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Persistent breeding-induced endometritis in mare: A review

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Abstract
Endometritis is a frequent adverse effect of reproduction, but ongoing inflammation puts pregnancy at risk. One of the main issues with a mare's reproductive health has been identified as persistent endometritis. Transient endometritis is required for the removal of dead spermatozoa and germs, however in a small percentage of mares, the inflammation does not go away quickly. The likelihood of developing persistent breeding-induced endometritis depends on many factors, including age, reproductive history, and endometrial quality. Seminal plasma mediates the immune response to mating because of its ability to protect live spermatozoa and because it aids the mare's immune system in identifying and attacking dead spermatozoa. Despite the fact that the adaptive immune response has been linked to the response to breeding, the innate immune response and mechanical clearance are the key mechanisms postulated to work to clear endometritis. Compared to mares who are resistant to chronic breeding-induced endometritis, vulnerable mares show a different post-breeding innate immune response. Numerous techniques and technologies are utilized to identify and treat chronic endometritis. Research on the ethology, pathophysiology, and therapies of persistent breeding-induced endometritis is compiled in this review of the literature.

Keywords: Breeding-induced, endometritis, mare

Introduction
Post-breeding endometritis, is endometrial infection in mares, within 48 hours of breeding, which is a natural reaction. Its goal is to clean the uterine lumen of seminal plasma, surplus sperm, Microbes, and debris in order to make room for the embryo. Persistent breeding-induced endometritis, or PBIE, affects mares that don't successfully undergo this removal and cleaning procedure. Mares are classified as susceptible or resistant to chronic PBIE depending on how well they are able to recover from this inflammation or infection 48 hours after giving birth. After a natural or artificial insemination, all mares exhibit this brief uterine inflammatory reaction within 30 minutes (Alghamdi *et. al.*, 2005; Troedsson, 1997) [2, 88]. In addition to threatening the viability of the conceptus, which migrates to the uterus about 5–6 days after breeding, this prolonged endometritis brought on by breeding reduces fertility. In addition to having a pendulous uterus and a poor vulvar conformation, susceptible mares struggle to remove inflammation (Scoggin, 2015) [79]. In addition, mature mares are more likely to be vulnerable (Woodward *et al.*, 2012) [97]. Mares with PBIE have a protracted uterine inflammatory response (Troedsson, 1997; Carnevale *et al.*, 2000) [88, 23], this causes an abnormal accumulation of polymorph nuclear neutrophils (PMNs) in intrauterine fluid up to 96 hours after breeding, impairing embryonic survival and the start of pregnancy. (Zent *et. al.*, 1998; Bucca *et. al.*, 2008; Woodward *et. al.*, 2013) [99, 20, 98]. Horse embryo moves from the uterine tube to the lumen between 144 and 168 hours after ovulation. (Freeman *et al.*, 1991) [40]. Inflammation can be resolved quickly because of this because progesterone production and cervical tone are both increasing at the same time. These mares' reproductive potential is diminished in both natural and artificial insemination because of persistent neutrophilia, excessive intraluminal fluid accumulation, and protracted pro-inflammatory cytokine release, they are all harmful to embryos (Robertson *et. al.*, 2018) [74]. Due to their frequent breeding and flushing throughout the breeding season, embryo donor mares are more likely to develop endometritis (Squires *et al.*, 2013) [82]. Between 6 and 12 hours after breeding, in mares that are PBIE-resistant, have balance of pro- and anti-inflammatory substances (Woodward & Troedsson 2015; Marth *et al.*, 2018a) [96, 60]. A possible infection can result from the ineffective elimination of germs introduced into the uterus during reproduction as a result of decreased innate immune response activation.

Endometritis has traditionally been treated using a variety of therapeutic modalities, such as a Mix of antibiotics, ecbolic drugs, anti-inflammatories, and uterine lavage. Sadly, a small percentage of mares do not react to conventional treatments (Canisso *et al.,* 2016; Scoggin, 2016) [22, 80]. For mares with PBIE, alternative medicines have been developed due to the ineffectiveness of conventional treatments and the rising prevalence of bacteria resistant to antibiotics (Scoggin, 2016) [80].

Etiology of Endometritis

Even though sperm and non-infectious factors like bacteria and fungus can both induce endometritis (Troedsson *et al.*, 1998) [99], they frequently co-occur in clinical practice. It is more likely for susceptible mares with poor reproductive anatomy, such as poor vulvar conformation, a torn vestibule-vaginal sphincter, ventral sacculation of the uterus, impaired uterine contractility, incompetent cervix, and atrophied endometrium folds, to aspirate air or collect fluid or urine in the vagina and uterus. This makes the mare more susceptible to both infectious and non-infect (Canisso *et. al.*, 2016; Trotter & McKinnon 1988) [22, 92]. Additionally, horses with healthy immune systems and functional reproductive systems are able to get rid of infections on their own (i.e., they are resistant to endometritis), whereas weak immune systems may leave horses vulnerable to infection or prone to chronic inflammation (Fumuso *et. al.,* 2007; Christoffersen *et. al.,* 2012; Christoffersen *et. al.,* 2015) [75, 97, 70].

Infectious endometritis

Both the innate and the adaptive immune systems make up the mucosal reproductive tract. T cells serve as the intermediary for adaptive immunity, which reacts to antigen detection progressively and selectively (Lieberman, 2003) [56]. The innate immune response, on the other hand, controls how an organism reacts to breeding (Nash, 2010) [65]. This is characterized by a non-specific, quick, and fleeting reaction (Muraille & Goriely 2017) [63]. It begins with Toll-like receptors (TLRs), immunoglobulins, and complement and leads to leukocytic digestion and elimination of foreign material, regardless of pathogenicity (Janeway & Medzhitov 2002; Medzhitov & Janeway 2000) [48, 61]. *Streptococcus* species, *Coliforms*, *Pseudomonas aeruginosa*, and Staphylococcus aureus are the most frequently isolated bacteria associated with endometritis in clinical situations (Canisso *et al*., 2016; LeBlanc & Causey 2009) [22, 54]. *Streptococcus zooepidemicus* and *Escherichia coli*, respectively, have been identified as the most frequent causes of acute and chronic endometritis by Frontoso *et al*. (2008) and Albihn *et al*. (2013) [41, 101]. Notably, *Streptococcus zooepidemicus* has been shown to cause latent, deeply ingested infections in the endometrium of mares, leaving them resistant to standard treatment (Petersen *et al*., 2015). According to Dascanio *et al*. (2001) [31], fungi can exist alone or in conjunction with bacteria and are less frequently (1–5%) linked to endometritis. The most prevalent genera are Aspergillus and Candida, while other species, as Mucorsp, have also been detected less frequently (Beltaire *et al*., 2012; Canisso *et al*., 2020; Dascanio *et al.*, 2001) [13, 21, 31]. It should be highlighted that fungal endometritis has been discovered following frequent use of intrauterine antibiotics. It often manifests as an opportunistic illness (Canisso *et al*., 2020; HINRICHS *et al*., 1992; Stout, 2008) [21, 46, 84]. Because bacteria and fungi can create biofilm, mares who are vulnerable to PBIE are more likely to have chronic infections (Ferris, 2017; Ferris, 2014) [36, 83]. According to Zigang *et al*. (2006) [100], biofilm is a complex collection of bacteria and their secretions that gives microbes the ability to elude the immune system (Mustoe, 2004; Thurlow *et al*., 2011) [64, 86]. Contrary to planktonic bacterial infection (bacteria without biofilm), which is when there isn't a biofilm, biofilm serves as a barrier for the diffusion of antibiotics, and this restricted penetration leads to resistance to antibiotic therapy (Mah & O'Toole 2001) [58]. The ability to form biofilms is possessed by 80% of the bacteria isolated from the horse uterus, according to research by Beehan *et al*. (2015) [12], Ferris *et al*. (2014) [83], and Ferris *et al*. (2016) [37]. According to Morse *et al*. (2018) [62] and Berger *et al*. (2018) [15], host immunity and the microenvironment are known to play a part in biofilm formation in various bodily systems, such as the oral cavity.

Non-Infectious Endometritis: Innate and adaptive immune systems make up the mucosal reproductive tract's immune system. T cells serve as a conduit via which adaptive immunity reacts progressively and selectively to antigen recognition (Lieberman, 2003) [56]. In contrast, the innate immune response controls how an organism reacts to breeding (Nash, 2010) [65]. This entails a quick, non-specific, and fleeting reaction (Muraille & Goriely 2017) [63]. It is initiated by Toll-like receptors (TLRs), immunoglobulins, and complement and results in the leukocytic digestion and elimination of foreign material independent of pathogenicity (Janeway & Medzhitov 2002; Medzhitov & Janeway 2000 [48, 61]. Repeated exposure to spermatozoa and embryos can "challenge" the immune system without causing the production of anti-sperm or anti-embryo antibodies because to the mostly innate immune response. A tolerogenic immunological milieu is created by regulatory T cells (Treg cells), which exclusively detect male antigens and reduce immune rejection and inflammation (Canisso *et al*., 2020; Schjenken & Robertson, 2015) [21, 78]. A subset of T lymphocytes known as "treg cells" has the capacity to inhibit the immune system. Treg cells typically inhibit macrophages, T, B, natural killer (NK), and dendritic cell cytokine production and effector activity (Sakaguchi, 2000; Shevach, 2002) [77, 81]. According to anecdotal evidence, it has long been hypothesized in clinical practice that mares that are regularly bred to the same stallion for cumulative breeding seasons, especially in embryo transfer programs, are more likely to acquire more severe PBIE or to become infertile.

Innate Immune Response to Endometritis: Following antigen identification and communication by mucosal epithelial cells in endometrium, the local innate immune response is promptly triggered (Marth *et al*., 2018; Nash *et al*., 2010 [60, 65]. Innate immune system, the first line of defense, is triggered by recognition of foreign particles. Innate immune system's primary duties include: (1) triggering cytokines, such as chemokines, to draw immune cells to infection sites; (2) encouraging the complement cascade to be activated in order to remove dead cells; (3) triggering the adaptive immune system's activation by antigen presentation; and (4) acting as a physical barrier to invaders.

Age: Age factor of the mare influences the sensitization of the uterus to oxytocin and prostaglandins (Rigby *et al.*, 2001) [73]. Many of the anatomical defects which occur as repeated mating and foaling as age progress such as vaginal stretching,

a pendulous uterus, or degenerative defects of the myometrium and/or endometrium, an incompetent cervix impair the mechanical pathway, makes mare susceptible to persistent breeding induced endometritis (Evans *et al.*, 1987; LeBlanc *et al.*, 1989) [34, 55]. As many as 25% of broodmares in a typical breeding program are under the age of 16, which is when mare fertility begins to diminish (Allen & Pycock 1988) [3]. According to Zent *et al*. (1998) [99], 10-15% of mares are thought to be at risk of developing PBIE.

Seminal Plasma (SP): Compared to other species, stallion semen in particular has high concentrations of SP. Although seminal plasma does temporarily increase endometrial inflammation, it also changes how long and how strongly the body reacts to spermatozoa. Studies conducted *in vitro* indicate that SP modifies and inhibits sperm-PMN binding and phagocytosis in horses. All *in vivo* research, however, has demonstrated that SP itself triggers neutrophilia in pigs (Bischof *et al.*, 1994) [16] and mares (Katila, 2005) [50].

Chemical factor: Expression of the inducible nitric oxide synthase (iNOS), which produces NO, is one of the immediate effects of the inflammatory response. Increased NO generated by iNOS aids in the elimination of infections but has the potential to be cytotoxic as well. As a recognized smooth muscle relaxant, NO prevented myometrial tissue from responding to electrical stimulation *in vitro* (Liu *et al*., 1997) [57]. Mares susceptible to PBIE also produced more NO and had higher iNOS activity 13 hours after fertilization compared to resistant mares (Alghamdi *et al.*, 2005) [2].

Physical defence factor: Prostaglandin F2a (PGF2a) is released as the PMNa passes through the endometrium of the mare; this PGF2a, coupled with the oxytocin secreted by the stimuli from AI or natural service, contribute to the constriction of the myometrium, which in turn affects the ejection of uterine fluids. Synchronized contractions of the longitudinal and circular myometrial tissues are necessary for effective uterine evacuation of fluid and debris. Using electrodes, Troedsson *et al*. found that susceptible mares for persistent endometritis showed less electrical activity than resistant mares in terms of length, frequency, synchronization, and intensity.

Pathology of persistent breeding induced endometritis

Immunoglobulin concentrations (IgA, M, and G) in uterine secretions are comparable during all phases of the oestrous cycle in healthy mares and are only increased in endometritis-prone mares (Waelchli & Winder 1987) [94]. After natural service or AI, the uterus begins to experience PMN penetration, which peaks 4–8 hours later (Katila, 1995) [49]. When the PMNs are activated, lytic enzymes are released, which cause bacteria to be phagocytosed. Pro-inflammatory cytokines also rapidly rise within the first 24 hours after breeding, and PMNs are activated to attach to and engulf germs and spermatozoa (Christoffersen *et al.*, 2012) [25]. According to Troedsson (1997) [88] and Troedsson & Liu (1991) [90], prolonged endometrial inflammation reduces the likelihood of a pregnancy and makes the uterine environment unfavorable for the embryo as it enters the uterus.

Timeline of the events in development of induced endometritis.

1. After AI or natural service the spermatozoa and bacteria are opsonised particullary the dead and damaged spermatozoa.
2. Once the opsonisation is done, the spermatozoa and bacteria induce the release of cytokines and PMNs recruitment.
3. PMNs released migrates through the endometrium which releases PGF2a along with the oxytocin secreted by the stimuli from AI or natural service.
4. The release of cytokines rises after 2hrs of AI or natural service where simultaneously uterine expulsion by contraction is taking place up to 6hr after breeding, with in this duration the spermatozoa are transported to the oviduct.
5. By 12-14 hrs. After breeding the cytokine immune response and uterine contractions subside to the baseline. And by 24hrs all the excess fluids which have remained are drained by lymphatics and the uterus is primed.

Diagnosis of persistent endometritis

For PBIE Even though the disorder might have originated with PBIE, microbes, most usually *S. zooepidemicus* and *E. coli*, are what cause chronic endometritis. The term "subclinical endometritis" is used when endometritis symptoms are relatively mild and only PMNs, not bacteria, are discovered. Pyometra is a severe form of endometritis where the uterus fills with a lot of pus. A severe uterine condition called acute post-partum metritis develops in the first week following delivery. The organism that causes Contagious Equine Metritis (CEM), *Taylorella equigenitalis*, is the most significant venereally transmitted organism. Therefore, the diagnosis becomes the primary factor affecting a mare's fertility. Endometritis must be diagnosed using a multimodal approach and a thorough clinical history. The most popular methods used to identify endometritis in mares are endometrial culture, cytology, and biopsy (Nielsen, 2005). The two primary categories of diagnostic techniques are clinical examination and laboratory examination.

History

Mares who are predisposed to PBIE may have a history of vulvar discharge, recurrent embryonic loss, early return to estrus, accumulation of intrauterine fluid both before and after breeding, and failure to conceive despite good breeding management.

Clinical examination

a) Transrectal

Transrectal palpation examination: Transrectal examination, performed 24 to 48 hours after breeding, is the earliest kind of evaluation that can confirm a diagnosis of PBIE. The condition can be identified by the size, tone, and location of the uterus, and the cervix is best inspected. The illness can be identified by the presence of free fluid in the uterine lumen.

b) Transrectal ultrasound examination: Used as a screening method to find IUF, which can be predictive of endometritis, and to measure its amount and visual appearance. However, not all endometritis-affected mares, particularly those with chronic endometritis, assemble IUF. It has been hypothesized that a mare is prone to endometritis if she has IUF more than 2 cm at any point during estrus. After childbirth, uterine fluid is also normal, but the inflammatory fluid can be distinguished by echogenicity. High echogenicity in a

fluid is a sign of inflammatory components. Fluid volume and echogenicity can help determine the need for additional diagnostic procedures and treatment plans.

Transvaginal examinations

Endometritis can manifest as vaginal discharge, however many affected mares do not. During insemination, a digital vaginal examination is always performed. If discharge is visible on the glove, endometritis is likely the cause. In order to detect the existence of abnormal discharges in the vagina or cervix, mares are subjected to speculum examination.

Hysteroscopy is transvaginal procedure, which involves utilizing a video-endoscope or a fiber-optic endoscope to visualize the endometrium. It is possible to find adhesions, retained endometrial cups, foreign substances, cysts, and fluid, focal sites of infection, pus and more localized lesions.

Laboratory/cytological examination

a) **Swab method:** Endometrial cytology is a quick and affordable method for determining whether a mare has endometritis. According to Hinrichs *et al*. (1992) [46], the number of microbe's decreases as it approaches the cranial reproductive canal (uterus, cervix).Therefore, a uterine cytology sample is obtained using a guarded swab or a brush. The completely guarded swab was positioned within the plastic cannula without a cap, whereas the partially guarded swab was enclosed in an outer plastic cannula with a distal gelatine capsule. After a 24-hour incubation period, only 25% of the cultures from fully guarded swabs and 54% of those from moderately guarded swabs were positive, respectively.

b) **Cotton-tip swab:** Quick, simple, and affordable method of sample collection for cytology and culture. Cytology and the results can be combined to determine the best course of treatment. However, because just a tiny portion of the uterus is sampled, it is possible to overlook localized infections that do not result in a widespread endometrial reaction. Less cells are retrieved and cells are slightly compressed compared to cytobrush, which makes evaluation more challenging.

c) **Cytobrush:** Fast, simple, and affordable method for gathering samples for cytology and culture, albeit it is more frequently used for cytology. However, when only a tiny portion of the uterus is sampled, localized infections may go undetected. Biofilm may prevent the detection of bacteria.

d) **Low volume lavage:** As a broader endometrial surface is covered, it is thought to be a more effective diagnostic tool than the swab method. As a result, this technique is more frequently used for the identification of challenging and chronic endometritis. Low recovery rate is seen, nonetheless, as a result of the fluid's unequal distribution. Through the use of a balloon catheter, Ball *et al.* (1988) [10] administered 60 ml of phosphate-buffered saline (PBS) and typically retrieved 35 ml. 10 ml of the recovered fluid can be centrifuged or allowed to decant prior to cytological examination. The caudal reproductive tract's commensal microbes, however, provide a risk of contamination. It calls for at least one qualified clinician and a support person. An excessive amount of fluid may undermine the cytological analysis by over diluting the sample, leading to a false-negative result. Poor fluid recovery can occur in mares who have a pendulous uterus.

e) **Endometrial Biopsy:** All of the aforementioned techniques can identify endometritis, but only a biopsy can tell the difference between acute and chronic endometritis. A histology slide with PMNs on it is a sign of acute endometritis. Endometrium biopsy is a sensitive and specific method for diagnosing endometritis in mares by histological evaluation and culture of the biopsy, even though this method is mostly employed for histological evaluation. Particularly beneficial for infections of the deep endometrium. Results could inform the chosen treatment plans. However, it necessitates a biopsy, a quick but intrusive process. Additionally, it calls for qualified laboratory staff who can conduct histological analyses and culture tests (Riddle *et al.*, 2007; Canisso *et al.*, 2016) [72, 22].

Treatment

Depending on the aetiology, there are many approaches to treating PBIE. Antibiotic-resistant bacteria are becoming more common, which has spurred the creation of non-traditional medicines in recent years due to the ineffectiveness of conventional therapeutic approaches.

a) **Ecbolic**: By using ecbolic medications such prostaglandin (PG) F2 and oxytocin (10–25 units), uterine clearance is enhanced. According to LeBlanc *et al.* (1994a) and LeBlanc & Causey (2009) [53, 54], oxytocin is active throughout oestrus but remains active for 48 hours after ovulation. However, age-related and anatomical aspects are unavoidable (Annandale *et al.*, 2018) [8]. 250g of cloprostenol, one of several PGF2 analogues tested for ecbolic effects, produced the most reliable uterine response (Combs *et al.*, 1996) [27].

b) **Antibiotics**: Antimicrobial resistance has been rapidly developing as a result of the inappropriate use of antibiotics. In order to properly treat endometritis and stop the emergence of antibiotic resistance, accurate identification of the microorganism(s), together with sensitivity to antimicrobials, is essential. *Streptococcus* spp, *Escherichia coli*, Klebsiellasp, Pseudomonas sp, and Staphylococcus spp are the most frequently isolated bacteria in the mare's reproductive tract (Canisso *et al*., 2016; LeBlanc & Causey 2009; Walter *et al.*, 2012; Beltaire *et al.*, 2012) [22, 54, 13], and -lactam (e. It is intriguing that the two most prevalent isolates from mares with endometritis, *Streptococcus zooepidemicus* and *Escherichia coli*, were discovered to be extremely resistant to conventional antimicrobials (Benko *et al*., 2015) [14].

c) **Uterine Lavage and Treatment for Biofilm**

In mares with severe intrauterine fluid buildup (e.g., >2 cm depth) and high echogenicity on ultrasonography, uterine lavage is advised (Brinsko *et al.*, 2003) [17]. The most popular methods for laving mares' uteruses include crystalloid solutions such lactated Ringer's solution (LRS) and 0.9% saline (Vanderwall & Woods 2003) [93]. Studies have shown that some bacteria, including *Escherichia coli*, can utilise the gluconate and lactate present in Plasmalyte and LRS, two crystalloid solutions that are less frequently employed for uterine lavages, respectively, as growth substrates (Eisenberg & Dobrogosz 1967) [33]. Infectious endometritis cannot be treated alone with uterine lavage with crystalloids. In cases of fungal endometritis, vinegar can alter the uterine microbiome. These solutions can also be enhanced with

mucolytics, such as N-acetylcysteine, dimethyl sulfoxide, and ethylenediaminetetraacetic acid-2-amino-2-hydroxymethyl-propane-1,3-diol alone or in combination with Tris. Although these products are frequently used to treat endometritis, it is not known how they impact the uterine microbiome that lives there or how to employ them to get the microbiome balance back in utero. In order to avoid harming the embryo or the sperm before or after fertilization, uterine lavage assists by physically eliminating germs, debris, inflammatory cells and mediators, and dead sperm from the lumen (Brinsko *et al*., 2003; Vanderwall & Woods 2003; Knutti *et al*., 2000). It may be carried out at any time before breeding or beginning four hours afterwards. The sperm must enter the uterine tubes within four hours of breeding in order for fertility to be unaffected (Brinsko *et al.*, 1991; Brinsko *et al.*, 1990; Fiala *et al*., 2007) [17, 19, 39]. Additionally, the use of uterine lavage can reintroduce live neutrophils to resume an active destruction of microorganisms in a stationary inflammatory state.

d) Immunomodulator: The immunological response to endometritis has also been observed to be altered by the use of bacterial extracts (Fumuso *et al.*, 2007; Christoffersen *et al*., 2012; Fumuso *et al.*, 2006; Rogan *et al.*, 2007) [75, 97, 43, 75]. A commercial immunomodulator called Mycobacterium phlei cell wall extract (MCWE) is used to treat *Streptococcus zooepidemicus*-induced equine endometritis. The innate humoral immune response is strengthened by this immunomodulator. It has been demonstrated that it increases the expression of the anti-inflammatory cytokine IL10 while decreasing the expression of the pro-inflammatory cytokines IL1, IL6, and TNF in sensitive mares after both breeding and a challenge with Gram-positive bacteria (Fumuso *et al.*, 2007) [75]. Additionally, mares receiving MCWE display a reduction in NO (Troedsson *et al.*, 1995) [95]. Despite the fact that MCWE had no effect on the expression of endometrial cytokines in response to *Escherichia coli* challenge, there was a significant decrease in bacterial growth and intrauterine fluid accumulation after treatment (Christoffersen *et al.*, 2012) [25]. MCWE was discovered to be bactericidal whether given intravenously or intrauterinally (Rogan *et al*., 2007) [75]. The immunostimulant Propionibacterium acnes (Neogen Corp, Lexington KY, USA) has also been reported to be able to increase mare pregnancy rates (Rohrbach *et al.*, 2007) [76]. This treatment elicits a non-specific cell-mediated response, mostly through the activation of macrophages and the release of cytokines. Propionibacterium acnes was administered intravenously to mares suffering from clinical endometritis in the only trial done on this immunostimulant (Rohrbach *et al*., 2007) [76]. Repeated administration of this drug as an adjunct to conventional therapy has increased the likelihood of live birth and improved pregnancy rates in mares with a cytologic diagnosis of endometritis.

e) Lactoferrin

The 80kDa protein lactoferrin is widely distributed throughout the body, including the immunological and reproductive systems (Suzuki *et al.*, 2005) [85]. Due to its capacity to chelate free iron, it is thought to be bactericidal (Ammons & Copié 2013) [7]. According to Kolm *et al.* (2006) [52], lactoferrin's endocrine dependence is demonstrated by the fact that its endometrial expression changes depending on the estrous cycle stage and increases during estrus. Recombinant lactoferrin can have a variety of effects, including a considerable reduction in endometrial IL6, as well as a trend toward a reduction in CXCL8, IL1, and TNF (da Silva *et al*., 2017) [29], showing its anti-inflammatory capabilities. In order to get a concentration that is similar to that of equine ejaculate, human recombinant lactoferrin should be delivered intrauterinally at a dose of 1 mL (50 g/mL) diluted in 10 mL of LRS. Although lactoferrin is given for its bactericidal and anti-biofilm properties in other species, horses have not been studied for these effects (Ammons *et al*., 2009; Ammons *et al*., 2011) [6, 5].

f) Platelet-Rich Plasma: (PRP) is entire blood plasma that has been concentrated (3-5 times) in platelets. It has frequently been applied to soft tissue injuries, such as tendinitis, tenosynovitis, and skin wounds, in horse therapeutic practice (de Fontoura Pereira *et al*., 2019; Georg *et al.*, 2009) [28, 102]. Additionally, autologous plasma and antibiotic medication have been shown to increase mare pregnancy rates (Pascoe, 1995) [69].

g) Stem cells: In both Veterinary and human medicine, the use of mesenchymal Stem cells (MSCs) to control inflammatory processes has seen a significant increase in attention (Timmers *et al.*, 2011; Barrachina *et al*., 2016) [87, 11]. These cells can develop into neuroectodermal cells (Grove *et al*., 2004) [45], renal parenchyma, hepatic epithelium, skeletal myoblasts, gut and skin epithelia, and even endometrial cells (Du & Taylor, 2007) [32]. They can be collected in diverse concentrations from different organs and contain anti-apoptotic, chemotactic, and immune-modulatory properties that can signal surviving cells. Endometrial fibrosis may be treated differently using MSC infusion or injection, according to Alvarenga *et al*.'s (2016) [4] 2016 study. Therapy with MSCs can cause an early (7 days) as well as a prolonged (60 days) remodelling of the endometrium in mares with chronic-degenerative endometriosis by controlling the expression patterns (such as smooth muscle actin, cytokeratin, vimentin, and laminin) linked to the development of pathological fibrosis in the horse endometrium and promoting glandular epithelial cell proliferation (Mambelli *et al*., 2014) [59]. Since no studies have yet evaluated the efficacy of MSCs in mares susceptible to PBIE, more study is necessary. Keep in mind that horses do not undergo decidualization, which entails morphological and functional changes to the endometrium in order to prepare for pregnancy, as do rodents and primates. Therefore, conclusions from studies conducted on these other species may not always be applicable to horses.

Conclusions

The main factor contributing to mares' subfertility is endometritis. Mares who are vulnerable to PBIE are usually aged, have a compromised uterine immunological response, and have inadequate physical barriers and other defence systems. Unbalanced pro- and anti-inflammatory pathways have a major impact on the immunopathogenesis of PBIE in mares. If the body's built-in defence mechanisms (physical and molecular) are weakened, infections of the endometrium may develop. It is also unclear how diseases and therapies change the composition of these microbe populations, the pathobiology of bacteria connected to endometritis in mares,

and the role of the uterine microbiome in preventing infection. Both conventional and alternative therapy for endometritis are centered on re-establishing the body's natural defense mechanisms (such as by repairing certain reproductive seals or immunomodulating inflammation). The ongoing efforts to create substitute antibiotic-free medicines to treat endometritis will help the present trend of avoiding the indiscriminate use of antibiotics in humans and animals.

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