Innate immunity and inflammation of the bovine female reproductive tract in health and disease

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Abstract

Mammalian reproductive physiology and the development of viviparity co-evolved with inflammation and immunity over millennia. Many inflammatory mediators contribute to paracrine and endocrine signalling, and the maintenance of tissue homeostasis in the female reproductive tract. However, inflammation is also a feature of microbial infections of the reproductive tract. Bacteria and viruses commonly cause endometritis, perturb ovarian follicle development and suppress the endocrine activity of the hypothalamus and pituitary in cattle. Innate immunity is an evolutionary ancient system that orchestrates host cell inflammatory responses aimed at eliminating pathogens and repairing damaged tissue. Pattern recognition receptors on host cells bind pathogen-associated molecular patterns and damage-associated molecular patterns, leading to the activation of intracellular MAPK and NFκB signalling pathways and the release of inflammatory mediators. Inflammatory mediators typically include the interleukin cytokines IL1β and IL6, chemokines such as IL8, interferons and prostaglandins. This review outlines the mechanisms of inflammation and innate immunity in the bovine female reproductive tract during health and disease condition.

Introduction

Inflammation is a cellular reaction triggered by noxious stimuli, including infection and tissue injury, which evolved as an adaptive response for restoring homeostasis in the cells and tissue of simple organisms (Medzhitov 2008). Before the appearance of receptors and signalling cascades, cytokines evolved as intracellular messengers in invertebrates, where they play a role in host defence and repair (Dinarello 2007). The general term ‘cytokine’ includes several families of proteins including interleukins (IL), chemokines, interferons, mesenchymal growth factors, adipokines and tumour necrosis factors (TNF), whilst typical lipid mediators of inflammation include prostaglandins and resolvins. Most of these inflammatory mediators are soluble factors that are produced by one cell and act on another target cell to change its function; some consider these mediators as the ‘hormones’ of the immune and inflammatory responses (Dinarello 2007). The systems of inflammation and innate immunity have co-evolved with reproductive physiology, and the development of viviparity over 340 million years. Several inflammatory mediators have been co-opted and used for signalling processes during evolution of the reproductive system, and have primary roles in physiology rather than inflammation. Inflammation is also part of maintaining tissue homeostasis during physiological changes in architecture, such as ovulation or parturition. However, innate immunity remains vital to protect the female reproductive tract from the adverse effects of microbial infections. These infections are particularly important in Bos taurus, where bacteria and viruses commonly cause infertility. This review outlines the mechanisms of inflammation and innate immunity in the bovine female reproductive tract during health and disease.

Inflammation

After infection or tissue injury, inflammatory mediators are released resulting in the cardinal signs of
Inflammation: redness, heat, swelling, pain and loss of function. Inflammatory mediators typically include cytokines such as IL1β, IL6 and TNFα; chemokines such as IL8 and prostaglandin E2. These mediators initially direct the inflammatory response by attracting and activating immune cells, particularly neutrophils and macrophages, to remove microbes and damaged host cells. Later, inflammatory mediators such as IL10 and resolvins foster the repair of tissue and coordinate the timely resolution of inflammation. However, multiple cytokines are involved in the inflammatory response, many have pleiotropic effects, and they often duplicate the function of each other.

**Innate immunity**

Innate immunity encompasses several facets of non-specific protection against microbial infection and tissue damage in the female reproductive tract. The obvious anatomical barriers comprising the vulva, vagina and cervix counter ascending microbial infections reaching the uterus. Other physical barriers in the genital tract include the stratified squamous epithelium of the vagina, the columnar epithelium of the endometrium, the basement membrane of ovarian follicles and the zona pellucida of the oocyte. Antimicrobial peptides and mucosal glycoproteins also cover the mucosa to neutralise the basement membrane of ovarian follicles and the zona pellucida of the oocyte. Antimicrobial peptides and mucosal glycoproteins also cover the mucosa to neutralise bacteria and prevent them reaching the epithelium. The principal cysteine-rich, cationic, antimicrobial peptides expressed in the bovine endometrium include β-defensins, lingual antimicrobial peptide (LAP), and tracheal antimicrobial peptide (TAP), and their transcripts are more abundant in the face of microbial challenge (Davies et al. 2008, Chapwanya et al. 2013). Other surface molecules that may help to protect the endometrium include the mucins; evidence for their role includes genetic deletion of Muc1 in mice, which is associated with chronic inflammation of the lower female reproductive tract by opportunistic bacterial infections (DeSouza et al. 1999). The expression of mRNA encoding acute-phase proteins in the uterus and ovary is also of interest, because they may provide further localised protection (Fischer et al. 2010, Lecchi et al. 2012, Chapwanya et al. 2013). Although, it is possible that the more usual hepatic production of acute-phase proteins in response to increased serum concentrations of IL6 may be more relevant in vivo. The complement system is also present in the female reproductive tract, and this series of related proteins opsonise infected cells to attract immunoglobulin and drive the formation of the membrane attack complex leading to cytolysis. However, normal cells in the reproductive tract are protected against formation of the complement complex by complement regulatory proteins including CD46, CD55 and CD59 (Jensen et al. 1995).

Whilst physical barriers and secreted proteins are important for passive defence against microbes in the female reproductive tract, there are some caveats. First, microbes have evolved strategies to avoid many host defences. For example, some bacteria produce enzymes that digest mucus to penetrate the protective layer, and many bacteria secrete proteases that can disrupt protective peptides and proteins produced by the host (Baxt et al. 2013). Second, the rapid inflammatory responses associated with microbial infections are associated with pattern recognition receptors of the innate immune system, rather than passive defence systems. Finally, there is also a role for adaptive immunity in the bovine reproductive tract, with the presence of plasma cells in the endometrium and accumulation of immunoglobulins (Anderson et al. 1996). However, the mechanisms of adaptive immunity in the female reproductive tract are not part of the present review.

Innate immunity is predicated on host cells proteins, called pattern recognition receptors that are able to sense pathogens or danger. Innate immunity is evolutionarily conserved across animal phyla, and the first pattern recognition receptor to be identified was Toll in Drosophila melanogaster (Lemaître et al. 1996). Toll-like receptors (TLRs) and Nod-like receptors (NLRs) are the two most studied families of pattern recognition receptors in mammals, and some of their ligands are listed in Table 1. The TLRs and NLRs principally bind pathogen-associated molecular patterns (PAMPs) found in prokaryotes, but not eukaryotes (Beutler 2009, Takeuchi & Akira 2010). However, danger signals and endogenous damage associated molecular patterns

**Table 1** Pattern recognition receptors, their localisation and the pathogen-associated molecular patterns they bind.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Localisation</th>
<th>Pathogen-associated molecular patterns</th>
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<tbody>
<tr>
<td>TLR1</td>
<td>Plasma membrane</td>
<td>Triacylated bacterial lipopeptides</td>
</tr>
<tr>
<td>TLR2</td>
<td>Plasma membrane</td>
<td>Bacterial lipopeptides, glycolipids, peptidoglycan</td>
</tr>
<tr>
<td>TLR3</td>
<td>Endosome</td>
<td>Viral double-stranded RNA</td>
</tr>
<tr>
<td>TLR4</td>
<td>Plasma membrane/endosome</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>TLR5</td>
<td>Plasma membrane</td>
<td>Flagellin</td>
</tr>
<tr>
<td>TLR6</td>
<td>Plasma membrane</td>
<td>Dicacylated bacterial lipopeptides</td>
</tr>
<tr>
<td>TLR7</td>
<td>Endosome</td>
<td>Viral and bacterial single-stranded RNA</td>
</tr>
<tr>
<td>TLR8</td>
<td>Endosome</td>
<td>Viral and bacterial single-stranded RNA</td>
</tr>
<tr>
<td>TLR9</td>
<td>Endosome</td>
<td>CpG-rich bacterial and viral DNA</td>
</tr>
<tr>
<td>TLR10</td>
<td>Plasma membrane</td>
<td>Unknown</td>
</tr>
<tr>
<td>RIG-I</td>
<td>Cytoplasm</td>
<td>Short double-stranded RNA</td>
</tr>
<tr>
<td>NOD1</td>
<td>Cytoplasm</td>
<td>O-glutamyl-meso-diaminopimelic acid</td>
</tr>
<tr>
<td>NOD2</td>
<td>Cytoplasm</td>
<td>Muramyl dipeptide</td>
</tr>
<tr>
<td>NLRP3</td>
<td>Cytoplasm</td>
<td>Uric acid crystals, cholesterol-dependent cytolysins</td>
</tr>
<tr>
<td>NLRC4</td>
<td>Cytoplasm</td>
<td>Flagellin, type III secretion system proteins</td>
</tr>
</tbody>
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(DAMPs), such as high-mobility group box 1 protein (HMGB1), ATP, nucleic acids and IL1α, released from damaged or dead cells, are also thought to bind pattern recognition receptors to initiate an inflammatory response (Chen & Nunez 2010). The most important TLRs for the recognition of bacteria are TLR1, TLR2 and TLR6, which form heterodimers to bind bacterial lipopeptides (Fig. 1), and TLR4, which binds the cell wall component of Gram-negative bacteria lipopolysaccharide (LPS, endotoxin), in complex with CD14 and MD2 (Takeuchi & Akira 2010). The NLRs, such as NODs and NLRP3, are intra-cytoplasmic receptors that recognise endogenous molecules as well as microbial molecules. Binding of PAMPs to pattern recognition receptors stimulates the production of inflammatory mediators, which recruit neutrophils and monocytes to the site of infection (Beutler 2009, Takeuchi & Akira 2010). In addition, NLR activation leads to the formation of the multi-protein inflammasome complex, characteristically leading to cleavage of caspase-1 to an active form and production of mature IL1β (Schröder & Tschopp 2010).

The innate immune response to PAMPs and DAMPs is most commonly driven by macrophages, neutrophils and dendritic cells. However, a range of cells other than ‘professional’ immune cells also express pattern recognition receptors. For example, endometrial epithelial and stromal cells express the TLR4–CD14–MD2 receptor complex necessary to detect LPS, and respond to LPS by secreting IL6, IL8 and prostaglandin E2 (Herath et al. 2009a, Cronin et al. 2012). So, the overall inflammatory response to pathogens associated with innate immunity depends on the sum of the actions of multiple cell types rather than just specialised immune cells. Furthermore, host cellular responses to pathogens are greater than when the same cells encounter commensal microbes. The impact of pathogens on the host is enhanced by a multitude of virulence factors including pore-forming toxins, bacterial secretion systems and bacterial proteases (Blander & Sander 2012). In addition, unlike commensal organisms, pathogens not only colonise tissues but also invade the cells of the female reproductive tract, often causing cell death (Donofrio et al. 2007, Sheldon et al. 2010). Therefore, the scale of the inflammatory response to microbes is dependent on multiple factors including microbial virulence effectors, host tissue factors, and on the regulation of intracellular signalling pathways associated with innate immunity.

The adaptor molecule MYD88 is essential for downstream signalling of all TLRs, with the exception of TLR3, although additional adaptor proteins are also involved with some of the TLR dimers (Fig. 1; Takeuchi & Akira 2010, Moresco et al. 2011). Once activated, MYD88 stimulates IL1R-associated kinases, which in turn activate TNFR-associated factor 6 (TRAF6) that catalyses a complex consisting of TGFβ-activated kinase 1 (TAK1) and the members of the TAK1-binding protein (TAB) family. This complex activates two important pathways in inflammation: the MAP kinase pathway and the NFκB pathway. The MAP kinase cascade (JNK, ERK1/2 and p38) activates the transcription factor AP1, which along with NFκB drives transcription in the nucleus (Fig. 1).
Typical genes transcribed by activation of these MAPK and NFκB signalling pathways from surface TLRs include cytokines (IL1A, IL1B, IL6 and TNFα), chemokines (CXCL8 and CXCL1), and lipid mediators (PLA2, PGHS and PGES). On the other hand, internal TLRs activate signalling pathways in response to microbial nucleotides that lead to the expression of type I interferons (Fig. 1).

**Inflammation and reproductive physiology**

There are extensive reviews about the role of inflammatory mediators and inflammation in reproductive physiology (Richards et al. 2002, 2008, Jabbour et al. 2009, Turner et al. 2012). In particular, physiological events within the ovary, including ovulation and corpus luteum formation and regression, are inflammatory-like events (Espey 1980). Whilst the luteinising hormone (LH) surge is the main trigger for ovulation, inflammatory mediators regulate several of the events around the time of ovulation. For example, IL6 regulates ovarian cumulus granulosa cell function, cumulus–oocyte complex expansion, and oocyte competence, at least in mice (Liu et al. 2009). The process of ovulation involves rupture of the dominant follicle, which causes tissue damage, and so it is not surprising that innate immunity and inflammation have roles in the maintenance of tissue homeostasis in the ovary (Richards et al. 2008). In the mouse, TLR4 may have a role in normal cumulus–oocyte complex expansion and ovulation by binding endogenous ligands such as hyaluronic acid (Shimada et al. 2008). After ovulation, the oocyte enters the uterine tube (oviduct), and although many neutrophils are present, prostaglandin E2 released by uterine tube epithelial cells after LH stimulation suppresses the phagocytosis of sperm by neutrophils, thereby supporting sperm survival in the uterine tube (Marey et al. 2014). As well as responding to LH, the uterine tube epithelial cells have innate immune capabilities, expressing TLR2 and TLR4, and generating inflammatory responses to LPS (Kowsar et al. 2013). The LH surge also stimulates localised production of IL8 and angiogenic factors, basic fibroblast growth factor and vascular endothelial growth factor A, which are essential for the development of the corpus luteum (Schams et al. 2001, Berisha et al. 2006, Jiemtaweeboon et al. 2011). Lymphangiogenesis is also important in the bovine corpus luteum, with lymphangiogenic factors expression particularly in the corpus luteum of mid-cycle as well as early pregnancy (Nitta et al. 2011). If cattle are pregnant, then interferon tau released by the trophoblast prevents the development of the luteolytic mechanism by inhibiting the expression of the oxytocin receptor gene in the endometrial epithelium, which prevents the release of luteolytic pulses of prostaglandin F2α (Domiak et al. 2013). Whereas, in the absence of an embryo, prostaglandin F2α induces luteolysis with the assistance of other inflammatory mediators such as TNFα (Skarzynski & Okuda 2010).

**Parturition and the postpartum period**

Progesterone maintains uterine quiescence throughout gestation, but before parturition the influence of progesterone diminishes rapidly. In several species, the reduction in the influence of progesterone is associated with an increase in inflammatory mediators, such as IL1β, IL6, IL8, and TNFα, and an influx of leukocytes (Challis et al. 2009). Similar mechanisms are likely important in cattle, as there is increased expression of genes such as CXCL8, CXCL2 and CXCL6 in the placentomes of healthy cattle during parturition (Streyl et al. 2012). Another feature of the parturial bovine uterus is the accumulation of CD14+ monocytes and macrophages in the caruncular stroma and septa, although not in the foetal part of the placentomes (Miyoshi & Sawamukai 2004, Oliveira & Hansen 2009). Activated macrophages enhance the local inflammatory response by attracting neutrophils and monocytes, which clear apoptotic cells, and remodel the caruncle, aiding the successful release of the foetal membranes. Whilst the distribution and concentration of CD14+ cells is similar in healthy cows and cows that develop retained foetal membranes, the latter have lower concentrations of acid phosphatases, a lysosomal enzyme of macrophages (Miyoshi et al. 2002). The uterus must recover rapidly after parturition in preparation for establishment of the next pregnancy. Recovery involves tissue repair, regeneration of the epithelium lining the endometrium, and elimination of bacteria that always contaminate the uterus around the time of parturition.

**Microbial infections of the female reproductive tract**

*Escherichia coli* infect the endometrium within a week of parturition, followed in subsequent weeks by *Trueperella pyogenes* and a range of anaerobic bacteria that include *Fusobacterium*, *Prevotella* and *Bacteriodes* species (Bonnett et al. 1991, Sheldon et al. 2002, 2010). Although not the focus of the present manuscript, it is important to note that bovine herpesvirus 4 is also tropic for endometrial stromal cells and causes *postpartum* uterine disease in some animals (Donofrio et al. 2007). The pathogens most consistently associated with uterine disease have been established by traditional microbiological culture methods. However, using molecular techniques, many more non-culturable microbes are identified in the uterus of *postpartum* cattle, with or without clinical signs of uterine disease (Santos & Bicalho 2012). Indeed, molecular markers for some of these species of bacteria may lead to diagnostic tests for a healthy or diseased uterus. The presence of several acid-producing strains of bacteria in the uterine lumen is interesting because *Lactobacilli* predominate in the healthy human vaginal microbiome, where they help to maintain a low pH to protect against pathogenic bacteria (Ravel et al. 2011).
Pathogens have evolved many virulence factors that may be important in the female reproductive tract, including toxins, proteases, neuramidases, secretion systems, fimbriae and iron acquisition systems. For example, endometrial pathogenic E. coli (EnPEC) adhere to and invade primary endometrial epithelial and stromal cells, cause inflammation, and recreate disease when infused into the uterus of mice (Sheldon et al. 2010). In particular, LPS appears to be the major virulence factor of EnPEC, stimulating TLR4-dependent inflammatory responses by endometrial cells (Sheldon et al. 2010, Cronin et al. 2012). On the other hand, the presence of T. pyogenes is associated with pus in the uterus, the severity of clinical signs and the extent of the subsequent subfertility (Bonnett et al. 2002, Karsch et al. 2010). The major virulence factor produced by all isolates of T. pyogenes is pyolysin, which is a heat-labile exotoxin that multimerises in cholesterol-rich domains of the plasma membrane of mammalian cells, creating transmembrane pores, 30–50 nm diameter, to disrupt ion balances and cause cytolysis (Jost & Billington 2005, Amos et al. 2014). Endometrial stromal cells are more sensitive to cytolysis by pyolysin than epithelial or immune cells, which explains how the bacteria act as pathogens, once the relatively resistant epithelium is denuded after parturition (Amos et al. 2014). Interestingly, the higher cholesterol content of stromal cells, compared with epithelial cells, explains the sensitivity of stromal cells to pyolysin, and reducing cellular cholesterol using cyclohexatin protects stromal cells against pyolysin (Amos et al. 2014).

Animal models of uterine infection and disease

The fundamental mechanisms of immunity and impact on the brain are most often studied by infusion of LPS into the uterus or into the peripheral circulation (Peter et al. 1989, Karsch et al. 2002). However, animal models of clinical uterine disease are also important because they are needed for the development of new vaccines or treatments, as well as uncovering the mechanisms of immunity in the female reproductive tract. Animal models of uterine disease have been generated in dairy and beef cattle, sheep and mice (Ayliffe & Noakes 1982, Karsch et al. 2002, Sheldon et al. 2010, Amos et al. 2014). Most of the animal models that replicate uterine disease rely on infusion of E. coli or T. pyogenes into the uterine lumen (Rowson et al. 1953, Ayliffe & Noakes 1982, Sheldon et al. 2010, Amos et al. 2014). A key step in generating models of uterine disease in cattle is mechanical debridement of the epithelium lining the endometrium (Ayliffe & Noakes 1982, Amos et al. 2014). Presumably, this allows T. pyogenes and pyolysin to access the stromal compartment of the endometrium to establish infection and cause tissue damage. The duration and severity of clinical endometritis following infusion of bacteria can be increased by administering exogenous progesterone to the animal, or by infusion of bacteria during the luteal phase of the oestrous cycle (Rowson et al. 1953, Lewis 2003). Similarly, early ovulation after parturition and establishment of a progesterone-dominated phase before pathogenic bacteria are eliminated predisposes to the development of pyometra (Olson et al. 1984). Conversely, administering oestrogens or oestrus often precludes the establishment of uterine disease (Rowson et al. 1953, Lewis 2003). However, the mechanisms linking the steroids to immunity are yet to be fully elucidated and are complex because there are interactions between the physiological effects of ovarian steroids and the inflammation caused by infusion of bacteria or LPS (Peter et al. 1989, Karsch et al. 2002, Lewis 2003).

Endometrial responses to bacteria in the uterus

Host responses to bacterial infection of the uterus are characterised by inflammation of the endometrium with infiltration by neutrophils and macrophages, and the accumulation of pus in the uterine lumen (Fig. 2). The diseases are defined as metritis, clinical endometritis or subclinical endometritis, depending on the signs and the time after parturition (Sheldon et al. 2006). The samples collected from the endometrium of diseased animals, compared with unaffected animals, have more abundant gene transcripts for the following: cytokines IL1A, IL1B, IL6 and TNF; chemokines CXCL8, CCL5 and CXCL5;...
calcium-binding proteins S100A8, S100A9 and S100A12 and receptors TLR4 and IL1R (Gabler et al. 2009, Herath et al. 2009b, Wathes et al. 2009, Fischer et al. 2010). Furthermore, there are temporal changes in the expression of transcripts for IL1B, IL6, CXCL8, TNF, CXCL5, HP and PTGS2 during the postpartum period, with peak expression on day 17 after parturition (Gabler et al. 2010). These temporal changes in gene expression reflect the histological evidence of inflammation during the first 3 weeks postpartum, which resolves by 40–60 days after parturition (Bonnett et al. 1991, Chapwanya et al. 2012). As well as increased gene expression, postpartum cows, particularly if the endometrium is infected, have increased protein abundance of S100A8 and S100A9 in the epithelium and stroma, as well as in immune cells, as determined by immunohistochemistry (Swangchan-Uthai et al. 2013). The findings at the transcription level are also supported by analysis of the proteome profile of cows with endometritis, which have more S100A9 and cathelicidin antimicrobial protein in the endometrium, compared with healthy cows (Ledgard et al. 2013). Taken together, these endometrial inflammatory responses are likely part of the physiological mechanisms of tissue homeostasis (Medzhitov 2008). However, stressors such as limitations on nutrient supply around the time of parturition may lead to excessive inflammatory responses and increased risk of disease (Wathes et al. 2009, Swangchan-Uthai et al. 2013).

The tissue responses following postpartum bacterial infection point to an important role for innate immunity. Endometrial biopsies collected from healthy and diseased postpartum dairy cows express mRNA for all ten TLRs (Herath et al. 2009b). In addition, ex vivo organ cultures of bovine endometrium accumulate IL1B, IL6, IL8, and prostaglandin E2 when challenged with E. coli or T. pyogenes (Borges et al. 2012, Amos et al. 2014). Furthermore, pure populations of endometrial epithelial and stromal cells express most TLRs and their co-receptors, such as MD2 and CD14 (Herath et al. 2006, Davies et al. 2008). Addition of LPS to epithelial or stromal cells in vitro stimulates phosphorylation of MAPK and the nuclear translocation of NFkB, secretion of prostaglandin F2α, prostaglandin E2, IL6 and IL8, and increased expression of antimicrobial peptides MUC1, LAP and TAP (Herath et al. 2006, Davies et al. 2008, Cronin et al. 2012). Intriguingly, the release of IL8 from polarised epithelial cells is vectorial and directed apically if the challenge is in the uterine lumen (MacKintosh et al. 2013). Similar cytokine and chemokine responses are also evident for cells treated with bacterial lipopeptides, with formal proof for the importance of innate immunity established using siRNA targeting TLR1, TLR2, TLR4 and TLR6 to suppress inflammation (Cronin et al. 2012, Turner et al. 2014). There are also increased peripheral blood concentrations of acute-phase proteins, such as serum amyloid A and haptoglobin, which are produced by hepatocytes in response to cytokines, such as IL6, from the infected uterus (Sheldon et al. 2001). Taken together, these data support the concept that the epithelial and stromal cells in the endometrium have roles in the initial sensing and response to pathogens (Fig. 2). A remaining question is the role of DAMPs in endometritis, because uterine infections are often associated with tissue damage including dystocia, retained foetal membranes and a large male calf.

Ovarian follicle responses to bacteria in the uterus

Follicle and oocyte development is a highly coordinated series of events starting with primordial-to-primary and secondary follicle transition, controlled by autocrine and paracrine signals including PTEN, C-KIT and FOXO3 (Parrott & Skinner 1999, Castrillon et al. 2003, Reddy et al. 2008). This is followed by gonadotrophin-dependent follicle growth, before formation of the follicle antrum and then selection of the dominant follicle. Whilst links between inflammation in the endometrium and infertility are intuitive, it was less obvious whether uterine infections might affect ovarian function. However, in dairy cattle, uterine disease is associated with LPS in follicular fluid, slower growth of dominant follicles, lower peripheral plasma concentrations of oestradiol and increased risks of anoestru or cystic ovarian disease (Opsomer et al. 2000, Sheldon et al. 2002, Herath et al. 2007). The mechanisms underlying these observations are now emerging.

During early stage of follicle development in the bovine ovary, LPS increases ex vivo primordial-to-primary follicle activation, which is associated with loss of primordial follicle PTEN and cytoplasmic translocation of FOXO3 (Bromfield & Sheldon 2013). The implication of this work is that multiple bacterial infections could deplete the ovarian follicle reserve, although LPS does not affect secondary follicle growth and function (Bromfield & Sheldon 2013). Antral follicles present a special situation because whilst the stroma of the ovary contains a wide range of immune cells including macrophages that might protect pre-antral follicles, healthy antral follicles are devoid of such cells within the basement membrane (Bromfield & Sheldon 2011). Intriguingly, mural granulosa cells lining emerged and dominant antral follicles express TLRs and secrete IL1β, IL6 and IL8 when treated with ligands that bind TLR2 or TLR4 (Bromfield & Sheldon 2011, Price & Sheldon 2013, Price et al. 2013). Treatment with PAMPS also reduces the expression of CYP19A1 mRNA and aromatase protein, as well as the secretion of oestriadiol from granulosa cells (Herath et al. 2007, Price et al. 2013). The responses of granulosa cells are rapid, with increased phosphorylation of p38 and ERK1/2, and expression of IL6, IL1β, IL10, TNF, CXCL8 and CCL5 mRNA (Bromfield & Sheldon 2011, Price & Sheldon 2013, Price et al. 2013). Furthermore, the innate immune response is attenuated using siRNA targeting TLR2 or...
TLR4, or by treating granulosa cells with inhibitors targeting MAPK or NFkB.

As physiological events within the ovary use several inflammatory mediators, events associated with infection of the female genital tract may disrupt the biology of reproduction. For example, expansion of the cumulus–oocyte complex is a key event during ovulation but, even in the absence of gonadotrophin, high concentrations of LPS cause inappropriate expansion, which would compromise fertility (Bromfield & Sheldon 2011). Even the oocyte is at risk from pathogens, and bovine virus diarrhoea and bovine herpesvirus 1 are amongst viruses that have been isolated from oocytes denuded of granulosa cells. Furthermore, in vitro maturation of oocytes in the presence of LPS reduces meiotic competence from 85 to 64%, and this failure cannot be rescued by the administration of exogenous gonadotrophins (Bromfield & Sheldon 2013). Therefore, alterations in the follicular environment due to pathogen exposure results in diminished oocyte quality, further affecting fertility.

Host responses in the brain associated with uterine disease

In postpartum cows, LPS in the uterus suppresses the LH surge and prevents ovulation (Peter et al. 1989). Also, during the follicular phase, LPS decreases LH pulse frequency, decreases oestradiol and interrupts the LH surge and ovulation in cattle (Suzuki et al. 2001, Lavon et al. 2008). Similar observations have been made in sheep, which is the species most often used to study the fertility (Bromfield & Sheldon 2011). Even the oocyte is at risk from pathogens, and bovine virus diarrhoea and bovine herpesvirus 1 are amongst viruses that have been isolated from oocytes denuded of granulosa cells. Furthermore, in vitro maturation of oocytes in the presence of LPS reduces meiotic competence from 85 to 64%, and this failure cannot be rescued by the administration of exogenous gonadotrophins (Bromfield & Sheldon 2013). Therefore, alterations in the follicular environment due to pathogen exposure results in diminished oocyte quality, further affecting fertility.

Administration of LPS during the follicular phase causes a delay in the onset of LH surge and this is positively correlated with the duration of oestradiol signal disruption (Fergani et al. 2012). Also, the expression of GNRH and GNRHR mRNA levels in the mPOA and median eminence is lower, but unexpectedly not in the mediod basal hypothalamus (Herman et al. 2012). These neuroendocrine disruptions are associated with an increase in C-FOS within the VMN, ARC, mPOA and diagonal band of Broca, but there is no anticipated activation of oestradiol receptor (ERα) containing cells in the ARC and mPOA (Fig. 3; Fergani et al. 2012). LPS also inhibits kisspeptin and dynorphin cell activation in the ARC and kisspeptin in the mPOA, and increases somatostatin activation in the VMN (Fergani et al. 2013); and C Fergani, J E Routly, D N Jones, L C Pickavance, R F Smith and H Dobson (2011), unpublished data). Somatostatin is one of the most potent inhibitors of GNRH neurone excitability so far identified (Bhattarai et al. 2010).

Not surprisingly, LPS treatment increases FOS and corticotrophin-releasing factor (CRF) mRNA levels within the paraventricular nucleus, the stress-control centre of the hypothalamus (Vellucci et al. 1995), as well as increasing CRF receptors (CRFR) in the ARC and median eminence (Fig. 3; Fergani et al. 2013). Therefore, there are two possible pathways for CRF suppression of GnRH, one being the direct association of CRF and GnRH cell terminals in the ME (Ghuman et al. 2010) and the other being the regulation of kisspeptin-neurokinin B-dynorphin cells and others in the ARC and median eminence (Fergani et al. 2013).

A cholinergic pathway is also involved in restraining the central response to LPS. Peripheral LPS increases the expression of IL1B and IL1R1 genes, as well as IL1β concentration in the mPOA and the hypothalamus, but the effect is reversed by concomitant rivastigmine, an
Indeed, acetylcholine itself reduces LPS-induced release of IL1β and TNFα. Furthermore, rivastigmine reverses LPS-induced suppression of GNRH and GNRHR mRNA levels in the ME as well as LHB and GNRHR mRNA levels in the pituitary (Herman et al. 2013b). A final caveat is that many of the GNRH/LH responses are not specific to LPS. Similar changes occur following other stressors, for example shearing, transport and isolation (Dobson et al. 2012). Thus, future hypotheses must take into account similarities and differences in responses, as well as issues relating to stressors of differing intensity.

Conclusions

The integration of innate immunity and inflammation in the female reproductive system has likely evolved...
over millennia. There appears to be adoption of inflammatory mediators in the physiology of reproduction in cattle, whilst innate immunity remains important for maintenance of tissue homeostasis and defence against pathogens. In dairy cattle, the need to control postpartum infections of the genital tract highlights the importance of understanding the mechanisms underlying the integration of innate immunity, inflammation and reproductive physiology. In addition, these interactions are modulated by stress, the environment and metabolism, which are likely to become more important with the need to feed the expanding human population and to counter climate change. An integrated approach to understand the interactions between innate immunity, inflammation and reproduction will be important for developing strategies to maintain animal health and welfare.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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References
Ayliffe TR & Noakes DE 1982 Effects of exogenous oestrogen and experimentally induced endometritis on absorption of sodium benzyll- penicillin from the cow's uterus. Veterinary Record 110 96–98. (doi:10.1136/vr.110.5.96)
Chen GY & Nunesz G 2010 Sterile inflammation: sensing and reacting to damage. Nature Reviews Immunology 10 826–837. (doi:10.1038/ nrri2873)

www.reproduction-online.org

Immunity and inflammation in reproduction

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Reproduction (2014) 148 R41–R51
IFNT in lymphangiogenesis in the corpus luteum during the maternal recognition period in the cow. Reproduction 142 879–892. (doi:10.1530/REP-11-0157)


Rowson LE, Laming GE & Fry RM 1953 Influence of ovarian hormones on uterine infection. Nature 171 749–750. (doi:10.1038/171749A0)


Schams D, Kosmann M, Berisha B, Amselgruber WM & Miyamoto A 2001 Stimulatory and synergistic effects of luteinising hormone and insulin like growth factor 1 on the secretion of vascular endothelial growth factor 1 on the secretion of vascular endothelial growth factor on ovulated COCs, which may alter global gene expression and immune responses in the uterus of the post-partum dairy cow. Reproduction 145 527–539. (doi:10.1530/REP-12-0513)


Vellucci SV, Parrott RF, de Costa AC, Obkura S & Kendrick KM 1995 Increased body temperature, cortisol secretion, and hypothalamic expression of c-fos, corticotrophin releasing hormone and interleukin-1β mRNAs, following central administration of interleukin-1β in the sheep. Brain Research 29 64–70. (doi:10.1016/0006-8993(94)00230-C)


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