Equine endometritis: a review of challenges and new approaches

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Abstract

Endometritis in the mare begins as a normal physiological inflammatory response to breeding that involves both a mechanical and immunological response pathway activated to rid the uterus of semen and bacteria. With successful resolution of this inflammation, the mare's uterus will provide a hospitable environment for the development of the semi-allogenic conceptus. If the mare fails to resolve this inflammatory response within 48 h of breeding, she will become susceptible to persistent breeding-induced endometritis (PBIE) which will have detrimental effects on her fertility. This condition can then predispose the mare to bacterial or fungal endometritis leading to further degeneration of the endometrium. Optimisation of the mare's fertility requires a fine balance between allowing the natural immune response of the endometrium to its exposure to allogenic semen to run its course, and yet preventing its progression to PBIE or the involvement of infectious agents. This review discusses the challenges presented by PBIE, latent infections, biofilms, fungal infections and the need to utilise diagnostic methods available and implement targeted treatments to optimise fertility in the mare.

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Background

'Equine endometritis' is often cited as one of the major causes of infertility in the mare (Troedsson 1999, Liu & Troedsson 2008, LeBlanc 2010, Troedsson & Woodward 2016, Canisso *et al.* 2020). More recently, however, endometritis itself has been defined as a normal immunological response to the introduction of semen into the mare's reproductive tract during breeding. In normal, fertile mares this physiological inflammation resolves with the successful removal of uterine fluid, semen and bacteria within 48 h of breeding. However, if this inflammation remains unresolved beyond 48 h, then the pathological condition of persistent breeding-induced endometritis (PBIE) ensues (Christoffersen & Troedsson 2017, Canisso *et al.* 2020). If PBIE remains unchecked, the condition results in immense inflammation and may progress to infectious endometritis or the development of endometrial fibrosis (Fig. 1).

Clinically, a presumptive diagnosis of persistent breeding-induced endometritis is made if more than 2 cm uterine fluid is detected by ultrasound during oestrus (Brinkso *et al.* 2003) or within 36 h after breeding (LeBlanc 2010). The incidence of uterine fluid retention within 30 h of natural service in a population of intensively managed thoroughbreds has been reported to range from 15% (Zent *et al.* 1998) to 30% (Pycock & Newcombe 1996). It is worth noting that this ultrasound evaluation

typically provides a 'presumptive diagnosis' made prior to the development of PBIE, and prophylactic treatment may be implemented. Furthermore, unless the mare fails to conceive, the diagnosis of PBIE under field conditions does not routinely involve detailed cytological, microbial or histological examination. In addition, the optimal time to administer uterine treatments, within 48 h postbreeding, is prior to closure of the cervix after ovulation. Consequently, culture and sensitivity results of uterine samples may not be available in time to make clinical decisions. Ultimately, these factors limit our ability to determine the actual incidence of PBIE in a commercial breeding population.

In the field, the first cycle pregnancy rate in thoroughbreds has been reported to range from 53 (Hanlon *et al.* 2012) to 68% (Nath *et al.* 2010), which translates to a failure of conception in some 32–47% of cycles. Mares that fail to conceive and older mares are more likely to receive uterine treatment within 48 h after breeding (Morris & Allen 2002, Allen *et al.* 2007, Riddle *et al.* 2007). In less than 10 years, in similar populations of mares, there has been an increase in the percentage of mares that receive intrauterine treatment, from 9 (Morris & Allen 2002) to 26.3% in 3- to 8-year-old mares (Allen *et al.* 2007), and from 24% (Morris & Allen 2002) to 51.3% in mares >18 years of age (Allen *et al.* 2007). Furthermore, Allen *et al.* (2007) reported that antibiotics comprised 24.6% of postservice treatments. To slow the

Figure 1 The success and failure of postbreeding endometritis in the mare.

emerging resistance to antibiotics, which has resulted from the overuse of antibiotics, the 'One Health' philosophy (<https://www.oie.int/en/>) to veterinary medicine has been developed. It is recommended that antibiotics only be used in cases of bacterial endometritis confirmed by positive bacterial culture. As such, the diagnosis and application of effective treatment of PBIE is now more important than ever.

A multitude of factors predispose mares to PBIE, the major ones being advancing age, poor perineal conformation, repeat breeding and dystocia (Watson 2000, Christoffersen *et al.* 2015b). Upon ruling out other causes of infertility, an effective breeding management strategy will reduce the risk of PBIE and implement evidence-based treatment to optimise fertility. To this end, this paper will review our current understanding of the physiology of endometritis and how treatments work to prevent and manage the development of PBIE.

Pathophysiology of persistent breeding-induced endometritis

After natural mating or insemination, normal mares typically exhibit some degree of physiological inflammation that resolves within 48 h of exposure to

semen (Katila 1995, Watson *et al.* 2001, Christoffersen *et al.* 2012, Fedorka *et al.* 2018). The resolution of this inflammation relies on the effective coordination of several response pathways, with (1) a mechanical pathway involving myometrial contractions to physically evacuate inflammatory debris, and (2) the innate immune response being the major mechanisms to eliminate excess spermatozoa and bacteria while (3) the classical immunological antigen–antibody response is minimal and thus of minor importance in mares (Fig. 2). Together, these pathways work to prevent the establishment of PBIE. Mares that are resistant to PBIE display efficient uterine clearance mechanisms and their transient endometrial inflammation ceases within 24–36 h after breeding, allowing an embryo-friendly uterine environment to become established prior to entrance of the conceptus into the uterine lumen some five days later. Mares which fail to resolve their physiological postbreeding endometritis are deemed to become 'susceptible' to PBIE which predisposes them to secondary uterine infection (Pycoc & Allen 1990).

Mechanical pathway

During mating or insemination, the mechanical stimulation of the cervix and vagina initiates a release of oxytocin which stimulates myometrial contractions that need to be maintained for effective expulsion of fluid from the uterine lumen (Campbell & England 2004, Risco *et al.* 2009) through the cervix and into the vagina during the ensuing 6–12 h (Troedsson 1997). After ovulation and cervical closure, any remaining uterine particulate matter, oedema and inflammatory cells are drained via the lymphatics within the endometrium (LeBlanc *et al.* 1995). This mechanical process may be impaired by the existence of acquired anatomical defects such as poor perineal conformation, vaginal stretching, an incompetent cervix, a pendulous uterus, or degenerative defects of the myometrium and/or endometrium. Such physical changes develop in association with repeated mating and foaling, or increasing age of the mare (Evans *et al.* 1987, LeBlanc *et al.* 1989) and increase the susceptibility of the mare to PBIE.

Mares susceptible to PBIE may also have delayed or reduced myoelectrical activity in the myometrium, which interferes with the clearance of uterine contents (LeBlanc *et al.* 1994b). This myometrial activity is influenced by a combination of mare factors and immunological, endocrine and chemical factors associated with inflammation. After deposition of spermatozoa and bacteria into the uterus, the PMN-phagocytosis of spermatozoa and/or bacteria results in inflammation that produces arachidonic acid metabolites, including $\text{PGF}_{2\alpha}$, which also causes myometrial contractions (Troedsson 1999). Mare age influences the response of the myometrium to oxytocin and prostaglandins by altering receptor-second messenger signalling mechanisms downstream of the intracellular

Figure 2 The pathogenesis of endometritis in the mare. Key: inhibition, modulation, stimulation. Red arrows indicate inhibition, blue arrows indicate modulation and green arrows indicate stimulation.

Ca(2+) release (Rigby *et al.* 2001). Then, superimposed on these age and endocrine factors, chemical factors such as the accumulation of nitric oxide (NO) in the uterus of susceptible mares (Alghamdi *et al.* 2005) have been suggested to contribute to the pathogenesis of impaired myometrial activity (Woodward *et al.* 2013b, Khan *et al.* 2017).

Any interference in the evacuation of uterine fluid through the cervix and lymphatics after mating contributes to the progression of endometrial degenerative changes (Woodward *et al.* 2012, Reilas *et al.* 2016). The ensuing endometrosis contributes to a vicious cycle involving severe inflammation, which causes fibrosis and subsequent fluid accumulation. Angiopathies impair blood circulation and lymphatic lacunae interfere with fluid circulation. Dysfunctional mechanical clearance of any uterine inflammatory by-products then undermines the mare's uterine defence (LeBlanc *et al.* 1994b, LeBlanc & Causey 2009, Katila 2012, 2016) and predisposes her to bacterial or fungal endometritis.

Immunological pathway

After the introduction of spermatozoa into the reproductive tract, the innate immune response provides the first

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natural, nonspecific defence response and involves phagocytic cells such as neutrophils, macrophages and dendritic cells. Troedsson (1999) suggested that the uterine defence system is not dependent on humoral factors until later when the antibody-mediated response is required to eliminate bacterial contaminants from the uterus. Indeed, the concentrations of immunoglobulins (IgA, M and G) in uterine secretions are similar throughout all stages of the oestrous cycle in normal mares and are only elevated in mares susceptible to endometritis (Waelchli & Winder 1987). However, during oestrus, the dominance of oestrogen contributes to the expression of immune-related genes required to manage the immune response to the uterine deposition of foreign material, spermatozoa and bacteria, during breeding (Asbury & Hansen 1987, Skarzynski *et al.* 2020).

Both spermatozoa and bacteria have similar allogenic, pathogen recognised receptors which activate the innate immune pathway that is mediated by toll-like receptors (TLR) on epithelial cells and serves to prevent the establishment of infection until antigen-specific cells are recruited to the local site (Schöniger *et al.* 2017, Ezz *et al.* 2019, Skarzynski *et al.* 2020). These responses are antigen-nonspecific, rapid, and are based on recognition of invariant molecular structures

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on pathogens known as pathogen-associated molecular patterns (PAMPS), which include the lipopolysaccharide (LPS) of Gram-negative bacteria and peptidoglycan and lipoteichoic acid of Gram-positive organisms (Quayle 2002). This innate immune response enables allogenic spermatozoa to challenge the immune system without developing anti-sperm antibodies (Canisso *et al.* 2020). The dendritic cells are the link between the innate and adaptive immune response. So when antigenic foreign proteins are present, both T and B cells are activated via dendritic presentation, followed by the complement cascade and initiation of polymorphonuclear leukocyte (PMN) chemotaxis (Troedsson 1999) which aids the removal of non-viable spermatozoa and pathogenic bacteria.

The PMN infiltration occurs in the uterus from 0.5 h after breeding to its peak within 4–8 h (Katila 1995). Once activated, the PMNs release lytic enzymes that result in phagocytosis of bacteria. This enzymatic activity ensures the release of DNA from the PMN and the production of neutrophil extracellular traps (NET) which have immunomodulatory and bacteriocidal effects (Rebordao *et al.* 2014).

During mating or insemination the mare's endometrium is exposed to seminal plasma which has an immunomodulatory role in the endometrial response to spermatozoa. Seminal plasma increases the mRNA expression of some pro-inflammatory cytokines (e.g. IL-8, IL-1 β) while suppressing others (e.g. TNF) (Fedorka *et al.* 2017) in the endometrium (Fig. 2). Although seminal plasma is not necessary for normal mares to achieve satisfactory pregnancy rates (Portus *et al.* 2005), when it is combined with spermatozoa it facilitates the uterine clearance of PMN by suppressing complement activation, PMN chemotaxis and phagocytosis (Fiala *et al.* 2007, Troedsson & Woodward 2016). This finely balanced immunomodulation by seminal plasma is made possible by the existence of one of its cysteine-rich secretory proteins, CRISP-3, which prevents PMN-binding and phagocytosis of viable spermatozoa, and enables the completion of sperm transit within a transitionally inflamed uterus over the next 4 h (Doty *et al.* 2011). Whereas lactoferrin in the seminal plasma appears to increase binding of dead spermatozoa to PMNs *in vitro* (Troedsson *et al.* 2014). Indeed, the removal of seminal plasma has a detrimental effect on the immune response to insemination (Sánchez-Rodríguez *et al.* 2018).

During the first 24 h after breeding, there is also a rapid increase in the pro-inflammatory cytokines, IL1 β , IL-8 and IFN- γ , as well as activation of PMNs to bind to and engulf bacteria and spermatozoa (Christoffersen *et al.* 2012). This physiological inflammatory reaction further serves to eliminate contaminating bacteria, debris, seminal plasma and excess spermatozoa from the uterine lumen (Kotilainen *et al.* 1994, Katila 1995, LeBlanc & Causey 2009, Christoffersen &

Troedsson 2017). In response to the inflammation, anti-inflammatory cytokines (IL1RA, IL10) are released to mediate the response.

The cessation of the physiological inflammatory endometrial response to mating requires a balance between both pro- and anti-inflammatory factors, which is observed between 6 and 12 h after breeding in mares that are resistant to PBIE (Woodward & Troedsson 2015, Marth *et al.* 2018a). In contrast, mares susceptible to PBIE fail to clear the endometrial inflammation within this timeframe and are also prone to bacterial infection (Hughes & Loy 1969, LeBlanc *et al.* 1989). In these susceptible mares, endometrial expression of pro-inflammatory cytokines is inadequate and prolonged, and they exhibit lower mRNA expression for the anti-inflammatory cytokines (ILRA, IL10, IL6) and higher mRNA expression for the pro-inflammatory cytokines (e.g. IL1 β , IL-8) after breeding. This cytokine imbalance leads to an increased number of PMNs observed within the uterus between 2 and 12 h after breeding (Christoffersen *et al.* 2012, Woodward *et al.* 2013a, Christoffersen & Troedsson 2017).

Infectious endometritis

Every time a mare is mated or inseminated her natural physical barriers to infection, the vulval opening, vagino-vestibular junction and cervix, are breached and bacteria are introduced. In the last 80 years, one of the earliest and most effective strategies to minimise the introduction of bacteria into the reproductive tract and improve conception rates has been the use of the Caslick vulvoplasty which contributes to improved pregnancy rates (Caslick 1937).

Bacterial factors in endometritis

If the mare fails to resolve her physiological endometritis and she develops PBIE, the immense inflammation, if combined with a concurrent challenge from pathogenic organisms, may progress to infectious endometritis (Hughes & Loy 1969, Christoffersen & Troedsson 2017). These pathogenic organisms may be latent, deep within the endometrial glands (Petersen *et al.* 2018), introduced at mating, or ascend from the caudal genital tract to cause infectious endometritis (Davis *et al.* 2013). The postbreeding physiological inflammatory response itself may activate latent bacteria (Petersen *et al.* 2015).

It has been reported that up to 60% of barren mares have been diagnosed with bacterial endometritis (LeBlanc & Causey 2009). The most common bacteria isolated from the uteri of mares include *Escherichia coli* and *Streptococcus equi* subsp. *zooepidemicus* (*S. zooepidemicus*) (LeBlanc & Causey 2009, Davis *et al.* 2013, Christoffersen *et al.* 2015b). Davis *et al.* (2013) isolated bacteria from 22 to 43% of more than 8000 uterine samples and from these, there was a similar

incidence of *S. zooepidemicus* (29%) and *E. coli* (28%). However, only 62% of Streptococci and 43% of *E. coli* positive cases had concurrent evidence of cytological inflammation, thus complicating the diagnosis as contamination remained a possibility (Davis *et al.* 2013).

S. zooepidemicus is one of several β -haemolytic Streptococci associated with infectious endometritis. Other species of Streptococci, including *S. dysgalactiae* subsp. *equisimilis* and *S. equi* subsp. *equi* (*S. equi*), have also been isolated from the equine endometrium (Albiñ *et al.* 2003, Christoffersen *et al.* 2015b). However, specific reproductive strains of *S. zooepidemicus* are opportunistic and reside in the caudal reproductive tract until they produce disease in response to tissue damage (Timoney 2004), as occurs during PBIE. These isolates belong to a genetically distinct subpopulation (Rasmussen *et al.* 2013, Christoffersen *et al.* 2015b), that differ from respiratory or abortive strains (Timoney 2004). In older, multiparous mares with poor vulval conformation, isolation of a single strain of *S. zooepidemicus* (clonal infection) can also occur (Christoffersen *et al.* 2015b).

Specific strains of *S. zooepidemicus* are highly pathogenic by virtue of the expression of super-antigens (Casagrande Proietti *et al.* 2011). These pathogenic strains of *S. zooepidemicus* (Rasmussen *et al.* 2013) persist deep in the uterine glands in the stratum compactum (Petersen *et al.* 2015) or intracellularly (Skive *et al.* 2017) in the endometrium in chronically infected mares. Here, the streptococci employ the anti-phagocytic hyaluronic acid capsule in addition to fibronectin-binding proteins to avoid the host immune response (Timoney 2004, Petersen *et al.* 2009). These dormant streptococci are not detectable by standard bacterial culture techniques, but can be activated by PBIE and acute infectious endometritis ensues (Petersen *et al.* 2015).

Unlike *Streptococcus* spp. and despite the frequency of isolation of *E. coli*, no uterine-specific strains of *E. coli* have been reported in the horse as occurs in cattle (Sheldon *et al.* 2010), suggesting that *E. coli* are most likely contaminants and do not reside in the equine uterus. In addition to *E. coli* and *S. zooepidemicus*, other bacteria, fungi and yeasts may also cause infectious endometritis. Some of these other infections are iatrogenically introduced environmental contaminants, venereal pathogens or secondary to over-treatment with antibiotics. These pathogens may be rare, but highly pathogenic. For example, *Pseudomonas aeruginosa* can be either an opportunistic environmental or venereal pathogen (Blanchard *et al.* 1992, Allen *et al.* 2011, Kidd *et al.* 2011).

Biofilm and biofilm-associated uterine infections

A biofilm comprises an aggregate of microbial cells adherent to a surface, embedded within a complex matrix of extracellular polymeric substances (EPS) which may also include nucleic acids, proteins,

polysaccharides and lipids of microbial origin, as well as host components (Costerton *et al.* 1999, Koo *et al.* 2017). Many Gram-negative bacterial pathogens (*E. coli*, *P. aeruginosa* and *Klebsiella pneumoniae*) isolated from the equine uterus can produce biofilm (Ferris *et al.* 2014b, 2016, Beehan *et al.* 2015, Brock *et al.* 2017). It is also possible that equine *S. zooepidemicus* can produce a biofilm, similar to that of *S. zooepidemicus* of porcine origin (Yi *et al.* 2014).

Bacteria living within a biofilm are physiologically distinct from free-floating (planktonic) organisms of the same species, which influences their susceptibility to treatment (Ferris 2017). Planktonic bacterial infections are readily detected and eliminated by the host immune system or when an appropriate antibiotic is used. However, in their biofilm state, bacteria exist in aggregates embedded within a complex matrix of extracellular polymeric substances, which may include nucleic acids, proteins, polysaccharides and lipids of microbial origin, as well as host components and they typically adhere to surfaces (Costerton *et al.* 1999, Koo *et al.* 2017). As such, bacteria in a biofilm are protected from the host immune system as white blood cells and antibodies have reduced ability to penetrate the thick layer of EPS within the biofilm (Thurlow *et al.* 2011). The bacteria within the biofilm survive with depressed metabolism and lower replication rates than planktonic bacteria (Stewart & Costerton 2001, Walters *et al.* 2003). In addition, bacteria in a biofilm may be 10–1,000 times more resistant to antibiotic treatment than planktonic bacteria (Mah & O'Toole 2001).

Antibiotics typically only act against metabolically active and/or rapidly multiplying bacteria, therefore, bacteria in the outer regions of the biofilm may be killed, but those deep within the biofilm survive and become the nidus for recurrence and development of a chronic infection (Olsen 2015). Their reactivation results in recurrence of clinical infection. It is of concern that horizontal transfer of antibiotic resistance genes may occur between bacteria while protected within a biofilm (Olsen 2015).

Biofilm-associated infections are typically difficult to diagnose and challenging to treat and rates of recurrence are high. Traditional antibiotic therapy, both systemic and local, are often ineffective against bacteria residing within a biofilm (Beehan *et al.* 2015) and so therapy includes biofilm disrupters in combination with antibiotics.

Fungal endometritis

Fungal organisms are reported to occur in 1–5% of mares diagnosed with infectious endometritis (Dasciano *et al.* 2001) and are predisposed by overuse of antibiotics and physical conditions such as pneumovagina, urovagina, decreased uterine fluid clearance, cervical defects, immunosuppression, necrotic foci and perineal defects

(Dascanio *et al.* 2001, Stout 2008). Overuse of antibiotics in the treatment and/or prevention of PBIE effectively alters the normal flora of the caudal reproductive tract, and facilitates fungal colonisation of the vaginal vault, clitoral fossa and clitoral sinuses (Zafraca 1975, Blue 1987). The most commonly cultured fungal organisms are the yeast, *Candida* spp., and mould/hyphate organisms, such as *Aspergillus* spp. and *Mucor* spp. (Coutinho da Silva & Alvarenga 2011). Fungal endometritis in mares is often chronic in nature, difficult to diagnose, difficult to eliminate and associated with a high incidence of recurrence.

Diagnosis of endometritis

The diagnosis of postbreeding endometritis in a clinical setting typically involves examination of the reproductive tract within 48 h after breeding (LeBlanc 2010). Clinically it is important to diagnose predisposing causes and to prevent the progression of PBIE to infectious endometritis. The physical signs of endometritis include a vulval discharge, cervical discharge visualised by vaginal speculum examination and uterine fluid accumulation as detected by ultrasound examination per rectum. Depending on the signalment of the mare, consideration of her age, parity, perineal conformation, cervical tone, reproductive status, foaling history, history of infection and the fertility of the stallion, after mating or insemination, the clinician may progress to laboratory-based tests to obtain evidence of a cytological response and identify any microbial organisms and their antimicrobial sensitivity. Further investigation may also include hysteroscopy or histological evaluation, and in some cases culture of an endometrial biopsy. New diagnostic methods are being developed that include confocal imaging for real-time 'biopsy' during hysteroscopy that will provide rapid diagnosis from detailed histological information about the inflammatory changes in the endometrium (Gallacher *et al.* 2018). Additional haematological and serum biochemistry tests are not considered useful indicators of equine endometritis due to the local nature of postbreeding endometritis (Katila 2016).

The sensitivity and specificity of laboratory diagnostic methods, including culture swabs, cytobrush, low volume lavage and uterine biopsy, have been reviewed (Katila 2016). Several studies conclude that low volume lavage is the most sensitive test for diagnosis of a bacterial endometritis (Christoffersen *et al.* 2015a, Katila 2016). Riddle *et al.* (2007) reported that the recovery of either 2–5 PMNs per 400x field, and/or a bacterial monoculture is deemed diagnostic of endometritis and has been associated with reduced pregnancy rates. The pathogenicity of the bacteria will influence the cytology results such as incurred by *E. coli* infections which can suppress the PMN response, or by the latency

of *Streptococci* spp., which have not induced a PMN response (LeBlanc 2010, Christoffersen *et al.* 2015a, Petersen *et al.* 2015). The opportunistic behaviour of pathogenic bacteria may also vary depending on the fragile balance of the normal flora or microbiome (Heil *et al.* 2019).

Recently, genome sequencing has identified Proteobacteria as the predominant species in the normal microbiome in fertile mares (Heil *et al.* 2018) and it is hypothesised that any shift in this population may lead to an increased risk of PBIE and bacterial endometritis. New qPCR diagnostic methods are also being developed which have a high sensitivity and specificity to identify mares that are susceptible to postbreeding endometritis by diagnosis of innate immune genes including equine β defensin 1 (EBD1), lysozyme (LYZ) and secretory leukoprotease inhibitor (SLP1) which are found in their endometrium (Marth *et al.* 2018a,b).

Diagnosis of fungal endometritis

If PBIE remains unresolved, there is an increased risk of mares developing fungal endometritis. Mares with fungal endometritis often have a history of chronic infertility and clinical signs may include a purulent vaginal discharge and an excess of echogenic fluid within the uterine lumen (Scott 2019). Currently, the standard techniques for detection of fungal infections are mycological culture, evaluation of endometrial cytology, uterine biopsy and detection of fungal DNA by PCR analysis (Dascanio *et al.* 2001, Stout 2008, Ferris *et al.* 2013, Scott 2019).

Fungal agents are often easiest to detect in cytology smears made after centrifugation of uterine effluent from a low-volume lavage. Yeasts are typically recognised by their 5–8 μ m diameter and their distinct cell wall (100–200 nm) forming a clear 'halo' around the organism. Hyphate fungi are narrower, 3–5 μ m in width and 8 μ m in length. These individual cells are commonly linked in long branching chains. In many instances, detection of a fungal organism on cytological examination may be the only evidence of fungal endometritis, since these organisms do not always grow in culture (Beltaire *et al.* 2012). Mares with fungal endometritis will often, but not always have inflammatory cells in the cytology sample. Fungal elements can also be detected within uterine biopsy samples stained with Gomori's methamine silver stain or periodic acid–Schiff (PAS) stain (Scott 2019). Analysis of uterine biopsies also provides useful information about the degree of inflammation associated with the infection (Coutinho da Silva & Alvarenga 2011).

Mycological culture is often reserved for difficult cases of infectious endometritis because it requires 3–4 weeks of culture to allow for the long growth phase of mycotic organisms and accurate identification. To optimise diagnosis, Sabouraud's dextrose agar is recommended to culture fungal organisms since a high percentage

do not grow under normal aerobic conditions in agar (Beltaire *et al.* 2012). Ideally, selection of an antifungal agent would be based on the results of susceptibility tests for the specific fungal organism (Dascanio *et al.* 2001). Unfortunately, due to the time involved in diagnosis, empirical treatment choices are usually implemented. A more rapid and accurate molecular PCR diagnostic method for fungal infections would be useful clinically (Ferris *et al.* 2013). However, qPCR is limited because it cannot be used to determine antimicrobial susceptibility for fungal organisms. After treatment, it is common for a secondary infection of bacteria such as *S. zooepidemicus* to develop. Consequently, it is often necessary to treat for bacterial endometritis along with, or after, treatment for fungal endometritis.

Management to prevent PBIE

In a well-managed breeding programme the incidence of PBIE in mares will be minimised and this will lead to a reduced reliance on antibiotics. Modern oestrous management aims to breed mares just once per cycle, not only to improve efficiency but also to allow time for the resolution of normal postbreeding endometritis without repeated antigenic stimulation from semen (Canisso *et al.* 2016). Nevertheless, multiple inseminations with fresh or frozen semen during oestrus have also been known to improve pregnancy outcomes in fertile mares without increasing the risk of postbreeding endometritis (Voss *et al.* 1979, Huber *et al.* 2019). Uterine fluid accumulation was not associated with the timing of insemination between 12 h before and 8 h after ovulation (Watson *et al.* 2001). The choice of insemination method might also contribute to minimising PBIE. Recently, it has been reported that deep uterine insemination results in less inflammation than conventional insemination into the uterine body (Cazales *et al.* 2018). Despite this, there appears to be an interaction between insemination technique and mare fertility as in another study conventional intrauterine insemination produced better fertility in problem mares and hysteroscopic insemination was better in normal mares (Sieme *et al.* 2004).

Treatment options

The objectives of any treatment of PBIE include diagnosis of the causative agent or predisposing factor and to ensure a nurturing uterine environment for embryonic development. Clinically, the dilemma is optimising the timing of treatment to prevent progression of physiological inflammation to the pathogenic condition of PBIE. The most popular strategies to prevent PBIE include a combination of ecboic and uterine lavage treatment within 48 h of breeding, which act on the mechanical response pathway and aid the physical removal of inflammatory debris and/or micro-organisms (Pycock &

Newcombe 1996, LeBlanc & Causey 2009, Rose *et al.* 2018). This fundamental strategy may be supplemented with treatments that act on the immunological response pathway such as anti-inflammatory medication (Rojer & Aurich 2010), antibiotics (Pycock & Newcombe 1996, Rose *et al.* 2018), mucolytics (Gores-Lindholm *et al.* 2013) and immunomodulation therapies (Woodward *et al.* 2015). Ultimately, the challenge of any therapy must enhance fertility and not cause harm to either the mare or public health (e.g. development of antimicrobial resistance), so an understanding of the indications, mechanism and role of the selected therapy is important.

Treatments targeting the mechanical response pathway

Ecboic agents

In oestrous mares, uterine clearance is improved by the administration of ecboic drugs, such as oxytocin and prostaglandin (PG) $F_{2\alpha}$. Injection of oxytocin at the recommended dose of 10 to 25 units results in high-amplitude myometrial contractions for a period of approximately 30 min in most mares. The effect is greatest during oestrus, but still present for 48 h after ovulation (LeBlanc *et al.* 1994a, LeBlanc & Causey 2009). During oestrus there is higher oxytocin receptor gene expression in the myometrium compared to the luteal phase (Annandale *et al.* 2018). Interestingly, factors often associated with impaired uterine clearance like mare age or chronic endometrial degeneration do not influence uterine oxytocin receptor expression (Annandale *et al.* 2018). Nevertheless, the efficacy of oxytocin is reduced if concurrent anatomical conditions exist like a pendulous uterus or closed cervix. Increasing the dose beyond recommended levels is not advisable and may induce inappropriate uterine contractions that do not allow for uterine drainage (LeBlanc & Causey 2009). However, the synthetic oxytocin analogue, carbetocin, has become an attractive alternative for oxytocin therapy due to its half-life being more than twice that of oxytocin (Schramme *et al.* 2008).

Among the $PGF_{2\alpha}$ analogues studied for their ecboic effects, 250 µg cloprostenol induced the most consistent uterine response (Combs *et al.* 1996). After cloprostenol treatment, uterine contractions were weaker than after oxytocin treatment, but they lasted for up to 4 h. Due to the detrimental effects of $PGF_{2\alpha}$ on luteal function and progesterone secretion required for pregnancy maintenance, it is not recommended as an ecboic postovulation (LeBlanc & Causey 2009).

Uterine lavage

The physical removal of fluids and inflammatory debris by uterine lavage after insemination underpins the prevention and treatment strategy for PBIE. Uterine

lavage in susceptible mares is indicated when there is hyperechoic intrauterine fluid accumulation or if free intrauterine fluid exceeds two cm in diameter (LeBlanc & Cousey 2009). The appropriate timing of post-breeding uterine lavage is considered more important than choice of the lavage fluid (Brinsko *et al.* 1991). Sterile saline or lactated Ringer's solution are commonly used for this purpose (Allen *et al.* 2007, LeBlanc & Cousey 2009). Neither uterine lavage performed immediately before insemination (Vanderwall & Woods 2003) or at 4 h after insemination was detrimental to fertility whereas lavage at 0.5 or two h after insemination decreased pregnancy rates (Brinsko *et al.* 1990). While uterine lavage with 0.05% povidone-iodine solution close to ovulation did not produce inflammation in one study (Brinsko *et al.* 1990), the addition of 1% povidone-iodine to uterine lavage fluids caused inflammation (Olsen *et al.* 1992) and also reduced expression of endometrial progesterone receptors which may have implications for fertility (Kalpokas *et al.* 2010).

Treatments targeting the immunological response pathway

As discussed, the physiological inflammation activated within the first few hours after breeding is fundamental to the success of the immunological balancing act required to allow passage of viable, but allogenic spermatozoa, whilst eliminating non-viable spermatozoa and contaminants in order to prepare the uterus for the safe arrival of a semi-allogenic embryo. A major challenge for our therapeutic strategy is that any treatment targeting this immunological pathway must preserve its delicate balance to optimise fertility.

Immunisation

Immunisation against bacterial infection during breeding would be the holy grail of preventative treatment for bacterial endometritis. By preventing infection, the balanced immunological response would be preserved and our dependence on antibiotics would be reduced. Ongoing studies in mice have identified cell surface protective antigens against *S. zooepidemicus* that may be useful in developing a vaccine to help prevent endometritis (Fu *et al.* 2013, Xie *et al.* 2018). In contrast to other species, the mare's uterine defence system relies predominantly on local uterine defence and IgA production more than systemic production of IgG (Widders *et al.* 1985), so a combined preventative approach may be warranted. However, the varied antigenicity and complexity of the virulence factors of *S. zooepidemicus* will complicate vaccine development (Timoney 2004). Immunisation of mares with a semi-purified extract of *S. zooepidemicus* was able to reduce bacteria shedding and improve the immune response of the treated mare to infection when administered

both systemically and locally. Reduction in bacterial shedding was greater in mares with higher serum IgG and uterine IgA titres (Widders *et al.* 1995).

Anti-inflammatory drugs

Seminal plasma's immunomodulatory role in facilitating sperm transit through the oestrogen primed uterus, make it or its component proteins like lactoferrin, a candidate for PBIE treatment. In mares susceptible to endometritis, supplementation of the inseminate with human recombinant lactoferrin (hrLF) suppressed the endometrial mRNA expression of tumour necrosis factor α at 6 h after breeding (Fedorka *et al.* 2017) and of interleukin 6 at 24 h after breeding (Coutinho da Silva *et al.* 2017). Subsequently, mares were treated with intrauterine hrLF at 6 h after mating, at which time the ratio of PMN to endometrial epithelial cells was suppressed (Fedorka *et al.* 2018). Further evaluation of seminal plasma proteins as therapeutic agents may be warranted for susceptible mares.

Iatrogenic modulation of either, the phospholipase A2 pathway with glucocorticoid treatment, or the cyclo-oxygenase pathway with NSAIDs treatment may be indicated in susceptible mares to prevent excessive inflammation. Glucocorticoids alter the expression of genes for complement, cytokines, TNF, TGF and major histocompatibility complex (MHC), while NSAIDs inhibit prostaglandin synthesis in response to endometrial inflammation (Wolf *et al.* 2012). This reduces inflammation and explains the improved fertility observed after glucocorticoid treatment with dexamethasone (Bucca *et al.* 2008) or prednisolone (Dell'Aqua *et al.* 2006) in mares susceptible to PBIE. Beneficial effects of NSAID treatment with vedaprofen (Rojer & Aurich 2010) or firocoxib (Friso *et al.* 2019) prior to breeding have also been observed. However, given the role of NSAIDs in inhibiting prostaglandin release and their potential to reduce the myometrial contractions required to evacuate uterine fluid, further evaluation of the efficacy of NSAIDs treatment is warranted (Cadario *et al.* 1995, Risco *et al.* 2009) and concurrent treatment with an ecboic such as oxytocin is recommended.

Stem cell treatment

There is potential for clinical application of stem cells to treat PBIE. The immunomodulatory effects of a uterine infusion of endometrial mesenchymal stem cells (eMSC) have been reported. This treatment reduced the influx of polymorphonuclear neutrophils and increased the induction of anti-inflammatory cytokines (IL-6, IL-8, MCP-1, CCL5, TLR4) in the uterus (Rink *et al.* 2018). In addition, these eMSC exhibited antimicrobial properties. Treatment of the uterus with stem cells can be performed by either hysteroscopic injection (Fumuso *et al.* 2014) or uterine infusion (Ferris *et al.* 2014a, Rink *et al.* 2018).

After infusion, the actions of these cells were exerted within the uterine lumen as the eMSC did not migrate into the endometrium itself.

Plasma

More recently, the use of plasma products has been directed towards immunomodulation rather than elimination of pathogens from the uterus. Platelet-rich plasma (PRP) produces a range of growth factors *in vitro* that may be useful in treating endometritis (Schar *et al.* 2015). Administration of PRP (257,000 platelets/ μ L) modulates the uterine inflammatory response to semen and results in reduced inflammation in mares resistant or susceptible to endometritis (Reghini *et al.* 2014, 2016). Indeed, clinical trials have recently demonstrated the beneficial effects of platelet enriched plasma on pregnancy rates in problem mares (Metcalf 2014). In humans the platelet granules exhibit bacteriocidal activity against *Staphylococcus* spp., *E. coli* and *Klebsiella* spp. (Canisso *et al.* 2020). Intrauterine treatment with autologous conditioned serum also reduced the infiltration of neutrophils in mares (Ferris *et al.* 2014a).

Mycobacterium cell wall extract (MCWE)

Mycobacterium cell wall extract is an immune modulator, comprising two primary ingredients, immunomodulatory trehalose dimycolate and muramyl dipeptide, which targets monocytes and macrophages and modulates the production of various cytokines. Clinically, treatment of susceptible mares with MCWE produced a cytokine profile with higher IL-8 and lower IL-10 than resistant mares, after artificial insemination, and similar to the effects of glucocorticoids (Fumuso *et al.* 2007). A subsequent study found that expression of IL1-beta mRNA was lower after treatment with either glucocorticoids or MCWE, but there was no effect on IL6, interferon gamma, IL10, or IL1RA cytokines (Woodward *et al.* 2015).

Anti-microbial treatment

Antibiotics

Judicious rather than routine use of antibiotics is now the objective of any treatment of bacterial endometritis (<https://www.avma.org/KB/Policies/Pages/Judicious-Therapeutic-Use-of-Antimicrobials.aspx>). The guidelines state that prescription of antibiotics should be based on clinical signs, cytological, culture and antimicrobial sensitivity tests as evidence of bacterial infection (<https://www.avma.org/KB/Policies/Pages/AAEP-Prudent-Drug-Usage-Guidelines.aspx>). One of the challenges in developing a treatment strategy is that identification of the infectious agent is not always consistent with cytological evidence of inflammation as Davis *et al.* (2013) reported that only

62% of *S. zooepidemicus* and 43% of *E. coli* infections show cytological evidence of inflammation.

Historically, there has been a preference for intrauterine antibiotic treatment of endometritis rather than a systemic parenteral approach (LeBlanc 2009), in part due to the small risk of side effects of antibiotic treatment on intestinal flora in the horse (Davolli *et al.* 2018). Intrauterine infusions produce high local concentrations of antibiotic in the uterine lumen (LeBlanc 2009). However, factors such as repeated invasion of the cervix during treatment, inhibition of the antibiotics by inflammatory debris, optimising treatment volume, and non-physiological pH of antibiotics all contribute to the potential for reduced efficacy, the risk of iatrogenic infection and inflammation as well as increased antimicrobial resistance. Homogeneous distribution of the antibiotic of choice at levels exceeding the minimum inhibitory concentration (MIC) for the target bacteria within the endometrium is required, and recently the efficacy of systemic antibiotic treatment and the relationship between plasma and tissue levels has been demonstrated for ceftiofur (Witte *et al.* 2010, Scofield *et al.* 2014), gentamicin, ticarcillin, neomycin, amikacin (Witte *et al.* 2018) and sulfadiazine and trimethoprim (Davolli *et al.* 2018). However, extrapolation of pharmacokinetic data for different antibiotics must be tempered with the knowledge that most *in vivo* clinical trials use healthy mares.

Performing antimicrobial sensitivity testing is a key diagnostic step in uterine infections which ensures that the bacteria are susceptible *in vitro* to the antibiotic is selected. The culture and sensitivity results of uterine swabs submitted to commercial laboratories have shown how bacterial susceptibility and resistance patterns vary by patient, by region and over time, with degrees of resistance to many of the commonly selected antibiotics, including gentamicin, penicillin, ticarcillin and trimethoprim sulphonamides, are evident (Albiñ *et al.* 2003, Davis *et al.* 2013, Mitchell *et al.* 2018). Furthermore, no statistical relationship was observed between the degree of inflammation based on a cytology score and the antibiotic resistance pattern in over 8000 cases (Davis *et al.* 2013).

Treatment of biofilm-associated uterine infections

Biofilm producing bacteria pose a challenge to effective treatment. If bacteria are avoiding the host immune system from within a biofilm then the biofilm must be disrupted to enable the antibiotics access to the bacteria. Therefore, the objective in eliminating a biofilm-associated bacterial infection is disruption of the biofilm and killing the associated bacteria (Ferris *et al.* 2017). Antibiotics alone, or non-antibiotic agents touted to disrupt biofilm alone, are often unable to completely eliminate biofilm-associated bacterial infections alone, and so these two therapeutic approaches are often combined (Fig. 3). Addition of Tris-EDTA (TrizEDTA®; Dechra Veterinary Products, Overland

1. Culture the mare's uterus to identify the causative bacteria
2. Perform an antimicrobial susceptibility test to determine the optimum antibiotic
3. Perform a uterine lavage to remove inflammatory debris, bacteria and fluid
4. Combine the optimal antibiotic with a non-antibiotic agent, such as Tris-EDTA or DMSO, in the same syringe
 - a. TrizEDTA[®], Dechra Veterinary Products, Overland Park, KS 66211, USA
 - b. DMSO 99 % purity, Valhoma Corporation, Tulsa, OK 74115, USA
5. Infuse the combination once per day for 3 consecutive days

Figure 3 A protocol for treatment of chronic uterine infections associated with a biofilm.

Park, Kansas, USA) or DMSO to most common antibiotics enhanced bacterial killing and disruption of biofilm, compared to treatment with the non-antibiotic agent or antibiotic alone (Ferris *et al.* 2016, Ferris 2017, Loncar *et al.* 2017). Selection of appropriate combinations of biofilm disrupters and antibiotics must also consider that some treatment combinations (e.g. N-acetylcysteine mixed with an antibiotic in the same syringe) may result in reduced activity of the antibiotic (Ferris *et al.* 2017).

Alternative non-antibiotic therapies for endometritis

To address the challenge to reduce our reliance upon routine antibiotic treatments for PBIE and infectious endometritis, non-antibiotic alternatives are being evaluated. However, not all alternative treatments are effective and the optimal concentration or dose must be determined (Loncar *et al.* 2017). Many of these non-antibiotic treatments claim to provide a multipronged approach on the treatment of PBIE by modulating inflammation in conjunction with some antibacterial properties.

DMSO

DMSO is well known for its anti-inflammatory actions (Jacob & Herschler 1983). Solutions of 2.5–30% DMSO are also reported to be bacteriostatic for *Staphylococcus aureus*, *E. coli*, *Streptococcus* spp. and many Gram-negative bacteria (Santos *et al.* 2003, Guo *et al.* 2016, Yahya *et al.* 2018). DMSO is capable of disrupting biofilm and acts as a synergistic agent in conjunction with some antibiotics (Yahya *et al.* 2018). For these reasons, DMSO may be considered a useful tool in the treatment of PBIE although there are no peer-reviewed reports which evaluate DMSO for this purpose. Furthermore,

some reports have shown that DMSO can inhibit the bacteriocidal activity of ampicillin, kanamycin and some quinolones (Mi *et al.* 2016). Systemic side effects of DMSO have been documented in mice and must be considered for both the horse and the veterinarian (Guo *et al.* 2016).

N-acetylcysteine

N-acetylcysteine is a powerful antioxidant, a precursor of L-cysteine and it also has mucolytic properties (Mokhtari *et al.* 2017). These characteristics make it a useful adjunctive treatment in bacterial or fungal endometritis. N-acetylcysteine (3.3% solution) decreased biofilm biomass and killed *E. coli* isolates but it was not as effective against *P. aeruginosa* or *K. pneumoniae* isolates (Ferris *et al.* 2016). In addition, N-acetylcysteine has anti-inflammatory properties which modulate the endometrial response (Melkus *et al.* 2013).

Hydrogen peroxide

Hydrogen peroxide is a by-product of metabolic processes. It is an acidic, water-soluble fluid with strong oxidative properties and has the ability to inhibit many enzymatic processes. Hydrogen peroxide produces oxidants such as singlet oxygen, superoxide radicals and a hydroxyl radical which can be cytotoxic at high levels. These properties may be exploited clinically and exposure to 1% hydrogen peroxide decreased biofilm biomass and reduced CFU of *E. coli* and *K. pneumoniae* isolates from horses (Ferris *et al.* 2016).

Hydrogen peroxide inhibits the growth of *E. coli* by inducing the formation of highly toxic hydroxyl radicals. However, it is not the panacea for infectious endometritis treatment. Bacteria like *E. coli* and

Staphylococci are 'catalase positive', that is, they utilise the enzyme, catalase, to break down hydrogen peroxide into hydrogen and water to protect themselves from cytotoxicity (Serra *et al.* 2008). Furthermore, *E. coli* is capable of adaptation and undergoing DNA repair in response to exposure to hydrogen peroxide and, therefore, there is the potential for mutation and the development of resistance (Min & Gu 2003). *In vitro* evaluation of hydrogen peroxide treatment has also revealed its ability to induce breakages in dsDNA and drive phenotypic diversity in yeasts (Qi *et al.* 2019) suggesting the potential for resistance to develop.

Generally, catalase-negative bacteria such as Streptococci, *Enterococcus* spp. and *Clostridia* spp. are susceptible to hydrogen peroxide. Other bacteria, like some Streptococci, are 'catalase negative' but actually produce hydrogen peroxide to protect their population (Serra *et al.* 2008). In healthy women, hydrogen peroxide producing *Lactobacillus* spp. predominate in the vaginal flora (Martin *et al.* 2012) which may be a reflection of the role of balance in natural immune systems in maintaining a healthy microbiome (Heil *et al.* 2018).

Tris-EDTA

Metal chelating agents such as Tris-EDTA may be useful for disrupting biofilm-forming agents, such as *P. aeruginosa* (Banin *et al.* 2006) or fungal infections (Lyle *et al.* 2011). However, their clinical efficacy is yet to be proven in controlled studies *in vivo*. Banin *et al.* (2006) explain that Tris-EDTA chelates divalent metal cations from their binding sites in lipopolysaccharide membranes to result in detachment, lysis and killing of cells. *In vitro* studies have reported a beneficial effect of Tris-EDTA as a potentiator of aminoglycoside activity against human strains of *Staphylococcus aureus*, *Enterococcus faecalis*, *P. aeruginosa* and *Enterobacteriaceae* spp. (Lebeaux *et al.* 2015). Banin *et al.* (2006) confirmed that Tris-EDTA in combination with gentamicin was the most effective disperser of biofilm cells, thereby effectively exposing bacteria to intense antimicrobial activity. Early studies in bovine endometritis demonstrated that Tris-EDTA may reverse antimicrobial resistance to some antibiotics (oxytetracycline, enrofloxacin, lincomycin-spectinomycin or amikacin) and produce good pregnancy rates after treatment (Farca *et al.* 1997). The infusion of Tris-EDTA (250 mM EDTA and 50 mM Tris, pH 8) into the mare's uterus has been considered a useful adjunct to antimicrobial treatment for endometritis caused by *Pseudomonas* spp. It is safe to use in the uterus-producing a mild inflammation that resolves within 7 days of treatment in a similar manner to saline infusion (Youngquist *et al.* 1984). Recently, *in vitro* studies of biofilm produced by equine uterine pathogens observed that Tris-EDTA was able to reduce biofilm mass by 10–40% (Ferris *et al.* 2016).

Kerosene

In New Zealand, kerosene is used as an intrauterine treatment to purge the uterine lymphatic glands during chronic bacterial endometritis. Treatment of the uterus with kerosene strips away mucus, cilia and, in some cases, endometrial epithelial cells and stimulates the apical portions of the endometrial glands (Bracher *et al.* 1991). This allows regeneration of the epithelium and mucociliary apparatus (LeBlanc & Causey 2009, Bradecamp *et al.* 2014). Inflammatory changes in the uterus resolve sufficiently within 21 days of treatment, so that 50% of treated mares conceived and carried a foal to term despite their predicted foaling rate being <50% based on their uterine biopsy scores (Bracher *et al.* 1991).

Treatment of fungal endometritis

Therapy for fungal endometritis is directed towards correcting a combination of predisposing factors such as uterine infusions with non-antibiotic agents, uterine lavage and systemic and/or intrauterine administration of anti-fungal agents (Stout 2008, Dasciano 2009). It may also be beneficial to apply topical antifungal medication to the vagina and clitoris as these areas may act as a reservoir or nidus for reinfection (Stout 2008). Prior to treatment it is beneficial to remove inflammatory uterine fluid by lavage with sterile saline or lactated Ringer's solution. Non-specific treatments are also often utilised to kill fungal organisms and/or disrupt biofilm. Those may include N-Acetylcysteine solution, DMSO, hydrogen peroxide, povidone-iodine, Tris-EDTA and acetic acid or vinegar.

Once diagnosed, yeasts and hyphate fungi are often susceptible to polyene class antifungal agents such as nystatin and amphotericin B, but their susceptibility to azole class antifungal agents such as ketoconazole, fluconazole, and miconazole may vary (Beltaire *et al.* 2012). Several anti-fungal agents are available for intrauterine therapy or systemic therapy. Fluconazole and itraconazole are available as oral formulations which can provide long term anti-mycotic activity and may be an important component of a multimodal treatment programme for fungal endometritis in the mare (Scofield *et al.* 2013). Treatment with more than one anti-fungal agent may be indicated in refractory or recurrent clinical cases. One recommended therapeutic protocol involves uterine lavage followed by intrauterine infusion of an antifungal agent (nystatin) once daily for five days and concurrent systemic administration of a second antifungal agent (fluconazole) for 21 days (McCue & Ferris 2018). Mixing an antifungal agent such as nystatin into a buffered chelator such as Tris-EDTA prior to uterine infusion may potentiate the effect of the antifungal agent. Lufenuron (Program, Novartis Animal Health) is an anti-flea treatment that inhibits chitin

synthesis in the cell wall. It has been reported to be effective to treat equine fungal endometritis if the fungal agent has chitin in its cell walls (Hess *et al.* 2002).

Conclusion

After breeding, all mares will experience some degree of physiological endometritis, which involves a mechanical and immunological response used by the mare to evacuate excess fluid, contaminants and dead spermatozoa, and ultimately creates a hospitable environment for embryo development. The challenge is to preserve and facilitate the balance between the complex pro- and anti-inflammatory uterine responses to the allogenic spermatozoa to ensure the uterine environment is hospitable at the time of arrival of the embryo 6 days after ovulation. This balance can be attained by careful management involving hygiene, exploiting the oestrogenic and immunologically primed uterus at the time of breeding, and minimising unnecessary invasion of the reproductive tract and application of treatments that may not be indicated. Blanket treatment with antibiotics is no longer a suitable strategy and needs to be evidence-based. An increased interest in non-antibiotic alternatives warrants further investigation, but more data is required to evaluate their efficacy and safety. It is also possible that resistance could develop to these alternative treatments and beware of their potential to actually induce mutations in some bacteria. New diagnostic methods that identify the genes involved in a mare's predisposition to endometritis, combined with the application of new immunological treatments, will help us address the prevention of PBIE and its detrimental consequences on fertility in the mare.

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Author contribution statement

L M conceived and coordinated the paper and wrote the sections on diagnostics, treatments, Figures 1 and 2 and non-antibiotic therapies. P M wrote the section on fungal endometritis and provided Figure 3 and provided review of the content. C A wrote the section on pathophysiology of endometritis and contributed to reference sourcing and provided review of the content.

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