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Original Research Article

Use of regenerative medicine in the treatment of endometritis in mares: A systematic review and meta-analysis

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ABSTRACT

Defining the optimal therapy for endometritis remains a significant challenge for clinicians. Given the public health threat posed by antibiotic resistance and the inconclusiveness of traditional therapies, regenerative medicine has been proposed as an alternative. The objective of this study was to conduct a comprehensive systematic review and meta-analysis, to investigate the efficacy of regenerative medicine products in the treatment of both post-breeding persistent and chronic degenerative endometritis (PBIE/CDE) in mares, following the PRISMA guidelines. This research could be a comprehensive scientific reference for determining appropriate treatments and clinical strategies.

All studies exploring the use of regenerative medicine therapies (i.e., plasma products, autologous conditioned serum, mesenchymal stem cells MSCs, and MSC derivatives) in the treatment of PBIE/CDE were included, regardless of the specific protocol used, the evaluated outcomes, or the diagnostic method employed. Two authors independently gathered data and evaluated the risk of bias for each study. Treatment effects were assessed using risk ratios for dichotomous data, accompanied by 95 % confidence intervals. Data were aggregated utilizing the fixed-effects model. The quality of evidence for each outcome was evaluated using GRADE criteria.

Eighteen studies were included in the systematic review, while fifteen trials were included in the meta-analysis. A sub-meta-analysis was conducted separately on platelet-derived products, as well as on MSCs and their derivatives. The results demonstrated an overall positive effect of regenerative therapies in treating PBIE/CDE, particularly those involving MSCs and their derivatives. The positive outcomes include an anti-inflammatory effect, characterized by reduced intrauterine fluid accumulation, neutrophils, and cytokine concentrations. Additionally, improvements in pregnancy, foaling, and embryo recovery rates have been observed in some cases. Despite the limited number of randomized controlled studies and the high variability among protocols, including the timing of treatment, type, and volume of products used, the use of regenerative products, especially MSCs and their derivatives, has promising results in terms of both efficacy and safety for treating PBIE/CDE in mares.

1. Introduction

Persistent breeding-induced endometritis (PBIE) and chronic degenerative endometritis (CDE) are recognized as significant contributors to reduced fertility in mares [1]. PBIE is a multifactorial condition referring to prolonged or persistent inflammation after the physiological response to breeding. Unresolved PBIE or weakened intrinsic uterine defenses can lead to a CDE, chronic inflammation of the endometrium with degenerative changes [2]. The exact mechanism underlying the persistent uterine inflammation in mares remains unidentified, and, although often observed, infectious causes are not always present [1,3]. An immunological balancing is necessary after breeding to allow the passage of normal spermatozoa, eliminate non-viable spermatozoa and debris, and prepare the uterus for the arrival of the embryo [2]. Generally, mares are defined as susceptible to PBIE when they exhibit an altered endometrial inflammatory status following exposure to bacterial

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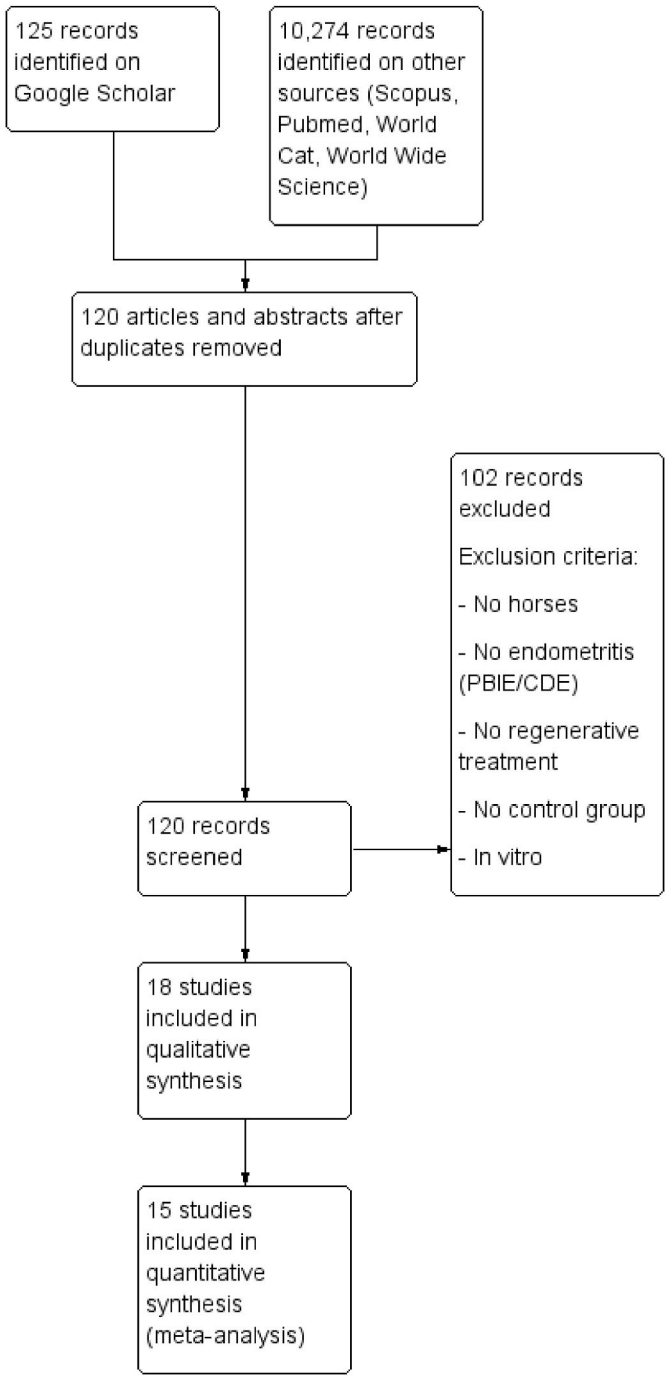

Fig. 1. Flow diagram for identification of published studies. Review Manager 5.4.1 (RevMan 5.4.1).

or seminal challenges. An imbalance of pro-inflammatory cytokines and inflammatory-modulating cytokines has been suggested in susceptible mares [1]. Although different approaches have been proposed, defining the best therapy for endometritis has remained a challenge. Traditional treatments typically involve uterine lavage, ecbolic agents, and systemic or intrauterine antibiotics [4]. Antimicrobic therapies can be beneficial for cases diagnosed with infectious endometritis, mainly when chosen on microbial sensitivity testing. However, since antibiotic resistance is a public health threat and the use of traditional therapies is not conclusive, alternative treatments have been proposed [5,6]. Among these, treatments targeting the immunological response pathway have been suggested as potential alternatives [2].

Regenerative medicine utilizing plasma products such as platelet-rich plasma (PRP) or platelet lysate (PL), as well as stem cells derived from various sources (bone marrow, adipose tissue, and human embryonic stem cells) along with their derivatives, constitutes the primary nontraditional approaches [7–9]. Regenerative medicine represents an emerging and challenging area of biomedical research, applicable to both human and veterinary contexts [10]. The utilization of regenerative therapies in veterinary medicine, particularly for orthopedic conditions in dogs and horses, has become widespread, despite a lack of conclusive evidence regarding its efficacy [11,12,30]. Since 2012, platelet products have been investigated as a therapeutic option for mare endometritis, due to their known anti-inflammatory, regenerative, and antimicrobial effects [13,14,21]. The therapies with stem cells and their derivatives have been considered promising candidates for uterine treatment due to their engraftment and immunomodulatory properties [15,16]. The adoption of MSC therapies for the inflammatory modulation of the endometrium has been slower to gain traction, albeit with good evidence.

Numerous studies, encompassing original research alongside case series and reports, have indicated the potential efficacy of regenerative therapies for PBIE, yielding promising albeit occasionally conflicting outcomes [7–9,14,16–29]. Accurately analyzing all articles could serve as a scientific reference point for establishing suitable treatments and clinical approaches. The use of meta-analysis to assess the current knowledge about the use of regenerative medicine in endometritis can provide more substantial data, mitigating the negative impact of the limitations and biases of individual studies. The aim of this study was to perform a comprehensive systematic review and meta-analysis investigating the efficacy of regenerative medicine in treating PBIE and CDE in mares.

2. Materials and methods

Systematic review was performed according to the PRISMA (Preferred Reporting Items for systematic Reviews and Meta-Analyses) statement, while the meta-analysis was performed using the program Review Manager 5.4.1 (RevMan 5.4.1).

A comprehensive literature search addressing the use of regenerative medicine for the treatment of PBIE and CDE in mares was conducted for all the articles in English, Portuguese, Spanish, and Italian, published between 2000 and 2023. Research was carried out between January and November 2023 by two authors (CDP, CM). Disagreement was resolved by discussion or arbitration by a third author (MMP). Terms “regenerative medicine treatments”, “PRP”, “platelet-rich plasma”, “MSCs”, “mesenchymal stem cells”, “MSC conditioned medium”, “endometritis”, “horse”, “mares” and “equine” were searched in PubMed, World Wide Science, Google Scholar, Scopus.

Studies evaluating other species than horses, other types of uterine conditions (different from PBIE or CDE), and other types of treatments (different from regenerative medicine) were excluded from the systematic review and meta-analysis. Because of their scarcity, clinical trials were included independently of their level of evidence or design and without distinguishing between studies with control groups and those without.

2.1. Data extraction and management

Two authors (CDP e CM) independently extracted data using a prepiloted data extraction form. When necessary, a third author (MPP) was involved in solving any disagreement. For each trial included in the systematic review, the following data were extracted: year of publication, authors, journal of publication, type and timing of the treatment, condition treated, studies classification randomized control trials (RCTs) or not (no-RCTs); original research or case report/series), sample size, control group, outcome measurements, main results (positive, negative, neutral), adverse events and bias.

Table 1
Treatment preparation, features and protocol of administration. In bold: studies included in meta-analysis.

Study	Type of treatment	Preparation methods	Concentrations/Features	Protocol
Abdelnaby et al., 2023 [17]	Exosomes derived from bone marrow MSCs	Ultracentrifugation of DMEM standard medium incubated with horse bone marrow-derived MSCs; lyophilization of CMC gel-loaded MSCs-derived exosomes	CMC gel-loaded MSCs-derived exosomes (200 μg/mL)	IU infusion of lyophilized exosomes solution diluted with 50 mL NaCl 0.9 % twice 21 days apart at the first and second ovulations.
Carluccio et al., 2020 [26]	Autologous PRP	Double centrifugations of 450 mL blood (1920 rpm for 10 min at 22 °C; 3960 rpm for 6 min)	1-1.2 × 10^6 platelets/μL	IU infusion of 15 mL 24 h after ovulation
Colombo et al., 2022 [19]	Autologous platelet lysate	Double centrifugations of 100 mL blood (535×*g* for 20 min at 20 °C; 2275×*g* for 15 min at 20 °C); Activation by freezing at −80 °C	239000 (55000–576000) platelets/μL	IU infusion of 10 mL 12 h after ovulation induction
Dawod et al., 2021 [18]	Autologous PRP	Double centrifugations of 100 mL blood (120×*g* for 10 min; 240×*g* for 10 min); Activation by calcium chloride solution (0.068 mEq/L of PRP)	2 × 10^6 platelets/μL;	IU infusion of 20 mL on the second day after the estrous phase
	Equine L-GF	Commercial product (Cairo Medical Centre Blood Bank) reconstituted in 20 mL of NaCl 0.9 %	L-GF equivalent to those found in platelets from 20 mL of whole blood	IU infusion of 20 mL on the second day after the estrous phase
de Oliveira Tongu et al., 2021 [20]	MSC-CM	Culture medium (Thermo Fisher Scientific) incubated with adipose Tissue-Derived MSCs (OMICS Animal Biotechnology)	IL-6: 81.77, mg/mL IL-10: 22.38 mg/mL TNF-α: 0.21 mg/mL	Supplementation of semen insemination dose at 3:4 (v:v, MSC-CM: semen) or IU infusion of 30 mL 24 h before AI
Ferris et al., 2014 [7]	ACS	Manufacturer's instructions (IRAP II, Arthrex Vet Systems, Bonita Springs, FL, USA).	IL-1Ra: 4447.9 ± 1191.4 pg/mL	IU infusion of 20 mL 24 h before sperm challenge (2 mL of dead spermatozoa)
	Allogeneic MSCs	Bone marrow-derived MSCs from a commercial source (Advanced Regenerative Therapies, Fort Collins, CO, USA)	1 × 10^6 MSCs/mL	IU infusion of 20 mL of Lactated Ringer's solution containing 20 million allogeneic MSCs 24 h before sperm challenge (2 mL of dead spermatozoa)
Ghallab et al., 2023 [23]	Autologous PRP	Double centrifugations of 100 mL blood (120×*g* for 10 min; 240×*g* for 10 min); Activation by calcium chloride solution (0.068 mEq/mL of PRP)	2 × 10^6 platelets/μL	IU infusion of 20 mL 6 h after natural breeding
Lange-Consiglio et al., 2020 [28]	AmnioticMVs	DMEM standard medium incubated with horse amniotic membrane-derived MSCs	20 billion MVs/50 mL of NaCl 0.9 %	IU infusion of 50 mL of NaCl 0.9 % containing 20 billion MVs at days 5 and 9 after ovulation for two cycles
Lange-Consiglio et al., 2023 [27]	Amniotic MSC-EVs	Ultraculture medium incubated with AMSCs was ultracentrifuged at 100000×*g* 4 °C for 1 h and the pellet resuspended in a serum-free medium	20 × 10^9 AMSC-EVs/30 mL of INRA96	Supplementation of semen insemination dose
Mambelli et al., 2013 [24]	Multipotent equine adipose tissue-derived -MSCs	Multipotent equine adipose tissue-derived -MSCs resuspended in 20 mL of fresh prewarmed NaCl 0.9 %	2 x 10^7/20 mL of NaCl 0.9 %	IU infusion of 20 mL (10 mL in each horn tip) during a synchronized estrus
Metcalf et al., 2012 and Metcalf 2014 [21,22]	Autologous PRP	Specialized blood fraction separating centrifuge system (Angel™ Cytomedix, Inc) from 180 mL of citrated whole blood	–	IU infusion of 10 mL (3 mL of PRP + 7 mL of plasma) at the moment of ovulation induction
Navarrete et al., 2020 [16]	Allogeneic adipose MSCs	Adipose samples collected from 3 Chilean Thoroughbred mares during the reproductive season (3 biological replicates)	2 x 10^7/20 mL of NaCl 0.9 %	IU infusion of 20 mL 24 h after IU infusion of 500 million dead sperm in saline
	Allogeneic endometrial MSCs	Endometrial samples collected from 3 Chilean Thoroughbred mares during the reproductive season (3 biological replicates)	2 x 10^7/20 mL of NaCl 0.9 %	IU infusion of 20 mL 24 h after IU infusion of 500 million dead sperm in saline
Pasch et al., 2021 [29]	Autologous PRP	Commercial platelet isolation device (Owl Manor): 60 mL blood loaded into the Restigen-L PRP Device and centrifuged at 3200 rpm for 15 min	9-fold concentration of recovered platelets in a volume of 6 mL	IU infusion of 15 mL (6 mL of PRP + 9 mL of plasma) 12–48 h before AI
Reghini et al., 2014 and Reghini et al., 2016 [9,25]	Autologous PRP	Double centrifugations of 100 mL blood (120×*g* for 10 min; 240×*g* for 10 min); Activation by calcium chloride solution (0.068 mEq/mL of PRP)	>250.000 platelets/μL	IU infusion of 20 mL 4 h after AI
Segabinazzi et al., 2017 [8]	Autologous PRP	Single centrifugation (120×*g* for 10 min) of 45 mL blood; no activation	354.236 ± 17.540 platelets/μL	IU infusion of 20 mL 24 h before AI or 4 h after AI
Segabinazzi et al., 2021 [14]	Autologous PRP and PPP	Double centrifugations of 450 mL (400×*g* for 15 min; 1000×*g* for 10 min); no activation	PRP: 622.9 ± 14 x10^3 platelets/μL PPP: 36.0 ± 25 x10^3 platelets/μL	IU infusion of 40 ml PRP or PPP before (48 and 24 h) and after AI (6 and 24 h)

Abbreviations: ACS: autologous conditioned serum; AI: artificial insemination; CMC: carboxymethylcellulose; DMEM: Dulbecco's modified Eagle medium; EVs: extracellular vesicles; IU: intrauterine; L-GF: lyophilized growth factors; MSCs: mesenchymal stem cells; MSC-CM: MSC-conditioned medium; MVs: microvescicles; PPP: platelet-poor plasma; PRP: platelet-rich plasma; TNF: tumor necrosis factor.

2.2. Types of studies

All studies (original research, case report and series) evaluating the effects of regenerative therapies for the treatment of endometritis in mares were included, regardless of the protocol, the outcomes, and the diagnostic method. Both PBIE and CDE were considered.

2.3. Types of interventions and outcomes

All studies exploring the use of regenerative medicine therapies, i.e., PRP, MSCs and their conditioned media, autologous conditioned serum (ACS), in the treatment of endometritis, were included. We categorized the outcomes as neutral, negative, or positive effects. Outcomes were considered positive if a significant reduction of uterine inflammation was demonstrated, as reflected by intrauterine fluid accumulation (IUF)

Table 2
Outcomes considered in studies involved in the review. In bold: studies included in the meta-analysis.

Authors and Year Evaluated	parameters
Abdelnaby et al., 2023 [17]	Histopathological examination, uterine morphometry, and hemodynamics by ultrasound, hormonal expression; pregnancy rates only in treated group.
Carluccio et al., 2020 [26]	Pregnancy and foaling rates.
Colombo et al. 2022 [19]	PMN count in uterine cytology; IUF and uterine edema observed by ultrasonography.
Dawod et al., 2021 [18]	Follicular growth, endometrial thickness, estrous cycle length, and pregnancy rate.
de Oliveira Tongu et al. 2021 [20]	PMN count in uterine cytology, cytokine concentrations (IL-6, IL-10, TNF-α), IUF.
Ferris et al. 2014 [7]	Presence of IUF, PMN, IL-1, and TNF-α concentrations in the LVF of the uterus.
Ghallab et al., 2023 [23]	Endometrial thickness and IUF evaluated by ultrasonographic scanning.
Lange- Consiglio et al., 2020 [28]	Histopathological examination.
Lange- Consiglio et al. 2023 [27]	PMN concentration, presence of IUF, and cytokine concentrations (IL-10, IL-6 and TNF-α).
Mambelli et al., 2014 [24]	Histopathological examination and expression of laminin, vimentin, KI-67-antigen, a-SMA and CK18.
Metcalf, 2014 [22]	Pregnancy rate and IUF.
Metcalf et al. 2012 [21]	mRNA expression of inflammatory biomarkers, such as IL-1b, IL1-Ra, TNF-α, IL-6, IL-8, IL-10, and NO.
Navarrete et al. 2020 [16]	Histopathological examination, TNF-α, IL-1α, 6, 8, 10, and COX-2 concentrations from uterine lavages and biopsies.
Pasch et al., 2021 [29]	IUF height and echogenicity, pregnancy rate.
Reghini et al. 2014 [9]	NO concentration, PMN count in uterine cytology, IUF observed by ultrasonography.
Reghini et al. 2016 [25]	PMN count in uterine cytology, IUF observed by ultrasonography and NO concentration of uterine fluid.
Segabinazzi et al. 2017 [8]	IUF, PMNs in uterine cytology and biopsy, COX-2 protein levels in the endometrial tissue, conception rate.
Segabinazzi et al. 2021 [14]	IUF, PMN count, uterine cytokines (Il-β, IL-6, IL-8, and IL-10), embryo recovery rates.

Abbreviations: a-SMA: a-smooth muscle actin; CK18: cytokeratin 18; IUF: intrauterine fluid accumulation; LVF:low volume flush; NO: nitric oxide; PMNs: polymorphonuclear cells.

and polymorphonuclear cells (PMNs) and cytokine concentrations.A significant increase in pregnancy/foaling and embryo recovery rates were also considered as good responses. The opposite results were classified as negative outcomes. No differences between treated and control groups were considered as neutral effects.

2.4. Measures of treatment effect

Risk ratios with 95 % confidence intervals (CI) for dichotomous outcomes were presented.

2.5. Risk of bias

The risk of bias in all included studies was assessed using PRISMA and Cochrane guidelines [30]. Each study was assigned as having a low, high, or unclear risk of bias, according to different parameters including random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of the outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias) and other biases. A correlation between the level of bias and the studies' outcome was speculated after analyzing both clinical and experimental trials.

2.6. Meta-analysis

To conduct a quantitative analysis, all the articles evaluated for the systematic review with a control group were included in the meta-analysis. The total events of the *in vivo* studies were considered as the total number of animals evaluated. All the data were analyzed using the software RevMan 5.4.1. The results were expressed as odds ratio (OR) and 95 % confidence intervals (95 % CI).

2.7. Assessment of heterogeneity

Significance tests were two-tailed, and significance level was set at $P < 0.05$. The Mantel-Haenszel (M − H) method was used to assess the study weight. Heterogeneity among studies was quantified using the I^2 statistics: percentages of around 25 %, 50 %, and 75 % would mean low, medium, and high heterogeneity, respectively [31].

3. Results

A total of 10 399 articles were identified after the initial electronic and manual research. Of the 120 articles screened, 18 studies respected the inclusion criteria (Fig. 1).

Of the selected studies, five were classified as RCTs [7–9,14,18]; 11 were classified as no-RCTs [16,17,19–27], one was a case report [28], and another was a case series [29].

All the studies were *in vivo* experiments: most of them used PRP for treatment of PBIE [8,14,18,19,21–23,25,29] and CDE [9,26], while fewer articles analyzed the efficacy of MSCs, MSCs conditioned medium or MSCs exosomes on PBIE [7,16,20,27] and CDE [17,23,28]. Only one study [7] evaluated the use of ACS to treat PBIE.

All the included articles demonstrated the positive effects of regenerative medicine in both PBIE and CDE. In the study of Mambelli et al. [24], only one mare, which presented a severe degree of CDE, showed no morphological improvement at the histological examination.

Most of the studies included a small number of animals, except for the articles of Carluccio et al. [23], Dawod et al. [18], and Ghallab et al. [23]. In the case report [28] and case series [29], the reduced number of included animals was associated with the nature of the study itself.

The criteria for inclusion of mares in most of the studies were related to reproductive history, i.e., unsuccessful inseminations, failed pregnancies, diagnosis of PBIE or CDE. Mainly, regenerative treatments were tested in the uterus of mares.

- With no apparent reproductive problems before/after the induction of PBIE by an intrauterine infusion of dead spermatozoa [7,16];
- With a previous history of infertility and repeat breeding, with normal conformation of the external genitalia and with apparently healthy reproductive organs and no IUF [18] or with persistent PBIE [23];
- Susceptible to PBIE, in case they had IUF, a positive endometrial cytology (≥3–5 PMNs per high-power field-HPF), and/or a positive aerobic culture 24/48/96 h after insemination or dead sperm challenge [8,14,19–22,25,27];
- Resistant to PBIE, with no IUF and <5 % PMNs in endometrial cytology 48 h after sperm challenge [20,25];
- With the diagnosis of CDE based on histological examinations of biopsy specimens [9,17,24,26,28];
- After failure to achieve pregnancy after one breeding cycle of artificial insemination with frozen semen [29].

Most of the studies compared two cycles (control vs treated) in the same group of mares [7–9,14,16,19–22,25], and only a few studies compared two groups of mares (control vs treated) [17,18,23,24,27]. One original article [26] and the case reports [28,29] have no control group/cycle.

Different methods have been used in the preparation and activation

Table 3
Risk of bias of selected studies. +: high risk of bias; -: low risk of bias; ?: undefined risk of bias; ↑: overall high risk of bias; ↓: overall low risk of bias. In bold the studies included in the meta-analysis.

Authors and Year	Random sequence generation	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Risk of bias
Abdelnaby et al., 2023 [17]	–	–	–	+	+	+	↓
Carluccio et al. 2020 [26]	–	–	–	–	+	+	↑
Colombo et al., 2022 [19]	–	–	–	–	+	+	↑
Dawod et al., 2021 [18]	–	+	–	–	+	+	↑
de Oliveira Tongu et al., 2021 [20]	–	–	–	–	+	+	↑
Ferris et al., 2014 [7]	+	+	–	–	+	+	↓
Ghallab et al., 2023 [23]	–	–	–	–	+	+	↑
Lange- Consiglio et al. 2020 [28]	–	–	–	–	+	+	↑
Lange- Consiglio et al., 2023 [27]	?	?	–	–	+	+	↑
Mambelli et al., 2014 [24]	–	–	–	–	+	+	↑
Metcalf, 2014 [21]	–	–	–	–	+	+	↑
Metcalf et al., 2012 [22]	?	?	–	–	+	+	↑
Navarrete et al., 2020 [16]	–	–	–	+	+	+	↓
Pasch et al. 2021 [29]	–	–	–	–	+	+	↑
Reghini et al., 2014 [9]	–	–	–	–	+	?	↑
Reghini et al., 2016 [25]	–	+	–	–	+	+	↓
Segabinazzi et al., 2017 [8]	+	+	–	–	+	+	↓
Segabinazzi et al., 2021 [14]	+	+	–	+	+	?	↓

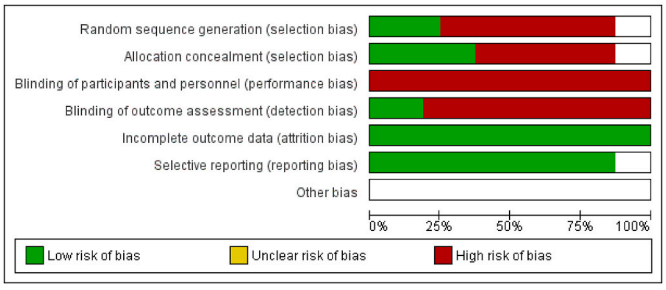

Fig. 2. Risk of bias of studies included in the meta-analysis.

of PRP, and they are summarized in Table 1. Double centrifugations and activation with calcium chloride solution (0.068 mEq/mL of PRP) are the most used methods [9,18,19,23,26]. In some cases, commercial kits for the preparation of PRP were used [21,22,29]. Platelet concentrates were always autologous products, while MSCs, MSCs-conditioned medium, or MSCs exosomes used heterologous cells. MSCs were produced from adipose, bone marrow, endometrial, or amniotic tissues [7,16,17,20,24,27,28]. In all studies, examiners were not reported to be blinded.

The timing of the intervention was different among studies: intrauterine infusions of regenerative medicine treatments were performed before [8,14,18–22,29], during, by supplementation of the semen extender [20,27], or after [8,9,14,16,23,26] natural breeding/insemination or sperm challenge (intrauterine infusion of dead sperm). Four studies [7,16,24,28] administered the treatment independently to the moment of insemination.

Clinical studies evaluated different variables to assess the effectiveness of different treatments, as shown in Table 2. Ultrasound examination of the uterus before and after treatment for the evaluation of the presence, height, and/or the echogenicity of the IUF was performed in 11/18 studies [7–9,14,19,20,22,23,25,27,29]. Only one study evaluated uterine morphometry (thickness and diameter/mm of uterine body and horns) and hemodynamics by B-mode and Doppler ultrasound of uterine arteries (pulsatility index, resistance index, peak systolic and end-diastolic points, and blood flow rate) [17]. Inflammatory cell count was performed in most of the studies; however, studies used different techniques to obtain endometrial samples: cytobrush [9,19,20,25,27], uterine fluid collection [7] or biopsy [17,24,28]. The concentrations of different pro- and anti-inflammatory cytokines, such as tumor necrosis factor α (TNF-α), interleukins (IL) 1α, 6, 8, 10, and cyclooxygenase-2 (COX-2), or nitric oxide (NO) in the uterine fluid were also tested to identify the mechanism of action of the used treatments. Some studies also assessed pregnancy and/or foaling rates [8,17,18,22,26] and embryo recovery rates [14]. Hormonal levels of estradiol and progesterone have been investigated only in one study [17]. Long-term follow-up after treatment was not described in any of the considered studies.

3.1. Risk of bias of selected studies

Table 3 summarizes the risk of bias in every single study included in the systemic review and meta-analysis. Figs. 2 and 3 illustrated and stratified the risk of bias for studies included in the meta-analysis. Most clinical studies were associated with a high risk of bias.

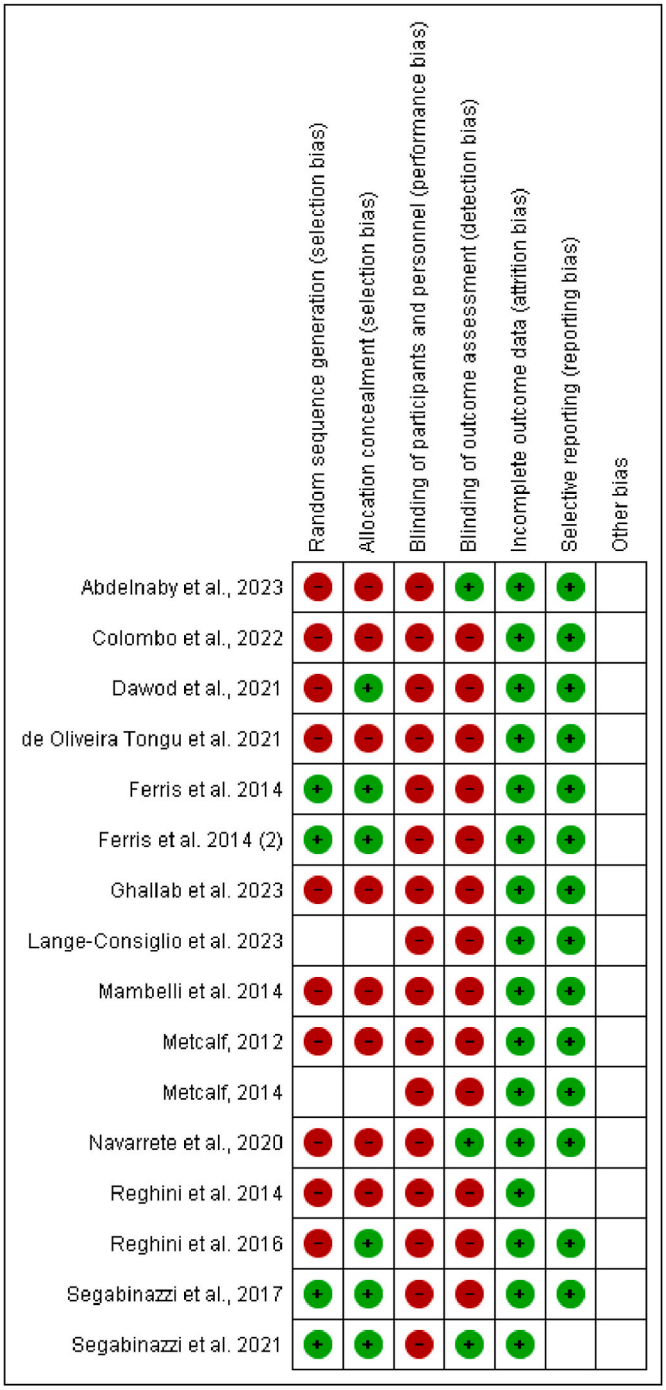

Fig. 3. Risk of bias of single studies included in the meta-analysis, evaluated by the authors.

3.2. Characteristics of the included studies

Table 4 summarizes the characteristics of each study included in the systematic review, including the type of study (methods), the characteristics of the animals involved, the intervention performed (type and timing of treatment), and the primary outcomes reported.

3.3. Meta-analysis

Only 15 of 18 trials were included in the meta-analysis. Exclusion criteria were as follows: studied species other than the horse; treated pathologies different than PBIE and CDE; used of non-regenerative medicine products; without a control group. Selected studies were reported in bold in Tables 2 and 3

Five trials were classified as RCTs [7–9,14,16] and ten as No-RCTs [16,17,19–25,27]. Animals included in the studies were mares of different ages, breeds, and reproduction status (multiparous, problem mares); nine studies compared a treated cycle with a previous non-treated cycle, in the same group of mares [7–9,14,16,19–22], while five articles compared control with treated among different mares [17,18,23,24,27]. Measured outcomes are shown in Table 2.

The PRP has been used for treating PBIE in 8 studies [8,14,19,21–23,25,27] and CDE in one article [9]; mares were infused with ACS in one study [7], while MSCs and MSCs derivatives were used in four studies for the treatment of PBIE [20,27] and CDE [17,24]. In two different experiments, Ferris et al. [7] induced PBIE in healthy mares, which were then treated with MSCs and ACS. Similarly, Navarrete et al. [16] induced endometritis in 9 healthy mares and treated them with an infusion of MSCs.

Animals were selected from patients referred to the hospital due to a history of infertility/repeat breeding [18,23,29], or PBIE [8,14,20–22,25], or presenting the disease [7,9,16]. Some studies included animals susceptible [8,11,19–22,25,27], or resistant [20,25] to PBIE or with the diagnosis of CDE [9,17,24,26,28].

As previously described, autologous PRP was obtained by double centrifugations and activation with calcium chloride solution in four studies [9,18,19,23]; commercial kits were used in two protocols [21,22].

Intrauterine infusions of PRP or MSCs or ACS were performed before insemination/natural breeding in seven studies [8,14,18–22], at the same time in two studies [20,27] and after insemination or natural breeding in five studies [8,9,14,16,23]. Three studies [7,17,24] administered the treatment independently to the moment of insemination.

Treatment effects are summarized in paragraph 3.2; no adverse effects of intrauterine deposition of regenerative products have been reported.

A fixed effect model was used to pool the data. The overall estimated effect showed a positive correlation between treatment with PRP/MSCs/ACS and positive outcomes (Fig. 4: OR 295.39–86.96 – 1003.35; $P < 0.05$), and I^2 index showed moderate heterogeneity among studies ($I^2 = 43\ \%$, $P = 0.03$). All the studies, except one [24], highlighted the positive influence of PRP, ACS, and MSCs or its derivatives in reducing uterine inflammation and improving pregnancy rates.

Fig. 5 shows the results of the sub-meta-analysis, which evaluated platelet-derived products and stem cells separately. There was a significant improvement in the outcomes for treated mares, which was more evident for the MSCs group (OR: 163.93–30.52 – 880.63; $P < 0.05$) compared to the PRP group (OR: 120.60–46.20 – 314.81; $P < 0.05$). As demonstrated in the last row of the forest plots, platelet-derived studies showed moderate heterogeneity with $I^2 = 60\ \%$ and $P = 0.007$, while MSCs showed no heterogeneity ($I^2 = 0\ \%$; $P = 0.6$) among studies.

4. Discussion

To the author's knowledge, this is the first meta-analysis evaluating the use of PRP, ACS, MSCs, and their derivatives for treating PBIE and CDE in the mare. The results of this meta-analysis showed an overall positive effect of regenerative therapies in PBIE/CDE. Despite the high risk of bias in the included studies and the variability among different types of products, the results of the current meta-analysis support the use of such products for treating PBIE and CDE. All studies reported a positive effect of regenerative treatments on the endometrium, showing no adverse effects, an improvement in the inflammatory status of the uterus, and in almost all cases, an increase in pregnancy rates was observed. Implementing standardized experimental designs with unified treatment preparation, timing, and outcome measures could help mitigate the biases identified in many studies. Moreover, it is challenging to

Table 4
Characteristics (methods, animals, interventions, and outcomes) of each study included in the systematic review.

Abdelnaby et al., 2023 [17]	
Methods	No- RCT
Animals	14 multiparous Arabian mares aged 13–15 years: 7 with PMNs to epithelial cells ratio ≤2 % (control group) and 7 with CDE.
Interventions	Mares were treated with an exosomes infusion twice 21 days apart at the first and second cycles; at third cycle, mares of the treated group were inseminated using frozen/thawed semen within 6 h after ovulation. Histopathological examination, hormonal expression, uterine morphometry and hemodynamics (by ultrasound), were evaluated. The pregnancy rate was assessed only in the treated group.
Outcomes	In mares treated with exosomes infusion, uterine body and horns thickness decreased, while uterine body-colored area and total uterine colored area were elevated. Uterine artery blood parameters did not change during treatment, but the blood flow rate increased after treatment. Estradiol level decreased, while progesterone level increased after treatment. Regression of fibrous tissue and restoration of healthy endometrial glands with normal epithelium after CDEtis, resulting in increasedpregnancy rate in infertile mares.
Carluccio et al. (2020) [26]	
Methods	No- RCT
Animals	60 mares affected by CDE).
Interventions	Mares were treated with an IU of 15 mL of autologous PRP 24 h after ovulation. Mares were inseminated with fresh semen from a stallion of proven fertility 24 h after ovulation induction. The pregnancy rate at 14 days post-ovulation was evaluated.
Outcomes	The pregnancy rate at 14 days post-ovulation was 75 %. Of the overall pregnancies, 69 % were achieved with the first infusion of PRP, while an additional 31 % was obtained with a second treatment.
Colombo et al. (2022) [19]	
Methods	No-RCT
Animals	12 mares susceptible to PBIE.
Interventions	All mares were randomly assigned to two different consecutive cycles: on untreated cycle, mares were not treated and on treated cycle, mares were treated with autologous PL 12 h after ovulation induction. Mares were inseminated with frozen-thawed semen 36 h after ovulation induction. PMN count in uterine cytology evaluated by optical microscopy, IUF, uterine edema observed by ultrasonography, and pregnancy rate (at 14 days) were evaluated.
Outcomes	A significant decrease was observed in cytology score, IUF, and edema score in treated mares. The pregnancy rate in PRP-treated cycles and control cycles were not significantly different.
Dawod et al. (2021) [18]	
Methods	RCT
Animals	73 purebred repeat breeder (3 successive cycles) Arabian Mares.
Interventions	Mares were randomly divided into three groups: control group did not receive IU infusion; mares of the PRP group received an IU of 20 mL of fresh PRP on the second day after the end of the estrous phase; and mares of the third group received an IU of 20 mL of L-GF on the second day after the estrous phase. All mares were subjected to natural mating, 12–24 h after the induction of ovulation. Follicular growth, endometrial thickness, estrous cycle length, and pregnancy rate (at 30 days) were evaluated.
Outcomes	No significant difference between control and treated groups in the diameter of the preovulatory follicles during the post-treatment cycle was found. The endometrium thickness increased significantly in the PRP group compared with the control group. PRP administration shortened the estrous cycle length and increased the pregnancy rate.
de Oliveira Tongu et al. *(2021)* [20]	
Methods	No-RCT
Animals	20 mares resistant or susceptible to PBIE
Interventions	Mares were inseminated with fresh semen 24 h post- induction of the ovulation in two (Control and CM1) and three cycles (Control, CM1 and CM2) in a crossover study: control, no pharmacologic interference; CM1, supplementation of MSCs at the semen, at a ratio of 3:4 (MSCs: semen); CM2, IU of 30 mL of MSCs in uterus 24 hbefore insemination. PMN count cytokine concentration (IL-6, IL-10, TNF-α) in uterine fluid and IUF were evaluated 6 and 24 h after the insemination.The pregnancy rate was evaluated at 14 days.
Outcomes	Resistant mares (13 mares) inseminated with MSCs-supplementedsemen had lower PMN counts in endometrial cytology at 6 h post-insemination. IUF was not detected before or 24 h after insemination in resistant mares. Fertility rates were similar between the control and treated cycles. Susceptible mares (7 animals) in the control cycle had higher PMN counts than in treatment cycles. There was a reduction in IUF post-insemination in treatment cycles compared with the control cycle. MSCs downregulated IL-6 and upregulated IL-10 concentrations in the uterus of susceptible mares after insemination.
Ferris et al. *(2014)* [7]	
Methods	RCT
Animals	6 healthy Quarter Horses mares.
Interventions	In the first experiment, mares were randomly assigned to three treatment groups: one treated with an IU of 20 mL of ACS or with 20 mg of dexamethasone in 20 mL of PBS; a third group which received 20 mL of PBS. Mares were treated 24 h before sperm challenge (2 mL of dead spermatozoa), once per estrus. The presence and depth of IUF, PMN and cytokine (IL-1 and TNF- α) concentration within the uterine flushing were evaluated 6 and 12 h after insemination. In the second experiment, 6 mares were randomly assigned to two different groups: a control group, which received an IU of 20 ml of PBS; a treated group, which received an IU of 20 mL of Lactated Ringer's solution containing 20 million allogeneic MSCs. Mares were treated 24 h before sperm challenge (2 mL of dead spermatozoa)n, once per estrus. The presence and depth of IUF, PMN and cytokine (IL-1 and TNF- α) concentration within the uterine flushing were evaluated 6 and 12 h after insemination.
Outcomes	Treatment with ACS significantly reduced PMN count in the uterine lumen 6 hafter the sperm challenge. There was no difference in IL-1Ra concentration in mares treated with ACS, dexamethasone, or a placebo. ACS was able to modulate the immune response to spermatozoa in normal mares. Treatment with MSCs significantly reduced PMN count in the uterine lumen 6 h after the sperm challenge. An increase in iIL-1Ra was observed after treatment with MSCs before exposure to spermatozoa. MSCs were able to modulate the immune response to spermatozoa in normal mares.
Ghallab et al. *(2023)* [23]	
Methods	No-RCT
Animals	39 repeat breeders with a history of PBIE.
Interventions	Mares were divided into three groups, according to bacterial culturing, ultrasonography, and endometrial cytology findings. Mares were treated 6 h after natural breeding with an IU of 20 mL of freshly prepared autologous PRP, or with three doses of systemic Enrofloxacin during the estrous period, or with nothing (control). The endometrial thickness and IUF assessed by ultrasonographic scanning on the 3rd day of the next estrus and the pregnancy rate (30 days) were evaluated.

(*continued on next page*)

Table 4 (*continued*)

Ghallab et al. *(2023)* [23]	
Outcomes	PRP resulted in a significant reduction in endometrial thicknessIUF and inter-estrus interval compared to the control. The pregnancy rate improved to 70 % in the PRP group.
Lange-Consiglio et al. (2020) [28]	
Methods	Case report
Animals	One 11-year old Fresian Horse with CDE.
Interventions	A mare was treated with an IU of 20 billion amniotic-derived MVs diluted in 50 mL of NaCl 0.9 % at days 5 and 9 post-ovulation. Biopsies were collected on day 10 after the second treatment, during diestrum, and after two consecutive ovulations. IUF and pregnancy diagnosis were carried out after AI with frozen semen.
Outcomes	Histological analysis showed an improvement of the degree of endometritis. No inflammatory IUF was detected after AI and an embryonic vesicle was detected at 14 days.
Lange-Consiglio et al. (2023) [27]	
Methods	No-RCT
Animals	16 mares susceptible of PBIE.
Interventions	Mares were inseminated with fresh semen alone (control) or with a supplementation of amniotic MSCs -EVs(treated.
Outcomes	Supplementation of amniotic MSCs-EVs to semen resulted in a reduction in PMN count, IUF and pro-inflammatory cytokine concentrations, such as TNF-α and IL-6. Anti-inflammatory IL-10 concentration increased in the EV group.
Mambelli et al. *(2014)* [24]	
Methods	No-RCT
Animals	6 mares affected by CDE.
Interventions	Mares were treated during a synchronized estrus with 2 million adipose tissue-derived MSCs diluted in 20 mL of NaCl 0.9 % (10 mL in each horn tip) or with 20 mL of NaCl 0.9 %. Homing of fluorescently labeled MSCs was observed by confocal microscopy of uterine biopsies collected from the uterine body and both uterine horns, including glandular and periglandular spaces, 7 and 21 days after transplantation.
Outcome	In 3 of 4 mares homing of fluorescently labeled MSCs was observed.
Metcalf et al. *(2012)* [21]	
Methods	No-RCT
Animals	9 barren mares with a history of PBIE.
Interventions	Mares underwent an untreated cycle followed by a treated cycle, with an IU of autologous PRP at the time of ovulation induction. AI was performed 24–36 h after ovulation induction. An endometrial biopsy was performed 24 h after insemination; mRNA expression of IL-1β, IL1-Ra, -α, IL-6, IL-8, IL-10, iNOS was evaluated.
Outcome	Expression of IL-1β, 6 and 8 and iNOS was significantly downregulated in the treated cycle compared to the non-treated.
Metcalf (2014) [22]	
Methods	No- RCT
Animals	16 barren mares with a history of PBIE
Interventions	Mares underwent an untreated cycle followed by a treated cycle with IU of autologous PRP at the time of ovulation induction. Mares were inseminated 24–36 h after ovulation induction The IUF 24 h post-insemination and pregnancy rate were evaluated.
Outcome	The pregnancy rate in the treated cycle was significantly higher than in the non-treated cycle and the IUF was significantly less.
Navarrete et al. (2020) [16]	
Methods	No-RCT
Animals	9 healthy mares.
Interventions	Mares were treated with IU of 20 mL of NaCl 0.9 % containing adipose MSCs, endometrial MSCs, or sterile NaCl 0.9 % after the IU of 500 million dead sperm. Inflammatory markers (PMNs,IL-6 and TNF-α, and immunostaining in biopsies; transcripts of IL-1a, −6, –8, −10, TNFα and COX-2) were analyzed in uterine lavages and biopsies immediately before, 3 h after sperm infusion, and 48 h later. Additional biopsies were taken 10 and 30 days after sperm infusion.
Outcome	A decrease of IL-6 and TNF-α was detected in the groups that received adipose-MSCs, while expression of IL-10 and COX-2 remained unchanged. In the mares that received endometrial MSC, IL-6 and 8 decreased significantly, IL-10 increased, while TNF-α, COX-2, and IL-1α did not significantly change their expression.
Pasch et al. (2021) [29]	
Methods	Case series
Animals	18 mares with one unsuccessful cycle of breeding.
Interventions	Mares received IU of PRP (6 mL) and plasma (9 mL) 12–48 h before AI with frozen semen. The IUF at 4 and 6 h after insemination and the pregnancy rate were evaluated.
Outcome	The IUF after insemination on the treatment cycle was absent in 67 % of mares, <1 cm in 28 % of mares and >2 cm in 5 % of mares. 14 mares were bred to carry a pregnancy, and 4 were bred as embryo donors. For those bred to carry, the treatment cycle pregnancy rate was 64 %; for embryo donor mares, the treatment cycle pregnancy rate was 50 %. The overall pregnancy rate for the treated cycle was 61 %.
Reghini et al. (2014) [9]	
Methods	No-RCT
Animals	23 mares susceptible (8 mares) or resistant (15 mares) to PBIE.
Interventions	Mares were treated with an IU of PRP 4 h after ovulation induction. The AI with fresh semen was performed from 24 h after ovulation induction . NO concentration, percentage of PMNs in uterine cytology, IUF were evaluated 24 h after AI.
Outcome	The resistant mares showed no differences between the cycles in NO concentration or in IUFn, but PMNs in cytology diminished during the treated cycle. In susceptible mares, a significant decrease was observed after PRP treatment in the percentage of PMNs, NO in uterine fluid and IUF.
Reghini et al. *(2016)* [25]	
Methods	RCT
Animals	21 mares with CDE.
Interventions	Each mare underwent one untreated cycle and one treated cycle. Mares were treated with the IU infusion of PRP 4 h after AI with fresh semen. The percentage of PMNs in uterine cytology, IUF observed by ultrasonography and NO concentration of uterine fluid were analyzed before and 24 h after insemination.
Outcome	The intrauterine inflammatory response decreased in CDE mares treated with PRP when compared with the no-treated cycle but did not modify NO concentrations in uterine fluid.

Segabinazzi et al. *(2017)* [8]	
Methods	RCT
Animals	13 mares susceptible of PBIE.
Interventions	All mares were randomly assigned to three different consecutive cycles, in a crossover study design. Cycles were classified in no treated cycle, pre-insemination PRP infusion (PRP administration 24 h before insemination), post-insemination PRP infusion (PRP administration 4 h after insemination). The IUF, PMNs in uterine cytology, and histology of the biopsies were evaluated 24 h after AI.
Outcome	PRP treatments resulted in a decrease of PMNs in the cytology after breeding compared to controls. The IUF did not differ among cycles; however, the pregnancy rates were significantly higher in the PRP mares. Mares positive for endometritis decreased in both treatment groups, and a more intense positive COX-2 labeling was observed in the control group compared to the two treatment groups.

Segabinazzi et al. *(2021)* [14]	
Methods	RCT
Animals	12 mares susceptible to PBIE.
Interventions	All mares were randomly assigned to three different consecutive cycles. Mares were treated with lactate ringer solution (control group) orPRP pre- (24–48 h before insemination) or post-breeding (6–24 h after insemination) with fresh semen. Every 24–96 h post insemination the IUF and endometrial PMNs were assessed. Uterine cytokines (IL-lβ, IL-6, IL-8, and IL-10) were evaluated before, 6, and 24 h post-breeding, and endometrial culture 3 and 9 days after breeding. Embryo recovery rate 8 days after ovulation was recorded.
Outcome	PRP treatment reduced endometrial PMNs, post-breeding IUF, and pro-inflammatory cytokines (IL-1β, IL-6, IL-8) compared to control-assigned cycles. Controls had a significantly higher percentage of positive bacterial cultures than PRP-assigned cycles. The PRP-assigned cycles had significantly greater embryo recovery rates than the control.

Abbreviations: 1RA: receptor antagonist 1; ACS: autologous conditioned serum; AI: artificial insemination; CDE: Chronic degenerative endometritis; COX: cyclo-oxygenase; CM: MSCs-conditioned medium; EVs: extracellular vesicles; IL: interleukin; iNOS: nitric oxide synthase; IU: intrauterine infusion; IUF: intrauterine fluid accumulation; L-GF: lyophilized growth factors; MSCs: mesenchymal stem cells; MVs: microvesicles; NO: nitric oxide; PBIE: persistent breeding-induced endometritis; PBS: phosphate-buffered saline; PL: platelet-lysate; PMNs: polymorphonuclear cells; PPP: platelet-poor plasma; PRP: platelet-rich plasma; RCTs: randomized control trials; TNF: tumor necrosis factor.

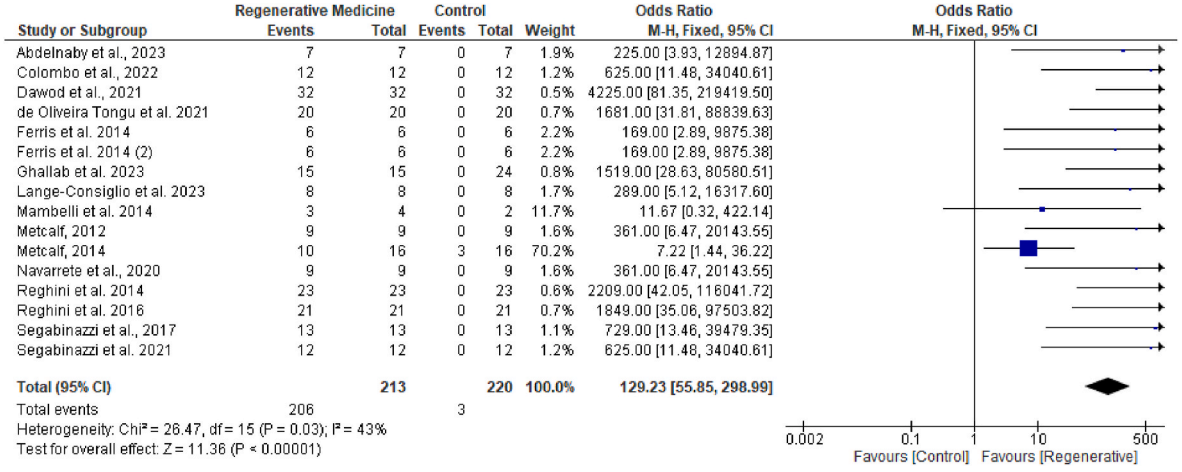

Fig. 4. Forest plot representing outcomes of selected studies, using a meta-analytic approach based on the fixed effects model. The Mantel-Haenszel method (M − H) was used to determine the studies' weight. Odds ratio (OR) > 1 indicates that the treatment with platelet-derived products or MSCs is associated with a positive outcome, whereas OR < 1 favors the association between lack of efficacy of treatment with platelet-derived (PD) products or MSCs. Confidence intervals (95 % CI) that overlap an OR of 1 suggest a lack of association between the treatment and the outcome. The box represents the positive outcomes of individual studies and the whiskers correspond to the 95 % confidence interval (CI). The box size is proportional to the weight of the study in relation to the pooled estimate. The placement of the centre of the diamond on the x-axis represents the point estimate, and the width of the diamond represents the 95 % CI around the point estimate of the pooled effect.

have an unbiased review of equine studies, considering that negative or neutral results are rarely published.

Intrauterine PRP has been shown to have an overall positive effect on mares experiencing fertility problems associated with PBIE or CDE. The studies analyzed in this meta-analysis demonstrated that PRP can moderate uterine inflammation by reducing PMNs concentration and improving uterine status, resulting in decreased endometrial wall thickness, edema, and IUF [9,14,18,19,22,23,29]. The improvement of the uterine environment is likely one of the major factors contributing to enhanced embryonic survival, which is considered the best outcome [8,14,22,23,26,29]. Despite variations in experimental designs, such as different timing of intervention, protocols for PRP preparation, and criteria for evaluating the inflammatory response, the anti-inflammatory effect remains evident. The mechanisms of action of PRP have not yet been fully understood; however, the intrauterine administration of PRP seems to be able to modulate the inflammatory mediators [8,14,21]. After PRP uterine treatment, an increase of the anti-inflammatory cytokine IL-10 and a downregulation of pro-inflammatory cytokines/chemokines (IL-1β, IL-6, IL-8), NO and COX-2 were observed. The improvement in immune response was also correlated with PRP's ability to inhibit nuclear factor kappa beta [32], metalloproteinase-3, TNF, and vascular adhesion molecules [33]. Moreover, the positive effect of PRP on CDE is likely due to the release of growth factors, including platelet-derived growth factor and transforming growth factor -β1. These factors are responsible for initiating or progressing tissue regeneration, which may be hindered during chronic conditions [34].

An antimicrobial effect of PRP has been reported in the literature. Platelet granules also contain antimicrobial peptides (such as platelet factor 4 and thymosin β-4), which contribute to PRP's activity against various bacteria [35]. For instance, *Escherichia coli* and *Klebsiella pneumoniae*, both isolated from mare uteri, are among the bacteria affected [14,36]. In the studies included in the meta-analysis, the antimicrobial

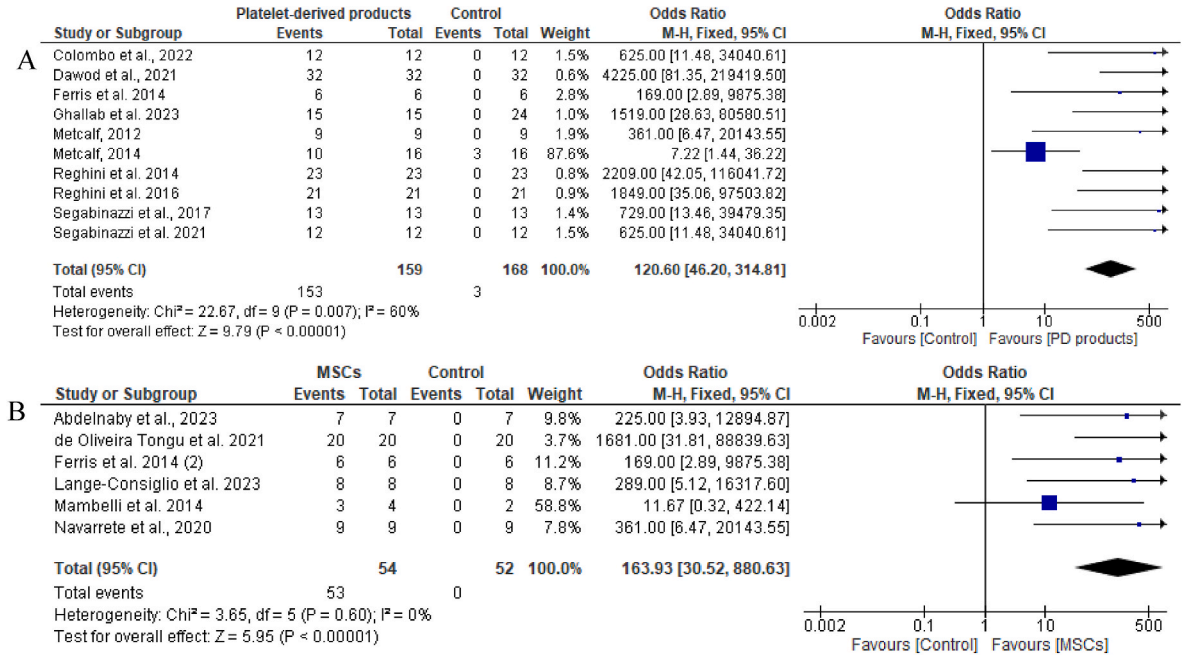

Fig. 5. Forest plot representing outcomes of the platelet-derived products (A) and MSCs (B) studies, using a meta-analytic approach based on the fixed effects model. Mantel-Haenszel method (M − H) was used to determine the studies' weight. Odds ratio (OR) > 1 indicates that the treatment with platelet-derived products or MSCs is associated with a positive outcome, whereas OR < 1 favors the association between lack of efficacy of treatment with platelet-derived (PD) products or MSCs. Confidence intervals (95 % CI) that overlap an OR of 1 suggest a lack of association between the treatment and the outcome. The box represents the positive outcomes of individual studies and the whiskers correspond to the 95 % confidence interval (CI). The box size is proportional to the weight of the study in relation to the pooled estimate. The placement of the centre of the diamond on the x-axis represents the point estimate, and the width of the diamond represents the 95 % CI around the point estimate of the pooled effect.

effect was not investigated thoroughly enough. Segabinazzi et al. [14] are the only ones who reported a dose-dependent antimicrobial property of PRP administered in the uterus.

The ACS, MSCs, and their derivatives have been tested and used to modulate inflammation in the uterus of mares [7,16,17,20,24,27,28]. The ACS contains a high concentration of anti-inflammatory molecules such as interleukin receptor antagonist 1 (IL-1Ra), fibroblast growth factor 2 (FGF-2) IL-10, and TGF-β [7]. The MSCs are stem cells originating from the mesoderm with the capacity for self-renewal and differentiation into various cell types. They can be extracted from various tissues, such as bone, cartilage, tendon, muscle, adipose tissue, and fetal membrane [24]. In case of tissue injury, MSCs also release a diverse array of bioactive molecules that regulate the inflammatory response [7]. The MSCs and their derivatives originating from bone marrow, amnios, and adipose tissue have been used in the trials included in this meta-analysis. However, the biological properties of MSCs vary among distinct subpopulations within a tissue-derived primary culture, exhibiting variation in function. Birth-associated tissues MSCs, such as the placenta and the umbilical cord/Wharton's jelly, may offer some advantages. These include an enhanced proliferative capacity *in vitro*, particularly under hypoxic conditions, compared to MSC populations obtained from adult tissues [37].

The MSCs derivative products, such as conditioned medium, or extracellular vesicles (including exosomes), have emerged as a cell-free therapy modality due to their high safety, ease of preservation, and transportation [38]. These products can alleviate inflammation-induced damage by suppressing the inflammatory response, inhibiting apoptosis, and promoting tissue repair, all facilitated by intercellular communication. Extracellular vesicles are integral components of the conditioned medium, which is a complex mixture of proteins secreted by MSCs, including cytokines, growth factors, and chemokines [39].

Until today the application of regenerative products has been considered safe. The most common adverse effects reported with PRP or MSCs were reported in orthopedic conditions, such as mild local swelling after inoculation or a local allergic reaction [40]. These effects resolve spontaneously within 24–48 h without intervention. The possible causes could include the presence of residual blood cell components in PRP, procedural-related reactions and contaminations [40].

Most of the trials included in these systematic review and meta-analysis had a control group or cycle. The inclusion of a control group in meta-analysis enhances the validity, reliability, and interpretability of findings, enabling more robust conclusions about the efficacy and safety of interventions under investigation [41]. Comparing outcomes between intervention and control groups facilitates the interpretation of study findings, helping to understand clinical significance and whether the intervention offers advantages over no treatment [41].

The primary reason for the high risk of bias in most of the involved studies is the absence of blinding in personnel responsible for treatment or assessment. No study was fully blinded, and only a few studies included a blinded evaluation of the outcomes. The absence of blinding, particularly in the evaluation of subjective outcomes, was linked to unwarranted conclusions [42,43]. The patients were undoubtedly unaware of the treatment to which they were subjected. However, it is challenging for operators not to discern the types of treatment they are administering, as they could be distinguished based on color or consistency. In most cases, the operators responsible for administering the treatment were also tasked with performing the uterine evaluation (e. g. IUF and endometrial thickness). It must also be noted that the papers do not mention whether the evaluators of cytology assessment were treatment-blinded. Furthermore, it is important to highlight that most of the outcomes evaluated in these studies, such as pregnancy rates and concentrations of cytokines, are objective parameters.

Another limitation observed was the small sample size in the trials, which could lead to type II errors. It is known that obtaining permission from owners to use a novel treatment can be challenging. Moreover, mares selected differ in age, breed, and phase of the pathology (resistant and susceptible to PBIE or with a diagnosis of PBIE or CDE). Breed and age could affect the composition of autologous products used [44,45].

Furthermore, the phase of the disease or uterine status (healthy, acute, or chronic inflammation) may differently influence the response to the same treatment and confound the association between treatment and outcome, potentially resulting in biased estimates of treatment effects [46]. However, positive outcomes have been reported in all included conditions, resulting in modulating and regenerative effects of PRP and MSC on endometrial inflammation.

Given this high risk of bias, it was anticipated that there would be moderate heterogeneity in the meta-analysis [47]. Effects estimates were still moderately heterogeneous among the studies (I^2 = 43 %), indicating that there are some differences in the results of individual studies, but these differences are not extreme. The analysis of subgroups (PRP and MSCs, along with their derivatives and ACS) reduced the heterogeneity for both treatments, reducing the I^2 MSCs to 0 %. The higher heterogeneity observed in meta-analyses of PRP studies could indeed be attributed to the different protocols used for preparing and activating PRP and to the different outcomes measured. Many protocols for the preparation and activation of PRP have been reported, resulting in different concentrations of platelets and consequently of growth factors and cytokines [48,49]. The outcomes evaluated in the studies included in this meta-analysis aim to understand the mechanism of PRP therapy by examining different aspects of PRP treatment. Moreover, the sub-meta-analysis revealed that treatment with MSCs in mares produced better results than those treated with platelet derivatives. Only a few meta-analyses on the use of regenerative therapies in horses divided the analysis into different treatment subgroups due to the low number of studies and the high heterogeneity [50,51]. In a previous meta-analysis on the use of regenerative medicine for tendinopathy and desmopathy in horses, it was already reported that MSCs alone or in combination with PRP showed better outcomes than PRP alone [51].

5. Conclusions

Regenerative medicine, particularly involving MSCs and their derivatives, has demonstrated promising outcomes in terms of both therapeutic effectiveness and safety in the context of endometritis. However, meta-analysis results in equine clinical studies should be treated with extreme caution given the inherently biased nature of reporting and lack of publication governance. Meanwhile, this analysis can support veterinarians in providing decision aids and facilitating shared decision-making, enabling owners to understand what they can expect from these treatments. Due to the limited number of randomized controlled study and high variability of protocols, further *in vitro* and *in vivo* studies are needed to establish the most appropriate product, preparation method, and dosage for intrauterine use.

Data availability

All data generated or analyzed during this study will be available on request.

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CRediT authorship contribution statement

Chiara Del Prete: Writing – original draft, Methodology, Conceptualization. **Chiara Montano:** Writing – original draft, Methodology, Formal analysis. **Natascia Cocchia:** Methodology. **Mariaelena de Chiara:** Visualization, Data curation. **Bianca Gasparrini:** Methodology. **Maria Pia Pasolini:** Writing – review & editing, Methodology, Conceptualization.

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