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Review Article

Intrauterine Therapy with Platelet-Rich Plasma for Persistent Breeding-Induced Endometritis in Mares: A Review

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Keywords: Platelet derivatives; Platelet lysate; Reproduction; Regenerative therapy; Uterus

Abstract

This review aims to emphasize the scientific focus on platelet therapies by presenting the results already obtained in mares susceptible to Persistent Breeding-Induced Endometritis (PBIE), as well as highlighting opportunities for further improvement. The recent publication demonstrating the absence of bacterial growth in susceptible mares treated with PRP underscores the potential of regenerative therapies to control infections without promoting the emergence of multidrug-resistant bacteria. Alternative therapies have gained prominence in the current public health context, with the World Health Organization listing antimicrobial resistance among the ten most significant global threats. Endometritis is the leading cause of subfertility in mares, and empirical antibiotic therapies are commonly used in the field due to market pressures related to the high financial value of embryos, along with logistical challenges in obtaining laboratory-dependent diagnostic results. Platelet-Rich Plasma (PRP) is an alternative therapy derived from whole blood plasma with a high concentration of platelets. Its anti-inflammatory, regenerative, and antimicrobial properties are particularly tested when traditional therapies fail to achieve the desired effect. In recent years, research on the use of PRP in equine reproduction has primarily focused on endometritis, with a particular emphasis on persistent breeding-induced endometritis (PBIE). However, there is a growing interest in other platelet derivatives, such as lyophilized platelet-rich plasma and platelet lysate, which offer practical field applications.

Abbreviations

PRP: Platelet Rich Plasma; PBIE: Persistent Breeding-Induced Endometritis; TLRs: Toll-Like Receptors; NF-kB: Nuclear Factor-Kappa B; IL: Interleukin; COX-2: Cyclooxygenase-2; TNFα: Tumor Necrosis Factor-Alpha; GFs: Growth factors; L-PRP: Lyophilized Platelet Rich Plasma; PL: Platelet Lysate.

Introduction

The methods for semen to reach the intrauterine environment in mares include natural mating and artificial insemination; however, even under ideal conditions, antigenic substances enter the uterus concurrently. To clear excess sperm, seminal plasma, uterine debris, and antigens, and to establish an optimal environment for embryo implantation, the uterus undergoes physiological post-breeding endometritis. The occurrence of physiological post-breeding endometritis is as crucial as its duration for achieving favorable fertility rates. Resolution within a physiological timeframe—around 48 hours—optimizes the uterine environment for embryo reception. A balance between pro-inflammatory and anti-inflammatory cytokines is essential for the inflammatory process to occur within the specific time window and appropriate intensity. Simultaneously, rising serum progesterone levels and cervical tone complete the uterine drainage function through the cervix, preparing for embryo arrival in the intrauterine environment approximately 144 to 168 hours after ovulation [1-3].

When the inflammatory response persists beyond 48 hours, Persistent Breeding-Induced Endometritis (PBIE) is

established. This condition is characterized by neutrophilia, excessive intrauterine fluid, and elevated levels of pro-inflammatory cytokines for an extended period—96 hours or more—without effective antigen clearance. These factors together create an embryotoxic environment, ultimately reducing fertility in affected mares [2,4]. Endometritis is the leading cause of subfertility in equines, and PBIE is the primary condition encountered in assisted equine reproduction programs [3,5].

In the 1990s, the role of platelets in inflammation and tissue healing was finally recognized. Since then, the clinical use of platelet-derived products (hemocomponents), such as Platelet-Rich Plasma (PRP), has grown significantly, especially in the medical management of musculoskeletal injuries due to its promise as a low-cost, autologous treatment option [6,7]. PRP is a plasma preparation with an increased concentration of platelets, featuring various concentrations of white blood cells and the lowest possible concentration of red blood cells [7,8].

Intrauterine platelet therapy for endometritis in mares modulates pathological inflammation and improves fertility rates [9-13]. Additionally, the antimicrobial therapeutic potential of platelets has been recently highlighted, with studies indicating a reduction of uterine infections in mares [14,15].

Developing and/or enhancing alternative therapies with antimicrobial properties that do not harm or even support the rebalancing of the affected organ's microbiome is essential, given the current antimicrobial resistance context highlighted by the World Health Organization [16]. The mammalian uterus has its microbiome, and while not all its functions are fully understood, maintaining its homeostasis is known to be crucial for fertility [17-19]. Therefore, the current review was designed to assess the immunophysiological uterine defense mechanisms following natural breeding or artificial insemination in mares, and their pathological imbalances, and to synthesize published findings on the application of platelet therapies as an alternative to modulate inflammation in PBIE. Furthermore, it addresses the recent evidence correlating the absence of microbial growth with treatment using platelet-rich plasma.

Literature review

Endometritis

Endometritis, an inflammation of the endometrium, was once thought to be caused solely by infectious agents, such as bacteria or fungi. However, pioneering studies have shown a similar neutrophilic response regardless of whether the mare was challenged with semen, saline solution, or bacteria [20,21]. Endometritis is a natural part of the physiological process following natural mating or artificial insemination when the endometrium encounters fresh, cooled, or frozen semen. Other substances, infectious (pathogenic or opportunistic microorganisms) or non-infectious (excess sperm, seminal plasma, diluents, cryoprotectants, debris), can also reach the endometrium. This is where physiological post-breeding endometritis, lasting approximately 48 hours, plays a role by clearing these substances and preparing the uterus for embryo implantation. Failure in this defense mechanism can lead to endometrial colonization by pathogens and/or retention of inflammatory substances, which, in turn, leads to persistent breeding-induced endometritis (PBIE). Consequently, an inhospitable uterine environment may then receive the embryo [3,5,21]. Mares are categorized as resistant or susceptible to PBIE based on their capacity for uterine clearance and resolution of inflammation 48 to 72 hours post-breeding [4].

Other causes of endometritis in mares stem from anatomical issues in the reproductive tract, which hinder the drainage of intrauterine fluids and/or facilitate the entry of air, feces, or urine reflux into the vaginal canal and uterus. In such cases, these anatomical problems, as primary factors, should be addressed before assessing treatment for endometritis, as resolving the primary cause can, in some instances, resolve the condition on its own [22,23].

Endometritis can be infectious or non-infectious. Non-infectious endometritis is often related to dysregulation in the inflammatory modulation mechanisms—either pro- or anti-inflammatory cytokines—following breeding [3] or from continuous or intermittent challenges by non-pathogenic exogenous substances (such as urometra or pneumovagina) [22]. Infectious endometritis is primarily associated with aerobic bacteria [24]. In clinical cases, the bacteria most isolated in association with endometritis are *Streptococcus zooepidemicus* and *Escherichia coli*, which predominantly cause acute and chronic endometritis, respectively, followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus* [25-27]. Notably, *Streptococcus zooepidemicus* has also been shown to cause latent and deep infections within the mare's endometrium, rendering them resistant to traditional therapy [28]. Fungal infections are less commonly associated with endometritis (1% - 5%) but can occur either independently or in conjunction with bacteria, often as opportunistic infections following repeated intrauterine antimicrobial treatments. The genera *Aspergillus* and *Candida* are most frequently observed in these cases [29-31].

Repeated episodes of endometritis, whether infectious or non-infectious, along with intrauterine therapies and advancing age, create a potential condition for the development of endometrial fibrosis [5,31,32].

Physiological post-breeding endometritis

The immune system of the mucosal reproductive tract consists of two branches: innate and adaptive immune responses. The response to reproduction is primarily governed by the innate immune response [33,34], which is characterized by a non-specific, rapid, and transient response

[35]. Following the deposition of ejaculate in the endometrium, the acute inflammatory process is triggered by the recognition of semen or foreign material through Toll-Like Receptors (TLRs), immunoglobulins, and the complement system. This activation leads to leukocytic digestion and the elimination of foreign materials, regardless of pathogenicity [3,36,37].

Following TLR activation, nuclear factor-kappa B (NF-kB), a key factor in initiating inflammation [38], is expressed in the endometrium [3,5]. NF-kB is a protein complex that activates genetic codes responsible for the release of pro-inflammatory cytokines, including chemokines and cyclooxygenase-2 (COX-2) [39,40]. Pro-inflammatory cytokines, primarily interleukin 1 (IL1), interleukin 6 (IL6), and tumor necrosis factor-alpha (TNFα), activate vascular endothelial cells. This activation leads to arteriole constriction and venule dilation in the affected area, increasing vascular permeability and causing exudate to leak into the interstitium, resulting in localized edema [41]. With changes in vascular endothelial permeability, cellular responses begin. Vascular endothelial cells increase the expression of P-selectin in response to inflammatory stimuli, which binds to L-selectin on the neutrophil surface, initiating chemotaxis [42]. Neutrophil chemotaxis is primarily mediated by the chemokine CXCL8 [43,44]. Neutrophils then produce integrins that bind to adhesion molecules on endothelial cells, allowing them to arrest and adhere to blood vessel walls [45]. Upon detection of foreign material, neutrophils migrate from the endometrium to the uterine lumen within 30 minutes, reaching peak activity between 6 and 12 hours [46]. Leukocytes subsequently release prostaglandins, which enhance myometrial contractility, aiding in the physical clearance of the uterus in healthy mares [47].

Within 48 to 72 hours post-breeding, anti-inflammatory interleukins IL10 and IL1RN play an important role in the resolution of post-breeding endometritis [4,48,49].

Persistent breeding-induced endometritis

Persistent Breeding-Induced Endometritis (PBIE) results from an inadequate immune response and/or compromised physical defense mechanisms that prevent the resolution of physiological post-breeding endometritis within the critical 48- to 72-hour window. Managing mares susceptible to PBIE presents a timing challenge, as the equine embryo migrates from the oviduct to the uterine lumen between 144- and 168 hours post-ovulation [3,50]. Persistent neutrophilia, excessive accumulation of intraluminal fluid, and prolonged production of pro-inflammatory cytokines are embryotoxic, reducing fertility potential in these mares during natural mating or artificial insemination [2]. Mares prone to PBIE are often older and/or frequently used embryo donors, subjected to multiple breeding interventions per season and over consecutive years. They may have a history of intrauterine fluid accumulation pre- and post-breeding, recurrent embryonic loss, early return to estrus, an inability to conceive despite optimal reproductive management, and may present with vulvar discharge [1,51].

The expression of various pro-inflammatory cytokines (e.g., IL1β, CXCL8, TNFα) is higher in mares susceptible to PBIE, even before exposure to an antigen, compared to resistant mares, and it increases further in response to challenges with pathogens or spermatozoa [44,52,53]. The onset of inflammation is gradual, followed by a prolonged immune response [54,55]. Mares susceptible to PBIE exhibit a failure in the expression of anti-inflammatory cytokines IL10 and IL1RN, as well as IL6, a cytokine with pleiotropic functions. This weak anti-inflammatory response contributes to prolonged endometritis [3,44]. Susceptible mares show increased neutrophilia at 2- and 12-hours post-breeding compared to resistant mares [4]. Neutrophils also secrete additional cytokines and chemotactic mediators, which further induce inflammation [45]. The excessive accumulation of neutrophils and intrauterine fluid over a period of 96 hours or more renders embryo survival impossible [4,56]. Furthermore, due to insufficient activation of the innate immune response, microorganisms introduced into the uterus during breeding are not efficiently eliminated, potentially leading to infection [3]. In addition to defective anti-inflammatory cytokine production, susceptible mares have been reported to exhibit impaired myometrial contractility. Mares with PBIE show a different myometrial response to bacterial challenges when compared to resistant mares, including variations in the frequency, duration, and intensity of contractions [57]. It is believed that this reduced smooth muscle activity interferes with uterine clearance in susceptible mares [58].

Traditionally, PBIE has been treated with multimodal therapies, including a combination of uterine flushing, ecbolic agents, anti-inflammatory medications, and antibiotics. Unfortunately, a subset of mares does not respond to traditional treatments. The failure of these therapies, along with the increasing incidence of antibiotic-resistant microorganisms, has led to the development of alternative therapies such as Platelet-Rich Plasma [3,25,59].

Platelet rich plasma

Platelet-Rich Plasma (PRP) is an autologous biological product derived from total blood plasma with a high concentration of platelets. It has become a popular alternative therapy in both human and veterinary medicine due to its anti-inflammatory, regenerative, and antimicrobial properties [60-62]. Platelet therapies are often employed when traditional treatments are costly or do not achieve the desired effect [7]. Furthermore, they are gaining prominence in the current context of human and veterinary public health and welfare [16], aiming to reduce the use of conventional medications while mitigating their side effects, resistance to antibiotic therapies, and imbalance of the uterine microbiome [19,59,63].

Platelets are derived from the cytoplasm of megakaryocytes and contain bioactive molecules stored in cytoplasmic granules, which play a crucial role in restructuring pathological

imbalances. These include fibrinogen; growth factors (e.g., transforming growth factor β, vascular endothelial growth factor, and hepatocyte growth factor); cytokines (e.g., CXCL8, RANTES, and TNFα); and antimicrobial peptides (e.g., platelet factor 4, connective tissue-activating peptide 3, basic platelet protein, thymosin beta-4, and fibrinopeptide A and B) [6,64,65]. Growth Factors (GFs) stored in alpha granules play a significant role in modulating inflammation, tissue repair, chemotactic properties, stimulation of mitosis, cell proliferation, and differentiation, neovascularization, and extracellular matrix deposition [6,66]. In tissue injury, substances are released that activate platelets, causing shape changes, internal transformation, and the release of GFs [67]. Equine platelets are particularly sensitive to ADP, collagen, and platelet-activating factor [68].

Among the main mechanisms of inflammation modulation present in PRP is Hepatocyte Growth Factor (HGF) [40], which inhibits the activation of NF-kB [40,69,70]. In addition to platelet-derived growth factors, other sources also play a role in modulating inflammation, including factors derived from plasma proteins, white blood cells, and red blood cells. These include pro-inflammatory and anti-inflammatory cytokines, anabolic growth factors, catabolic enzymes, hormones, acute-phase proteins, and immunoglobulins, among others [62].

The antimicrobial potential of PRP may be attributed to the platelets, plasma, white blood cells, or the complex mixture of all these constituents. The most relevant bioactive components associated with immune functions against bacteria and fungi include cytokines (e.g., CXCL4, CXCL5, CXCL7, and RANTES), antimicrobial peptides (e.g., platelet factor 4), and the complement system [64,71,72]. It is also suggested that platelets generate reactive oxygen species that can bind to and internalize microorganisms, participating in antibody-dependent cellular cytotoxicity [73,74]. Activated platelets detect signals from the site of injury and microbial threats, expressing a wide range of antibacterial proteins and potential bacterial receptors. Through various signaling pathways, they also have the capacity to alter the host's defense mechanism and trigger the recruitment of leukocytes, which help in identifying, sequestering, and combating invading microorganisms [75,76]. Activated platelets release platelet factor 4 (PF4) stored in their alpha granules, which binds to polyanions (P) on bacteria, undergoing a conformational change and exposing neoepitopes. These neoepitopes induce the production of anti-PF4/P antibodies. Since PF4 binds to a variety of bacteria, the anti-PF4/P IgG can bind and opsonize diverse bacterial species [72].

The anti-inflammatory and antimicrobial properties of PRP may result from a synergism of its constituents. It remains unclear whether the alteration of inflammatory cytokines, used as markers, is due to molecules present in the PRP or if the control of microorganisms by PRP leads to regulated signaling [52,62,77]. Some authors suggest that intentionally increasing the leukocyte concentration in PRP would improve product stability, regulate the inflammatory response, and enhance the immune complex [76,78], further boosting its ability to phagocytize microorganisms [75]. However, other researchers report that during regenerative processes, there could be an exacerbation of the local inflammatory response due to metalloproteinases, pro-inflammatory proteases, and acid hydrolases secreted by white blood cells, which may delay the healing process [76,79].

In PBIE, PRP is recognized as an effective inflammatory modulator, and its antimicrobial potential in infectious endometritis in mares is beginning to be described [14,15,80].

Intrauterine therapy with platelet-rich plasma in mares

In recent years, the primary focus of research related to the use of PRP in equine reproduction has been on PBIE [9–11,14,15]. The effects of intrauterine therapy with autologous PRP in mares with a history of PBIE have been described by researchers analyzing various aspects (Table 1).

The understanding of the antibacterial action of PRP in equine reproduction is still limited. However, combined with other lines of research, both in vivo and *in vitro*, in equine and human medicine, it demonstrates its relevance in the potential control of bacterial infections [8,15,81].

It is worth noting that intrauterine infusion of PRP is often performed without platelet activation. Activation occurs when platelets interact with the uterine lumen; however, this has not yet been confirmed [11,82]. Similarly, intra-articular infusion of PRP is conducted without platelet activation under the assumption that platelets are activated upon exposure to the joint lumen [83]. For intrauterine infusion in mares, a larger volume (10 to 60 mL) is presumably required to cover the entire surface of the endometrium [82]. The most studied and widespread platelet derivative in equine reproduction to date is autologous PRP. However, there is a growing trend toward using allogeneic pooled lyophilized platelet-rich plasma (PRPL) and platelet lysate (PL) [8].

Therapies with autologous platelet derivatives are safe but limited due to the influence of intrinsic and extrinsic factors of the platelet donor on the product [84,85]. The cellular content and growth factor release of PRP is influenced by the donor's breed, age, and sex [85]. The use of varying protocols for PRP preparation and the variability in the blood constituents of donors complicates the systematic analysis of results across different studies [7,76,86].

Considering individual platelet variability, the allogeneic pool may be advantageous for producing a more consistent, standardized, and effective platelet derivative [87-89]. Therapies utilizing an allogeneic pool allow for qualitative and quantitative improvements in biological products, expanding their production and application potential [89,90]. Allogeneic PRP products processed through lyophilization, such as

Table 1: Effects of intrauterine therapy with autologous PRP in mares with a history of PBIE.

Evaluated Parameters	Metcalf, et al. 2012	Metcalf. 2014	Reghini, et al. 2014	Segabinazzi, et al. 2017	Segabinazzi, et al. 2021	Pasch, et al. 2021	Ghallab, et al. 2023
FERT.	-	▲	-	▲	▲	▲	▲
P.I.C.	▼	-	-	-	▼	-	-
IUF	-	▼	▼	N.D.	▼	▼	▼
PMN	-	-	▼	▼	▼	-	-
COX-2	-	-	-	▼	-	-	-
NO	-	-	▼	-	-	-	-
P.T.B.G.	-	-	-	-	Ø	-	-

FERT: Fertility; P.I.C.: Pro-inflammatory cytokines; IUF: Intrauterine fluid; PMN: Polymorphonuclear cell concentration; COX-2: Positive cyclooxygenase-2 staining; NO: Nitric oxide in intrauterine fluid; P.T.B.G.: Post-treatment bacterial growth;
▲: Increased; **▼:** Decreased; **-:** Not evaluated by the author; **Ø:** Absent; **N.D.:** No difference.

Lyophilized Platelet Rich Plasma [91], or lysis, such as Platelet Lysate [88,92], offer enhanced standardization, storage, transportation, and handling, improving logistics for field professionals [89-91]. Furthermore, allogeneic administration is advantageous over autologous administration in cases where the animal suffers from a medical condition that precludes blood collection or when blood collection is simply not feasible [61].

Comparative proteomic analysis of PRP from mares resistant to PBIE and those susceptible to the condition revealed that four immune response-related proteins were more abundant in resistant mares. These findings suggest that allogeneic PRP prepared from resistant mares may be more effective in modulating endometritis in susceptible mares. However, this hypothesis still requires in vivo testing [93].

Intrauterine therapy with lyophilized platelet-rich plasma

The possibility of a platelet derivative with improved logistics for field use is appealing. Lyophilized platelet-rich plasma (L-PRP) can be used after reconstitution with sterile saline or water, eliminating the need for repeated blood derivative collections and specialized equipment [94,95]. Lyophilized platelets have been tested for wound repair with promising results [96,97]. However, little is known about their biological effects in treating PBIE. Mares with a history of PBIE treated with L-PRP exhibited a shorter estrus phase duration and higher pregnancy rates compared to the control group [95]. Additionally, *in vitro* studies have demonstrated that the most important growth factors are preserved during the lyophilization process of PRP [98].

Intrauterine therapy with platelet lysate

The interest in using platelet lysate (PL) has been growing in equine clinical practice [99,100] due to its improved storage capabilities [92,101,102] and its nature as a cell-free product with lower immunogenic potential [61].

Platelets are lysed, releasing growth factors (GFs), cytokines, and other related proteins [103] through cryogenic platelet rupture via multiple freeze-thaw cycles [92], without affecting the original properties of the platelet concentrate [104]. Theoretically, all blood cell membranes are removed during the preparation protocol, minimizing immunogenic reactions [61], and thereby enhancing the safety of allogeneic platelet concentrates. In equine reproduction, pioneering in vivo studies using autologous PL are beginning to emerge [39].

Mares susceptible to PBIE treated with intrauterine autologous PL and inseminated with frozen semen showed a significant reduction in cytology scores, uterine edema, and intrauterine fluid. However, pregnancy rates between treated and control cycles were not significantly different [39].

In vitro, the broad-spectrum antimicrobial potential of the equine platelet lysate pool has been demonstrated, with bacterial growth decreasing as Gram-negative and Gram-positive bacteria were exposed to increasing concentrations of platelet lysate. Specifically, the growth of Escherichia coli and Pseudomonas aeruginosa was affected in a concentration-dependent manner, with higher PL concentrations producing greater effects [87].

Methods

The literature search was conducted in PubMed, Scopus, Web of Science, and Google Scholar databases, using the keywords “Platelet”, “Platelet Rich Plasma”, “Lyophilized Platelet-Rich Plasma”, “Platelet Lysate”, “Post-Breeding Endometritis”, “Persistent breeding-induced endometritis”, and “Mare”. Keyword combinations were applied to ensure comprehensive coverage of studies related to the use of platelet derivatives in regenerative therapies targeting the reproductive system, particularly the uterus. Inclusion criteria focused on original articles and reviews published in English that were relevant to veterinary reproductive medicine.

Authors perspective

The literature on the use of PRP in PBIE is not extensive, and many mechanisms remain unexplained. The inflammatory modulation potential is strongly reported, while the

antimicrobial effects still face significant reservations related to platelet concentration in the therapeutic dose and pathogen specificity.

Limitations

In other medical fields, the actual therapeutic efficacy of platelet derivatives is debated due to inconsistent results across studies, a point echoed in this review. The lack of standardization and criteria for PRP production—both in terms of technique and the influence of the platelet donor—along with studies addressing conditions where traditional therapies have failed, introduce variables that prevent consistent outcomes among researchers.

Recommendations

Combined with other positive aspects highlighted in this review, studies aimed at improving platelet therapies should be encouraged. More detailed information on the production and composition of products used in research should be strongly requested in future studies on platelet derivatives.

Future directions

The methodological advancement of platelet products, particularly in standardization, storage, transport, and scalable production, represents a significant barrier to overcome. Consequently, their use is expected to gain broader acceptance among field professionals, thereby enhancing their benefits, such as reducing the empirical reliance on traditional antibiotics.

Conclusion

The effectiveness of intrauterine platelet therapy in equine reproduction is undeniable when fertility parameters improve in mares with a history of multiple infertile cycles. The literature on the use of PRP in PBIE is not extensive, and many mechanisms remain unexplained. The inflammatory modulation potential is strongly reported, while the antimicrobial effects still face significant reservations related to platelet concentration in the therapeutic dose and pathogen specificity.

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