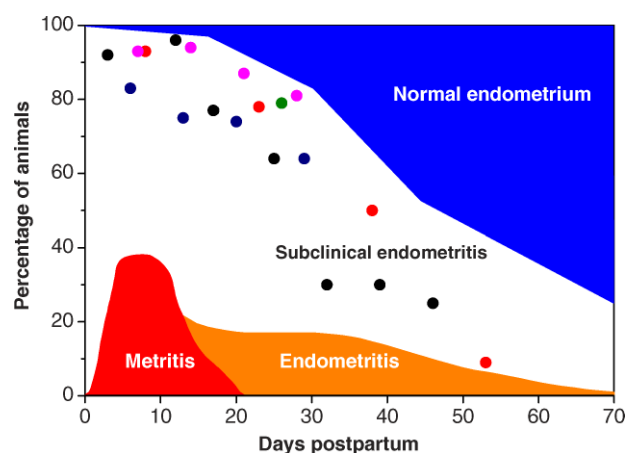


FIG. 1. The incidence of uterine bacterial infection and disease in postpartum dairy cattle. Bacteria can be isolated from the uterus of most cows during the postpartum period; each marker (circle) indicates the percentage of animals with bacteria isolated from the uterine lumen [10–14]. The shaded areas represent estimates of the proportion of animals with metritis (red), clinical endometritis (orange), or a normal uterus (blue); the remainder of animals have subclinical endometritis [15].

depression, and/or collapse are classified as having grade 3 metritis, which has a poor prognosis.

Clinical endometritis is defined in cattle as the presence of a purulent uterine discharge detectable in the vagina 21 days or more postpartum or mucopurulent discharge detectable in the vagina after 26 days postpartum [2]. A simple grading system based on the character of the vaginal mucus (Fig. 2A) is readily used to evaluate cows with clinical endometritis [2]. The endometritis grade correlates with the presence of pathogenic organisms associated with uterine disease (Fig. 2B) and is prognostic for the likely outcome of treatment (Fig. 2C) [11, 22].

Subclinical endometritis is characterized by inflammation of the endometrium that results in a significant reduction in reproductive performance in the absence of signs of clinical endometritis. The inflammation is presumably associated with recovery of the tissues after clinical endometritis, trauma, or other nonmicrobial disease. Subclinical disease is defined by polymorphonuclear neutrophils (PMNs) exceeding between 5.5% of cells [23] and 10% of cells [24] in samples collected by flushing the uterine lumen or by endometrial cytobrush, in the absence of clinical endometritis, about 5 wk postpartum. The incidence of subclinical endometritis is dependent on the cutoff for diagnosis and the time after parturition but is in the order of 37%–74% of animals (Fig. 1) [15].

REPRODUCTIVE AND ECONOMIC CONSEQUENCES OF UTERINE DISEASE

The placenta should be expelled within a few hours of parturition in cattle. During the first week postpartum, the uterus contracts rapidly, and lochia is discharged containing remnants of fetal membranes and fluids. During the second to fourth weeks, any damaged endometrial tissue regenerates, a wave of ovarian follicles develop, a dominant follicle is selected, and estradiol secretion leads to ovulation and formation of a corpus luteum to recommence ovarian cycles [25]. The genital tract should have little evidence of the previous pregnancy by 6 wk after calving and be capable of establishing the next pregnancy. However, about 50% of dairy cows have irregular ovarian cycles during the postpartum

period, and animals with abnormal vaginal discharge are more likely than normal animals to have delayed resumption of ovarian cycles after calving (anovulatory anestrus [odds ratio, 4.5]) or prolonged postpartum luteal phases (odds ratio, 4.4) [26]. Conception rates are about 20% lower for cows with endometritis, the median calving to conception interval is 30 days longer, and there are 3% more animals culled for failure to conceive [20, 21]. Cows are less fertile even after successful treatment of clinical endometritis than age-matched counterparts in the same herds that had no clinical uterine disease postpartum [20]; this is probably because subclinical endometritis persists after the clinical signs have resolved. Animals with subclinical disease also have more days open, take longer to conceive, and have conception rates about half those of normal animals [24].

The financial effect of uterine disease is derived from infertility, increased culling for failure to conceive, reduced milk production, and the cost of treatment. The economic cost of a single case of metritis has been calculated to be about €292 [18]. Studies report 24 146 000 dairy cows in the European Union [27] and 8 495 000 dairy cows in the United States [28]. Using a conservative incidence rate of 20% for metritis [18, 19], we calculate that the annual cost of uterine disease in the European Union is €1.4 billion and in the United States is \$650 million. The costs of endometritis are an additional burden on the dairy industry and need to be quantified.

PATHOGENESIS OF POSTPARTUM REPRODUCTIVE TRACT DISEASE

During pregnancy, the uterus is sterile, but after parturition the uterine lumen is almost always contaminated with a wide range of bacteria (Fig. 1). However, development of clinical disease is dependent on the balance between host immunity and the pathogenicity of the bacteria. This balance can be tipped in favor of disease by risk factors such as retained placenta, dystocia, twins, and stillbirth [29, 30]. Unfortunately, these factors are not particularly amenable to intervention to reduce the incidence of disease, and the factors that could be addressed (such as the cleanliness of the animal or environment) are less important [31].

Bacterial Infection

Escherichia coli and *Arcanobacterium pyogenes* are the most prevalent bacteria isolated from the uterine lumen of cattle with uterine disease, followed by a range of anaerobic bacteria such as *Prevotella* species, *Fusobacterium necrophorum*, and *Fusobacterium nucleatum* [10–14]. Bacteria are also isolated from the uterus of animals that do not develop clinical disease. Indeed, the presence of coagulase-negative staphylococci and α -haemolytic streptococci decreases the risk of endometritis [11], so probiotics may be considered in the future for prevention of disease. Infection of the uterus with *E. coli* appears to pave the way for subsequent infection with other bacteria or viruses [32–34]. Furthermore, *E. coli* infection during the first days or week after parturition is associated with negative effects on the ovary, hypothalamic-pituitary axis, and general health, as well as uterine disease [32]. However, the most severe endometrial lesions are caused by *A. pyogenes* [14]. The strains of *A. pyogenes* isolated from the uterus all express the virulence gene *plo* [35], which encodes a cholesterol-dependent cytotoxin called pyolysin [36]. Cholesterol-dependent cytotoxin molecules are attracted to cholesterol-rich domains in cell membranes, where they aggregate to form a pore, leading to osmotic death of the cell [36], and

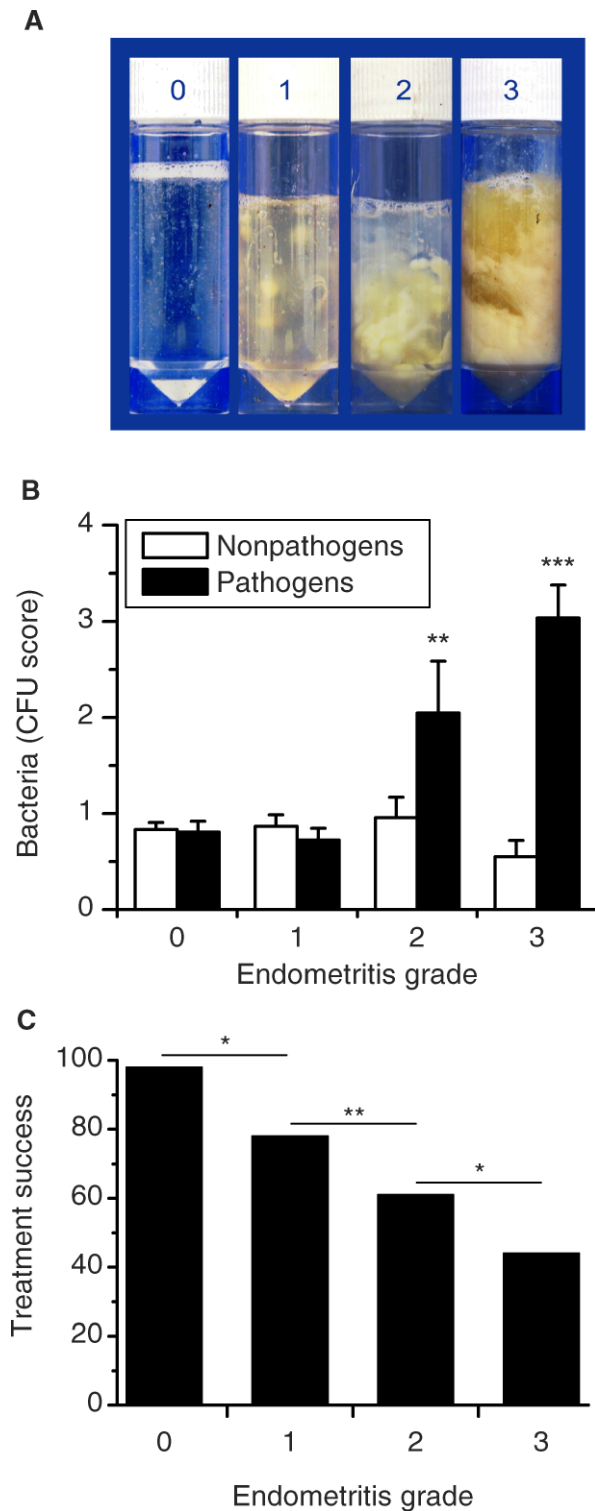


FIG. 2. Grading scheme for clinical endometritis. **A**) Vaginal mucus character is graded as 0 (clear or translucent mucus), 1 (mucus containing flecks of white or off-white pus), 2 (exudate containing <50% white or off-white mucopurulent material), or 3 (exudate containing \geq 50% purulent material, usually white or yellow but occasionally sanguineous) [2]. **B**) Endometritis grades reflect the number of pathogenic (black bars) but not opportunist nonpathogenic (white bars) bacteria isolated from the uterus of cattle [11]; data are presented as semiquantitative scores of the number of colony-forming units (CFU) from uterine swabs, where CFU is scored as 0 (no growth), 1 (<10 CFUs), 2 (10–100 CFUs), 3 (101–500 CFUs), or 4 (>500 CFUs). Values differ from endometritis grade 0, $**P < 0.01$ and $***P < 0.001$. **C**) Endometritis grade is prognostic for treatment success [22]; treatment success rates were determined as the percentage of

pyolysin readily kills endometrial epithelial and stromal cells in vitro [37]. Furthermore, *A. pyogenes*, *F. necrophorum*, and *Prevotella* species act synergistically to enhance the likelihood and severity of uterine disease [38, 39]. *Fusobacterium necrophorum* produces a leukotoxin, *Prevotella melaninogenica* produces a substance that inhibits phagocytosis, and *A. pyogenes* produces a growth factor for *F. necrophorum*. Presumably, the necrotic lochia associated with retained placenta provides an excellent media for bacteria. Trauma to the tissues during parturition also likely facilitates adhesion and invasion of microbes. Finally, suppressed or dysregulated immune mechanisms around the time of parturition (discussed herein) probably also perturb host defense against microbes.

Viral Infection

Bovine herpesvirus 4 (BoHV-4) is the only virus consistently associated with uterine disease after parturition in cattle [40, 41]. Like other herpesviruses, BoHV-4 can establish latent infections in cattle, particularly in macrophages [42], and the viral infection is often identified concurrent with bacteria that cause uterine disease [43, 44]. So, the association between BoHV-4 infection and uterine disease has been hard to establish, although the contribution of BoHV-4 to uterine disease in which the virus is endemic in cattle will become clear when a vaccine is developed. The virus is highly tropic for endometrial cells, rapidly replicating and killing epithelial or stromal cells [42, 45]. BoHV-4 replication is driven by host cellular factors transactivating the viral immediate early (IE2, also known as *UL122*) gene promoter. A luciferase reporter for the *UL122* promoter was transactivated in a concentration-dependent manner when transfected bovine stromal cells were treated with prostaglandin E_2 (PGE), *E. coli*, or its lipopolysaccharide (LPS [endotoxin]), and PGE and LPS acted cooperatively [34]. Furthermore, viral replication was reactivated in latently infected macrophages when cocultured with stromal cells [45]. We suggest that there may be a vicious circle composed of bacterial endometritis, leading to secretion of PGE and then stimulation of viral replication by PGE and LPS, which causes further endometrial tissue damage and inflammation (Fig. 3). Identifying the specific host cellular transcription factors that transactivate the BoHV-4 *UL122* gene to drive viral replication will inform strategies to prevent herpesvirus genital tract disease in cattle and other species.

Uterine Immunity

Mammalian pregnancy involves regulation of uterine immunity to facilitate implantation and survival of the semiallogeneic fetus. The classic view is that immunity is suppressed during gestation, although evidence is emerging that some immune and inflammatory mechanisms are also critical for implantation in mammals [51]. If the immunosuppressive mechanisms associated with pregnancy persist in the endometrium after parturition, they would likely predispose to uterine disease. So, some of the roles of the uterine immune system during pregnancy may be at odds with the need to respond in a coordinated way to pathogenic organisms in the uterus after parturition.

During mid and late pregnancy, lymphocytes and macrophages are found in the intercaruncular endometrium, although

animals ($n = 300$) with normal vaginal mucus 2 wk after initial endometritis grading and treatment 21–28 days postpartum. Values differ between endometritis grades, $*P < 0.05$ and $**P < 0.01$.

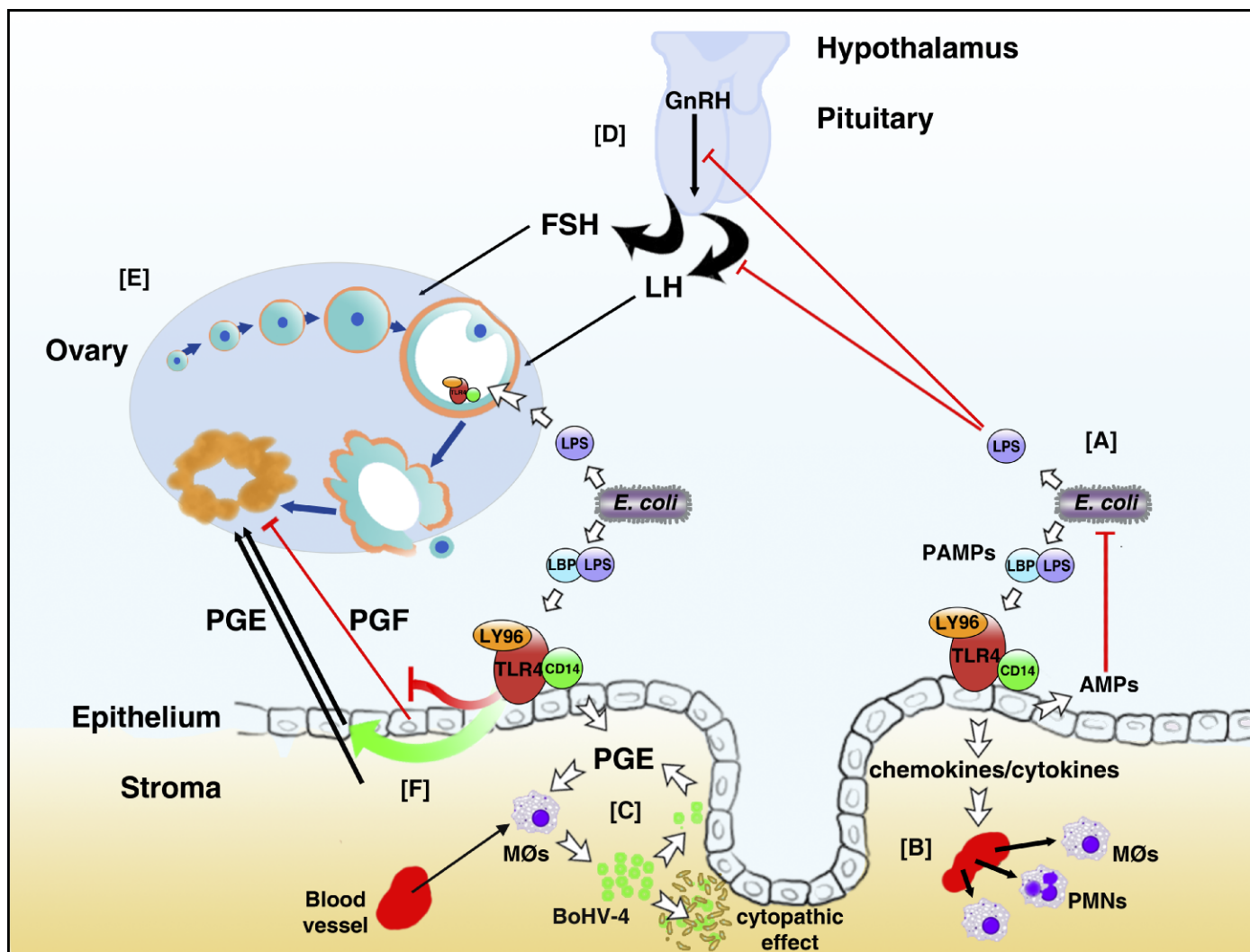


FIG. 3. The mechanisms underlying infertility associated with uterine disease. **A)** Bacterial infection with *E. coli* and *A. pyogenes* is common after parturition [10]. The innate immune system is alerted by endometrial cell TLRs detecting pathogen-associated molecules (such as bacterial DNA and lipids) and *E. coli* LPS, which is bound to LPS-binding protein (LBP) [4, 5]. The bovine endometrial cells secrete cytokines and chemokines to direct the immune response, increase the expression of AMPs, and secrete principally PGE rather than PGF [9, 46]. Bacterial infection causes endometrial damage and inflammation, reducing the chance of conception. **B)** Cytokines and chemokines direct the immune response. Chemokines attract neutrophils (PMNs) and macrophages (MØs) to eliminate the bacteria. However, neutrophil function is often compromised in cattle around the time of parturition [47]. Persistence of PMNs in the endometrium in the absence of bacteria is thought to be the primary characteristic of subclinical endometritis [15, 24]. **C)** It is thought that viral replication may be stimulated in macrophages that are persistently infected with BoHV-4 by PGE and LPS [34]. The BoHV-4 can then infect the endometrial stromal and epithelial cells, causing further tissue damage [45]. **D)** Follicle-stimulating hormone (FSH) concentrations from the pituitary are unaffected by uterine disease, and so waves of ovarian follicles emerge in the first weeks after parturition [10]. However, the release of GnRH from the hypothalamus and LH from the pituitary can be suppressed by LPS, reducing the ability to ovulate a dominant follicle [48, 49]. **E)** Cows with endometritis have slower growth of dominant follicles in the ovary and lower peripheral plasma estradiol concentrations and so are less likely to ovulate [10]. Follicular fluid contains LPS in animals with endometritis, granulosa cells express the TLR4/CD14/LY96 (MD2) complex required to detect LPS, and LPS perturbs estradiol secretion from granulosa cells by reducing aromatase expression [7]. **F)** If cows with endometritis ovulate, they form a corpus luteum secreting progesterone and reinitiate ovarian cycles. However, the peripheral plasma concentrations of progesterone are lower than those in normal fertile animals [32]. Cytokines may perturb luteal cell steroidogenesis [50]. Luteolysis is probably disrupted, and luteal phases are often extended because bacteria switch the endometrial epithelial secretion of prostaglandins from the F series to the E series [9].

not in the caruncular endometrium of cattle [52–54]. The subepithelial uterine stroma contains more CD4⁺ T cells, B cells, CD14⁺ macrophages, and mast cells compared with other regions of the endometrium and the myometrium [55, 56]. Mast cells have a prominent sensor and effector function during bacterial infections in mammals [57], but their role in response to intrauterine bacterial contamination in cattle is not clear. Specialized uterine natural killer cells are important for normal pregnancy in many mammals, but these cells are not common at the end of gestation [58] and are sparse in the bovine endometrium [59]. Whether these immune cells are still

present in the tissues after labor in cattle and whether they regulate the inflammatory process after pathogen contact are largely unknown.

Innate Immunity in the Endometrium and Pathogen Recognition

The initial defense of the mammalian endometrium against microbes is dependent on innate immune systems, including toll-like receptors (TLRs), antimicrobial peptides (AMPs), and acute-phase proteins [60]. Bacteria are detected by pattern

recognition receptors on mammalian cells binding molecules specific to microbial organisms, often called pathogen-associated molecular patterns (PAMPs) [3–5]. The most important group of such receptors comprises the TLRs, and 10 members of the receptor family are widely encoded in the mammalian genome and are most often found in a broad range of immune cells [3, 4]. TLR1, TLR2, and TLR6 recognize bacterial lipids such as lipoteichoic acid, whereas TLR3, TLR7, TLR8, and TLR9 recognize nucleic acids, often from viruses. Lipopolysaccharide from Gram-negative bacteria such as *E. coli* is bound to LPS-binding protein and is recognized by TLR4 in complex with CD14 and LY96 (MD2), TLR5 binds flagellin, and TLR9 also recognizes bacterial DNA. Activation of TLRs initiates signalling cascades, resulting in the synthesis and production of proinflammatory cytokines and chemokines that mobilize and activate immune cells [4, 5], which in the case of bovine uterine disease is particularly associated with the influx of PMNs into the uterus [61].

Whole endometrium from normal nonpregnant cattle expresses TLR1 through TLR10 [46]. Before and after parturition, TLR2, TLR3, TLR4, TLR6, and TLR9 are expressed in the caruncular and intercaruncular endometrium, and TLR expression was greater in the caruncular endometrium than in the intercaruncular endometrium 4–6 h postpartum [62]. Purified populations of epithelial cells express TLR1 through TLR7 and TLR9, and stromal cells express TLR1 through TLR4, TLR6, TLR7, TLR9, and TLR10 [46]. These TLRs appear to be functional, as epithelial cells secreted PGE in response to bacterial PAMPs. Pure populations of epithelial or stromal cells (not contaminated with leukocytes, as determined by the lack of expression of the protein tyrosine phosphatase, receptor type, C [PTPRC, formerly CD45] panleukocyte marker) express the specific receptor complex comprising TLR4/CD14/LY96 (MD2) to bind LPS [6, 9]. Heat-killed *E. coli* or LPS provokes an inflammatory response by the endometrial cells, characterized by the increased expression of transcripts for tumor necrosis factor, nitric oxide synthase, and prostaglandin-endoperoxide synthase 2 (PTGS2, formerly COX-2) and the secretion of prostaglandins $F_{2\alpha}$ (PGF) and PGE [6]. Heat-killed *E. coli*, LPS, *A. pyogenes* pyolysin, BoHV-4, bacterial DNA, and lipids also influence endometrial cell prostaglandin secretion, particularly stimulating the secretion of PGE rather than PGF in cattle [9, 45, 46, 63]. This may explain why animals with uterine infection have higher concentrations than normal animals of LPS and PGE in the uterine lumen and peripheral plasma [32, 64]. Endometrial explants and epithelial and stromal cells also secreted predominantly PGE in response to LPS, and this effect was not reversed by oxytocin [9]. This LPS-induced PGE secretion by endometrial cells is important for fertility because prostaglandins have multiple roles in endometrial function, and luteolysis is initiated by PGF from oxytocin-stimulated epithelial cells [65]. In addition, PGE has an important role in the mammalian immune response, acting through prostaglandin E receptors 2 and 4 (PTGER2 and PTGER4) to control inflammation [66]. The bovine endometrial cells express the PTGER2 and PTGER4 necessary to respond to PGE [9, 67]. The endometrial prostaglandin switch induced by LPS appears to be early in the prostaglandin synthetic pathway. Arachidonic acid is liberated from cell membranes by phospholipase A2 group IV and group VI enzymes (PLA2G4 and PLA2G6) and is converted to prostaglandin H and then PGE or PGF by synthase enzymes [68]. Treatment of endometrial cells with LPS stimulated increased levels of PLA2G6 but not PLA2C protein in epithelial cells but did not change the levels of PGE or PGF synthase enzymes [9].

The AMPs are an ancient component of the immune system, and the defensins family is particularly important for mucosal immunity [69]. Bovine uterine tissue expresses lingual AMP (LAP), tracheal AMP (TAP), bovine neutrophil β -defensins (BNBD4 and DEFB5), and bovine β -defensins (BBD19, BBD123, and BBD124) [70]. Furthermore, pure populations of endometrial epithelial cells express LAP, TAP, BNBD4, and DEFB5, and expression was increased when cells were treated with LPS [46]. Mucin 1 (MUC1) is an epithelial cell glycosylated transmembrane protein that may also have a role in microbial defense of the endometrium in mammals [71]. MUC1 is expressed by epithelial cells of the bovine endometrium, and expression was increased when the cells were treated with LPS [46]. Acute-phase proteins are produced in the liver in response to proinflammatory cytokines, and peripheral plasma concentrations are increased during the first few weeks postpartum in cattle [72]. However, no acute-phase proteins were detected in bovine endometrial cells in vitro [46].

Effector Cell Immigration into the Uterus after Pathogen Contact

Blood-derived PMNs are the main effector cells for removing bacteria from the uterus after calving. However, endocrine and metabolic changes around the time of parturition in cattle modulate PMN phagocytic function and gene expression [47, 73]. Furthermore, blood PMNs obtained from cows with endometritis were significantly less phagocytic [74]. The process of transmigration into the uterine lumen also modulates PMN function. For example, interleukin 8-induced attraction of PMNs into the uterine lumen increased the generation of reactive oxygen species by these cells [61]. However, when PMNs are in the uterine lumen, their function is further modulated by soluble factors in lochial secretions. Whereas lochial secretions of healthy cows only moderately affected the function of PMNs, the secretions of infected cows severely depressed the generation of reactive oxygen species [75].

Regulation of Uterine Immunity

Changes in hormone concentrations around the time of parturition may influence the risk of peripartur infections [76]. Progesterone and estrogen have immunomodulatory properties, changing the repertoire and expression density of hormone receptors in immune cells from cattle [77]. In addition, estradiol and especially progesterone reduce the secretion of prostaglandins by epithelial or stromal cells stimulated with LPS [6]. The somatotrophic axis also influences the course of the bovine puerperium, mediated by changes in plasma and endometrial levels of insulin-like growth factor 1 (IGF1) [78, 79]. Indeed, IGF1 has immunomodulatory properties in addition to its growth-promoting function in mammals [80]. Finally, there are several proteins found in the endometrium that could influence the immune response directly or affect the steroid or IGF1 pathways in endometrial cells. The uterine serpins are progesterone-induced members of the serpin superfamily of serine proteinase inhibitors and, at least in the sheep, inhibit lymphocyte proliferation to mediate the immunosuppressive effects of progesterone on uterine immune function [81]. A family of glycan-binding proteins, the galectins, may also regulate uterine immunity by interacting with multiple galactose- β 1,4-*N*-acetylglucosamine units on cell surface glycoproteins [82, 83]. Lectin, galactoside-binding, soluble, 1 (galectin 1 [LGALS1]) controls mammalian cell proliferation, the survival of effector T cells and neutrophils,

and their extravasation in vivo [83–85]. One of the counter-players of galectin 1 is lectin, galactoside-binding, soluble, 3 (galectin 3 [LGALS3]), which modulates the adhesion of T cells to endothelial cells and the adhesion between T cells and dendritic cells or macrophages [86]. LGALS1 is expressed in the murine and human female reproductive tracts, as well as by immune cells [87, 88]. In humans, LGALS1 expression is strongly enhanced in late-phase endometrium and in the decidua [87], and LGALS1 is differentially expressed between normal and pathologically altered placentas [89, 90]. In cattle, LGALS3 is detected in the ovary, oviduct, uterus, and cervix and is postulated to be involved in mucosal defense [91]. However, the role of galectins in postpartum uterine disease requires further exploration.

UTERINE INFECTION AND OVARIAN FUNCTION

Cows with postpartum uterine infection had slower growth of the first postpartum dominant follicle and lower peripheral plasma estradiol concentrations around the time of maximal follicle diameter, and in those animals that did ovulate, peripheral plasma progesterone concentrations were lower 5–7 days after ovulation (<2 vs. >5 ng/ml) [10, 32]. These effects of uterine microbes on ovarian function could be caused by PAMPs or inflammatory mediators acting on the hypothalamus, pituitary, or ovary.

Hypothalamic and pituitary function is critical for directing ovarian cycles. Follicle-stimulating hormone concentrations are not affected in animals with uterine disease, so follicle waves emerge in diseased animals as in normal animals [10]. However, LPS suppresses hypothalamic release of gonadotropin-releasing hormone (GnRH), pituitary secretion of luteinizing hormone (LH), and the sensitivity of the pituitary to GnRH in sheep [49, 92]. The consequences of these changes would be that animals are less likely to ovulate, and this appears to be the case in cattle administered LPS [48]. However, intrauterine infusion of a lower concentration of LPS in cattle did not disrupt LH secretion [93].

The follicular fluid of cattle with uterine inflammation also contains LPS [7]. Animals with clinical disease had concentrations of LPS that ranged up to 0.8 µg/ml; normal animals did not have measurable concentrations of LPS in their ovarian follicular fluid, while animals with subclinical disease had intermediate concentrations about 40–60 days after calving. Theca cells convert cholesterol to androstenedione, which then passes across the basement membrane of the ovarian follicle and is converted to estradiol by the granulosa cells. Treatment of bovine theca cells from any stage of follicle development with LPS did not affect androstenedione production or cell survival, but granulosa cells collected from growing or dominant follicles secreted less estradiol when treated with LPS [7]. As with endometrial cells, LPS does not affect theca cell or granulosa cell survival. The effect of LPS on bovine granulosa cells appears to be a direct one, as the granulosa cell cultures were free of contaminating leukocytes [7], and the granulosa cell compartment within the basement membrane of the ovarian follicle is devoid of immune cells in vivo, at least in mice [94]. Furthermore, granulosa cells from cattle express the TLR4/CD14/LY96 (MD2) complex required for binding LPS [7]. Aromatase transcript expression was reduced by LPS treatment of granulosa cells collected from dominant follicles [7]. So, granulosa cells have a mechanism for direct action of LPS in the ovarian follicle to impair ovarian function and ovulation. The effect of uterine disease on follicular function may be further enhanced by cytokines released by the endometrial cells because granulosa cell steroidogenesis is

also impaired by proinflammatory cytokines [95]. If animals ovulate, the cytokines secreted by the infected endometrium may also partly explain the reduced progesterone secretion from the corpus luteum because bovine luteal cells are highly responsive to a range of cytokines and cytokines are also important in luteolysis [50, 96].

The extended luteal phases in some cows with uterine disease could be associated with effects on luteolysis or on luteal cell function. Certainly, the switch in endometrial prostaglandin to PGE from PGF could disrupt the luteolytic mechanism [9]. In ruminants, PGE is luteotropic, while PGF is luteolytic [65]. Using endometrial explants, the ratio of PGE:PGF concentration was 0.45 in response to oxytocin and 2.75 following LPS treatment [9]. Furthermore, administering oxytocin after treatment of endometrial cells with LPS did not reverse the propensity to secrete PGE [9].

CONCLUSIONS

In conclusion, uterine infections are common after parturition in dairy cattle, causing infertility. The working model that links the mechanisms of infection and immunity with infertility is summarized in Figure 3. Bacterial infection with *E. coli* precedes infection with other microbes that disrupt endometrial structure and function. The innate immune system is alerted to the presence of pathogens by endometrial cell TLRs detecting pathogen-associated molecules such as LPS, DNA, and bacterial lipids. The endometrial cells secrete cytokines and chemokines to direct the immune response and increase the expression of AMPs. Chemokines attract PMNs and macrophages to eliminate the bacteria, although neutrophil function is often perturbed in postpartum dairy cows. Persistence of PMNs in the endometrium in the absence of bacteria is thought to be the primary characteristic of subclinical endometritis. Uterine disease also affects ovarian function. Cows with uterine bacterial infections have slower growth of dominant follicles in the ovary and lower peripheral plasma estradiol concentrations and so are less likely to ovulate. The release of GnRH from the hypothalamus and LH from the pituitary can also be suppressed by LPS, further reducing the ability to ovulate a dominant follicle. Follicular fluid contains LPS in animals with endometritis, granulosa cells express the TLR4/CD14/LY96 (MD2) complex required to detect LPS, and LPS reduces estradiol secretion. If cows with uterine infections ovulate, the peripheral plasma concentrations of progesterone are lower than those in normal fertile animals, and luteal phases are often extended. Luteolysis is probably disrupted because bacteria switch the endometrial epithelial secretion of prostaglandins from the F series to the E series. The regulation of endometrial immunity depends on steroid hormones, somatotrophins, and possibly local regulatory proteins such as galectins. Advances in knowledge about infection and immunity in the female genital tract should be exploited to develop new treatments and prevention strategies for uterine disease.

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