CARLOS MATTOS TEIXEIRA SOARES

DESAFIOS DIAGNÓSTICOS E TERAPÊUTICOS DA ENDOMETRITE EM ÉGUAS: ESTUDOS EXPERIMENTAIS E META-ANÁLISE

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-graduação em Medicina Veterinária, para a obtenção do título de *Doctor Scientiae*.

Orientador(a): Mariana Machado Neves

Coorientador(a): Yamê Fabres Robaina Sancler da Silva

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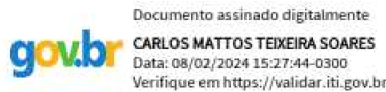

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Carlos Mattos Teixeira Soares
(Autor)

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Mariana Machado Neves
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RESUMO

TEIXEIRA-SOARES, Carlos Mattos, M.Sc., Universidade Federal de Viçosa, julho de 2023. **Desafios diagnósticos e terapêuticos da endometrite em éguas: estudos experimentais e meta-análise.** Orientador(a): Mariana Machado Neves. Coorientador(a): Yamê Fabres Robaina Sancler da Silva.

A endometrite é um desafio contínuo na reprodução equina e suas biotecnologias associadas, resultando em prejuízos significativos para a indústria de criação de cavalos. Apesar de décadas de pesquisa, ainda existem lacunas no entendimento dos mecanismos envolvidos no seu desenvolvimento, o que enfatiza a necessidade de novos métodos diagnósticos e terapêuticos mais eficazes. Com o objetivo de compreender melhor a endometrite em éguas, foram realizadas pesquisas experimentais para comparar e avaliar diferentes abordagens diagnósticas e terapêuticas. O primeiro estudo teve como objetivo realizar o exame ginecológico abrangendo diversos métodos diagnósticos a fim de caracterizar os principais achados em receptoras de embriões consideradas subférteis. Os resultados revelaram que a endometrite é a principal causa de subfertilidade em éguas receptoras removidas de programas de transferência de embriões, com 60% de prevalência. O fluido intrauterino detectado via ultrassonografia foi o principal achado clínico; a bactéria *Escherichia coli* a mais frequentemente isolada; e o exame histopatológico a partir da biópsia uterina demonstrou ser o método mais eficaz para o diagnóstico da endometrite. No segundo estudo, o intuito foi avaliar e comparar as características do proteoma do fluido uterino de éguas saudáveis e com endometrite. As éguas saudáveis exibiram um perfil proteômico caracterizado por proteínas envolvidas na manutenção da integridade uterina, éguas com endometrite infecciosa exibiram um perfil proteômico distinto com proteínas associadas a resposta inflamatória e imune, enquanto éguas com endometrite pós-cobertura apresentaram proteínas relacionadas principalmente à resposta imune inata. O terceiro estudo consistiu em uma avaliação meta-analítica do efeito dos tratamentos para endometrite pós-cobertura sobre parâmetros inflamatórios uterinos. Os tratamentos com agentes imunomoduladores biológicos e as terapias individuais com células-tronco, firocoxib e plasma sanguíneo foram os mais eficazes no controle da inflamação, reduzindo significativamente a proporção de células inflamatórias uterinas. Esses achados abrem novas perspectivas para a compreensão da endometrite e pesquisas futuras, especialmente no aprimoramento do diagnóstico e na validação de possíveis biomarcadores, bem como no desenvolvimento de abordagens terapêuticas.

Palavras-chave: Reprodução equina. Avaliação ginecológica. Tratamentos não tradicionais

ABSTRACT

TEIXEIRA-SOARES, Carlos Mattos, M.Sc., Universidade Federal de Viçosa, July 2023. **Diagnostic and therapeutic challenges of endometritis in mares: experimental studies and meta-analysis.** Adviser: Mariana Machado Neves. Co-adviser: Yamê Fabres Robaina Sancler da Silva.

Endometritis is a continuous challenge in equine reproduction and its associated biotechnologies, resulting in significant economic losses for the horse breeding industry. Despite decades of research, there are still gaps in understanding the mechanisms involved in its development, which emphasizes the need for new, more effective diagnostic and therapeutic methods. With the objective of better understanding endometritis in mares, experimental research was carried out to compare and evaluate different diagnostic and therapeutic approaches. The first study aimed to perform a gynecological examination covering several diagnostic methods in order to characterize the main findings in embryo recipients considered subfertile. The results revealed that endometritis is the main cause of subfertility in recipient mares removed from embryo transfer programs, with a 60% prevalence. Intrauterine fluid detected via ultrasound was the main clinical finding; the bacteria *Escherichia coli* the most frequently isolated; and the histopathological examination from the uterine biopsy proved to be the most effective method for the diagnosis of endometritis. In the second study, the aim was to evaluate and compare the characteristics of the proteome of the uterine fluid of healthy mares and mares with endometritis. Healthy mares exhibited a proteomic profile characterized by proteins involved in maintaining uterine integrity, mares with infectious endometritis displayed a distinct proteomic profile with proteins associated with the inflammatory and immune response, while mares with post-breeding endometritis showed a proteomic profile mainly related to the innate immune response. The third study consisted of a meta-analytical evaluation of the effect of treatments for post-breeding endometritis on uterine inflammatory parameters. Treatments with biological immunomodulatory agents and individual therapies with stem cells, firocoxib and blood plasma therapies were the most effective in controlling inflammation, significantly reducing the proportion of uterine inflammatory cells. These findings offer new perspectives for understanding endometritis and future research, particularly in improving diagnostics, validating potential biomarkers, and developing therapeutic approaches.

Keywords: Equine reproduction. Gynecological evaluation. Non-traditional treatments.

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CAPÍTULO 1

REVISÃO DE LITERATURA

1. Introdução

A reprodução animal e suas biotecnologias aplicadas às fêmeas equinas vêm ganhando destaque no cenário mundial. Técnicas como transferência e produção *in vitro* de embriões aceleram o melhoramento genético, atendendo às demandas de um mercado cada vez mais exigente (Alvarenga, 2010; Claes et al., 2018; Felix et al., 2022). No entanto, é indispensável o uso de animais férteis para o sucesso dessas biotecnologias e consequente desenvolvimento da equideocultura (Heap et al., 1982; Troedsson, 2011). Os equinos são conhecidos por apresentarem taxas de fertilidade mais baixas em comparação com outras espécies domésticas (Griffin et al., 2019). Isso ocorre devido à seleção dos indivíduos com base em aspectos genéticos e sucesso esportivo em detrimento de características reprodutivas (Palmer & Chavatte-Palmer, 2020). Além disso, patologias reprodutivas têm um importante impacto negativo no processo, sendo consideradas fatores determinantes para alcançar índices satisfatórios.

Dentre essas patologias, a endometrite é considerada a principal causa de subfertilidade em éguas e representa um grande desafio para a reprodução equina (Morris et al., 2020). A inflamação do endométrio ocorre como processo fisiológico transitório, após a cobertura ou a inseminação artificial, visando proteger contra substâncias que atuam como antígenos, tais como microrganismos e excesso de espermatozoides (Schuberth et al., 2008; Christoffersen & Troedsson, 2017). Porém, essa inflamação pode persistir no endométrio por um longo período devido a falhas nos mecanismos de defesa uterina em eliminar esses antígenos, caracterizando então a endometrite (Troedsson, 2011). Esta patologia tem um impacto negativo na fertilidade da égua por causar luteólise prematura, tornar o ambiente uterino hostil para sobrevivência e implantação do embrião, além de afetar o reconhecimento materno da gestação (LeBlanc & Causey, 2009; Swegen, 2021). Éguas acometidas necessitam de um manejo clínico reprodutivo

intensivo, resultando em custos adicionais e consideráveis perdas econômicas (Diel de Amorim et al., 2016).

Tanto o diagnóstico quanto o tratamento da endometrite são pontos críticos no processo de resolução da doença para manutenção da fertilidade da égua. No primeiro caso, o diagnóstico deve ser baseado em uma avaliação ginecológica mais completa possível, utilizando múltiplos achados clínicos associados a exames laboratoriais (Teixeira-Soares et al., 2022). Embora existam vários métodos de avaliação, a etiopatogênese multifatorial da endometrite e as diferentes interpretações dos resultados dificultam o diagnóstico (Kozdrowski et al., 2015). Apesar de décadas de pesquisa, há uma crescente demanda pelo desenvolvimento de novos métodos de diagnóstico mais sensíveis e associados a técnicas de coleta de amostras rápidas e seguras (Buczkowska et al., 2014; Morrell & Rocha, 2022). Já a falta de eficácia no tratamento da endometrite usando as modalidades terapêuticas tradicionais, aliada à crescente incidência de microrganismos resistentes a antibióticos e formadores de biofilme, impulsiona o desenvolvimento de terapias não tradicionais (Buczkowska et al., 2015). Novos produtos biológicos estão sendo testados por sua capacidade de modular a resposta inflamatória endometrial. Porém, ainda existem poucos resultados sobre sua eficiência (Herrera et al., 2020). Portanto, a incapacidade de desenvolver um modelo que possa servir como padrão de tratamento torna essa área da reprodução equina de grande interesse científico (Liu e Troedsson, 2008; Scoggin, 2016).

Neste contexto, a endometrite é uma condição que representa um desafio contínuo na prática da reprodução equina e um potencial causador de prejuízos econômicos para a indústria de criação de cavalos. Apesar de décadas de pesquisa, ainda há lacunas no entendimento dos mecanismos que levam ao seu desenvolvimento. Essa falta de conhecimento destaca a necessidade de entender melhor os métodos diagnósticos e terapêuticos para melhorar as taxas de fertilidade nesses animais. Portanto, é fundamental investir em pesquisas e soluções que possam minimizar os impactos negativos da endometrite, promovendo a saúde reprodutiva das

éguas e a indústria equina. Com isso, nosso objetivo foi realizar estudos experimentais sobre a endometrite, enfatizando sua prevalência em éguas retiradas de programas comerciais de transferência de embriões (Capítulo 2) e aspectos do proteoma do fluido uterino de éguas sadias e com endometrites pós-cobertura e infecciosa (Capítulo 3). Além disso, realizou-se um estudo meta-analítico sobre a eficácia dos tratamentos sobre parâmetros inflamatórios uterinos de éguas com endometrite pós-cobertura (Capítulo 4).

2. Etiopatogenia da endometrite

A predisposição à endometrite pode ocorrer por falhas nos mecanismos de proteção e depuração uterina que torna as éguas susceptíveis a inflamação persistente (Katila & Ferreira-Dias, 2022). Tais mecanismos de proteção compreendem barreiras anatômicas e mecânicas, bem como mecanismos imunológicos (Troedsson, 2011). Lábios vulvares, dobra vestíbulo vaginal e cérvix são barreiras físicas que, uma vez comprometidas, podem permitir a chegada de bactérias ascendentes ao útero e causar endometrite (Troedsson, 2011; Canisso et al., 2016). Já contrações uterinas, relaxamento cervical e drenagem linfática atuam mecanicamente na limpeza e eliminação de contaminantes no útero (LeBlanc, 2010; Canisso et al., 2016). Por fim, a mobilização de células polimorfonucleares e imunoglobulinas também estão envolvidas na proteção do útero contra a endometrite (LeBlanc, 2010; Troedsson, 2011). Todos esses processos de defesa desempenham um papel crucial na criação de um ambiente favorável para chegada do embrião (Christoffersen & Troedsson, 2017). Acredita-se que falhas nesses mecanismos sejam o principal fator contribuinte para o desenvolvimento da endometrite (Troedsson, 2011).

Animais com mecanismos de proteção ineficientes são chamados de suscetíveis a endometrite, por permitirem uma predisposição a inflamação recorrente (Troedsson, 2011). A possibilidade de ocorrência de algum fator predisponente à endometrite foi confirmada por Hughes e Loy em 1969, quando observaram que éguas virgens eliminavam a inoculação

bacteriana intrauterina de forma mais rápida do que éguas multíparas mais velhas, as quais permaneciam cronicamente infectadas. Fatores de risco individuais podem predispor à suscetibilidade, como aumento da idade associado a alterações na resposta imunológica sistêmica, má conformação perineal, deficiência na contratilidade miometrial com atraso na depuração uterina, retenção de fluido uterino e número de partos (Brinsko et al., 2003; Woodward et al., 2012; Christoffersen et al., 2015).

A resposta inflamatória no útero de animais acometidos pela endometrite envolve uma complexa interação entre diversos fatores, incluindo a presença dos antígenos, a ativação de células imunes, a liberação de citocinas e a regulação dos mecanismos pró e anti-inflamatórios (Morris et al., 2020). A imunidade inata é considerada a primeira linha de defesa não específica que deve responder à introdução de antígenos no útero (Abbas et al., 2015; Morris et al., 2020). A quimiotaxia de células de defesa, principalmente de neutrófilos, é induzida do sangue para o lúmen uterino podendo ser alterada por diferentes antígenos, influenciando a característica e o grau da reação inflamatória (Fiala, 2017). A resposta imune predominantemente inata permite a repetição do desafio do sêmen e embrião para o sistema imunológico, sem o desenvolvimento de anticorpos para uma fertilização bem-sucedida (Iijima et al., 2008; Canisso et al., 2020). Durante as primeiras 24 horas após o acasalamento, também ocorre um rápido aumento das citocinas pró-inflamatórias, como interleucinas (IL)-1β, IL-6, IL-8, fator de necrose tumoral (TNF)-α e interferon (IFN)-γ, que auxiliam no processo de eliminação de antígenos (Christoffersen & Troedsson, 2017). Em resposta, citocinas anti-inflamatórias, como ILRA, IL-1, IL-4, IL-10, IL-13, são liberadas para modular a inflamação e reestabelecer o equilíbrio, que é observado entre 6 e 12 horas após o acasalamento ou inseminação artificial (Woodward & Troedsson, 2015).

A resposta inflamatória uterina pode ser influenciada pela presença de componentes seminais e meios diluidores de sêmen, podendo induzir eventos que estão envolvidos no equilíbrio pró e anti-inflamatório e atuando como antígenos para o sistema imune uterino

(Morrel & Rocha, 2022). Os espermatozoides, quando presentes no lúmen uterino, geram uma resposta inflamatória transitória semelhante àquela provocada pela inoculação bacteriana, com quimiotaxia de células de defesa e aumento das citocinas inflamatórias (Pycock & Allen, 1990; Troedsson et al., 1995; 2001). O plasma seminal recruta e ativa células do sistema imunológico, além de promover com a ação de citocinas um equilíbrio entre as respostas pró-inflamatórias e anti-inflamatórias (Robertson, 2005 Woodward & Troedsson, 2013; Morelli et al., 2021). As proteínas presentes no plasma seminal também impedem a ligação de neutrófilos e a fagocitose de espermatozoides viáveis, permitindo assim a progressão dos espermatozoides dentro de um útero transitoriamente inflamado (Alghamdi et al., 2004; Doty et al., 2011). Meios diluidores de sêmen e seus constituintes como o leite e antibióticos, também podem interferir na resposta inflamatória no útero das éguas (Palm et al., 2008; Fiala, 2017). Portanto, deve existir um equilíbrio inflamatório entre os componentes seminais e a resposta imune (Woodward & Troedsson, 2013; Sánchez-Rodríguez et al., 2018).

Os microrganismos patogênicos são considerados antígenos para o sistema imune quando presentes no útero, seja na forma latente nas profundezas das glândulas endometriais, introduzidos durante o acasalamento ou ascendendo a partir do trato genital caudal (Petersen et al., 2018; Morris et al., 2020). Esses patógenos possuem padrões moleculares associados, como o lipopolissacarídeo de bactérias Gram-negativas e o peptideoglicano e ácido lipoteicóico de Gram-positivas, que desencadeiam a resposta imune (Quayle, 2002; Morris et al., 2020). Alguns microrganismos uterinos ainda possuem a capacidade de formar biofilmes, que são estruturas que dificultam o reconhecimento e penetração dos neutrófilos (Ferris, 2014; Koo et al., 2017). Além disso, é importante reconhecer que estudos podem ter subestimado a complexidade do microbioma uterino e, portanto, a etiopatogênese da endometrite pode não estar completamente elucidada (Amann et al., 1995; Knudsen et al., 2016; Heil et al., 2018).

3. Classificação da endometrite

A endometrite é considerada uma patologia multifatorial, podendo ser classificada de diferentes formas de acordo com sua etiopatogênese (Troedsson, 2011). Ela pode ser dividida em não infecciosa e infecciosa dependendo do antígeno. Na prática clínica essas causas frequentemente ocorrem em associação, tornando os sinais clínicos não específicos (Canisso et al., 2020). Por exemplo, bactérias podem ser introduzidas no útero durante o processo natural de reprodução e em inseminações artificiais, existindo o risco de infecção uterina mesmo que se tomando precauções para evitar contaminação (Woodward & Troedsson, 2015). Os mecanismos de resolução da inflamação podem variar devido a antígenos específicos, como no caso de microrganismos, que continuam a se reproduzir no trato reprodutivo e podem desenvolver mecanismos de defesa (Woodward & Troedsson, 2013). Assim, a endometrite é geralmente classificada de três formas: endometrite persistente induzida pós-cobertura causada principalmente pela presença de substâncias do sêmen, endometrite infecciosa provocada por microrganismos patogênicos e endometrite degenerativa crônica, caracterizada principalmente pelo acúmulo de tecido fibrótico no útero (Troedsson, 2011; Tuppits et al., 2014).

Os mecanismos fisiológicos de limpeza e a resposta imune promovem a resolução da inflamação dentro de 24 a 36 horas após a reprodução, preparando o útero para a chegada do embrião no dia 5 a 6 após a fertilização (LeBlanc & Causey, 2009; Christoffersen & Troedsson, 2017). No entanto, quando essa inflamação persiste por mais de 48 horas e afeta negativamente os mecanismos de limpeza uterina e a sobrevivência do embrião, ocorre a endometrite persistente induzida pós-cobertura (Katila, 2016). Esta forma de inflamação persistente do endométrio é o tipo mais comum de endometrite e é desencadeada por espermatozoides, plasma seminal e diluente de sêmen (Alghamdi et al., 2005; Fiala, 2017). O desequilíbrio entre os mecanismos pró-inflamatórios e anti-inflamatórios desempenha um papel importante na imunopatogênese da endometrite persistente pós-cobertura, podendo comprometer a fertilidade da égua (Canisso et al., 2020).

A endometrite infecciosa já ocorre quando as barreiras de proteção são superadas ou ineficientes. Assim, microrganismos acessam o útero e causam disbiose do microbioma comensal uterino (Heil et al., 2018). A endometrite infeciosa por bactérias é causada principalmente por *Streptococcus* spp., *Escherichia coli*, *Pseudomonas aeruginosa* e *Klebsiella pneumoniae* (Troedsson, 2011). Apesar de menos diagnosticada, a endometrite fúngica, principalmente causada por *Candida* spp. e *Aspergillu*s spp., também tem sido reconhecida como uma causa importante de queda na fertilidade, visto que, geralmente são mais difíceis de tratar e de diagnosticar (Dascanio et al., 2010; Silva e Alvarenga, 2011). Além disso, a inflamação do útero pode ser aguda ou crônica, onde a aguda é caracterizada por um grande influxo de neutrófilos, enquanto a crônica, mais comum em animais susceptíveis e por bactérias altamente patogênicas produtoras de biofilme, geralmente evolui para endometrite degenerativa pelo dano tecidual prolongado (Vanderwall e Newcomb, 2007; LeBlanc & McKinnon, 2011).

A inflamação persistente e crônica do endométrio desencadeia a resposta imunológica, resultando em um influxo contínuo de células de defesa, liberação de radicais livres e secreção de metaloproteinases que levam à deposição de colágeno e à formação de tecido fibroso (Causey et al., 2006). Esse acúmulo de tecido fibroso é conhecido como fibrose endometrial e está associado a alterações degenerativas crônicas, resultando em um processo chamado endometrose ou endometrite crônica degenerativa (Schoon et al., 1992). As alterações endometriais causadas geralmente são irreversíveis e incluem fibrose ao redor das glândulas e no tecido endometrial, formação de lacunas nos vasos linfáticos, atrofia do endométrio e diminuição das glândulas uterinas (Schoon et al., 1992; de Holanda et al., 2019). Ademais, a diminuição da atividade mucociliar, que leva a uma maior adesão bacteriana ao endométrio, e a degeneração dos vasos sanguíneos, que compromete a entrega de hormônios ao endométrio e o fluxo venoso de retorno, também contribuem para danos (Morrell & Rocha, 2022).

4. Diagnóstico da endometrite

O diagnóstico da endometrite pode ser desafiador devido a sua natureza multifatorial, utilização de métodos inadequados e interpretação incorreta dos achados (Kozdrowski et al., 2015; Canisso et al., 2016). A busca por novos métodos e possíveis biomarcadores tem sido objeto de pesquisa com a finalidade de aumentar a eficiência do diagnóstico (Diel de Amorim et al., 2020). Atualmente, o diagnóstico baseia-se na realização de uma avaliação ginecológica incluindo a coleta de dados, avaliações clínicas e métodos laboratoriais (Teixeira-Soares et al., 2022). Os métodos de diagnóstico apresentam vantagens e desvantagens, ainda não existindo um que combine facilidade de execução, baixo custo e resultados rápidos para os profissionais (Buczkowska et al., 2014; Ferris 2016). Até o momento, o exame histológico tem sido considerado o mais completo, mas a utilização de múltiplos achados pode ser a forma mais eficaz de diagnosticar a endometrite (Diel de Amorim et al., 2016).

Para uma avaliação ginecológica eficiente, que facilite o diagnóstico preciso e a tomada de decisão, é crucial coletar o máximo de dados possível, incluindo idade, escore de condição corporal e histórico reprodutivo (Zent, 2011). A idade desempenha um papel significativo na fertilidade das éguas (Katila & Ferreira-Dias, 2022). Éguas idosas a partir de quinze anos apresentam uma redução comprovada na fertilidade devido uma maior incidência de problemas anatômicos perineais, bem como falhas nas contrações uterinas e na drenagem linfática, que contribuem para uma predisposição a infecções uterinas recorrentes (Morley & Murray, 2014). O escore de condição, além de ser um fator determinante para fertilidade, também reflete a saúde geral das éguas destinadas à reprodução, onde pontuações indesejáveis podem acarretar falhas nas defesas físicas do útero e doenças metabólicas (Vick et al., 2007; Morley & Murray, 2014). O histórico reprodutivo também desempenha um papel fundamental na identificação das causas atuais de subfertilidade e na prevenção de problemas futuros (McCue, 2008). A análise de alterações, diagnósticos e tratamentos anteriores podem fornecer uma visão mais abrangente sobre a origem do problema e oferecer informações valiosas a serem consideradas no diagnóstico (LeBlanc, 2008).

A ultrassonografia transretal é o principal método clínico de diagnóstico para endometrite, com melhor sensibilidade e especificidade (Teixeira-Soares et al., 2022). A visualização do acúmulo de líquido resultante de uma falha na depuração uterina e do edema endometrial anormal, resultado de uma inflamação persistente, estão associados a redução de fertilidade (Liu & Troedsson, 2008; Canisso et al., 2016). Porém, Leblanc & Causey (2009) relataram a ocorrência de endometrite subclínica, sem sinais evidentes na ultrassonografia, que podem ocorrer devido a processos inflamatórios crônicos (Katila & Ferreira-Dias, 2022). Portanto, a ultrassonografia deve fornecer um diagnóstico presuntivo e não definitivo para endometrite, ressaltando a inclusão de múltiplos achados de diferentes métodos diagnósticos (Diel de Amorim et al., 2016; Morris et al., 2020).

Outro exame clínico é a avaliação da conformação do períneo, um método realizado por meio de palpação, visualização e mensurações da área que envolve o ânus e, principalmente, a vulva (Brandecamp, 2011). Durante o exame, são realizadas medições do comprimento efetivo e do ângulo de inclinação da vulva em relação à vertical (Caslick, 1937). Com base nesses resultados, é possível calcular o índice de Caslick, onde valores acima de 150 indicam a necessidade de realizar vulvoplastia (Brandecamp, 2011). Outro método adicional de avaliação perineal envolve a separação dos lábios vulvares para verificar o fechamento adequado desses e do esfíncter vestíbulo-vaginal, identificando a presença de ruído de aspiração de ar na vagina (Canisso et al., 2016). Uma boa conformação perineal é caracterizada por lábios vulvares promovendo um fechamento adequado para evitar a entrada de contaminantes, e a vulva posicionada verticalmente, com pelo menos dois terços do seu comprimento localizados abaixo da borda pélvica (Pascoe, 1979; Troedsson, 2011; Canisso et al., 2016). Alterações anatômicas nessa região podem predispor o animal a condições como pneumovagina ou urovagina, o que pode resultar em alterações de fertilidade, especialmente devido a infecções uterinas ascendentes (Brandecamp, 2011).

O exame clínico de visualização da vagina com o auxílio de um espéculo, a vaginoscopia, fornece informações sobre a presença de anormalidades, como traumas, o estado fisiológico do ciclo estral (Fig. 1A), a integridade do esfíncter vestíbulo-vaginal e a presença de secreção (Fig. 1B) (LeBlanc, 2008; McCue, 2008; Zent & Steiner, 2011). Além da vaginoscopia, o exame manual da cérvix, que consiste na palpação do orifício externo e do canal cervical, também fornece informações úteis durante a avaliação ginecológica (LeBlanc & McKinnon, 2011). Anormalidades cervicais como fibroses, aderências, divertículos e tortuosidades cervicais podem comprometer a proteção física e a eficiência na limpeza uterina, levando a tratamentos repetidos de infecções uterinas sem identificar a verdadeira causa primária da subfertilidade (LeBlanc, 2008). Manipulações reprodutivas repetidas ao longo dos anos ou com técnicas inadequadas são as principais causas de problemas cervicais, juntamente com complicações obstétricas durante o parto (McCue, 2008).

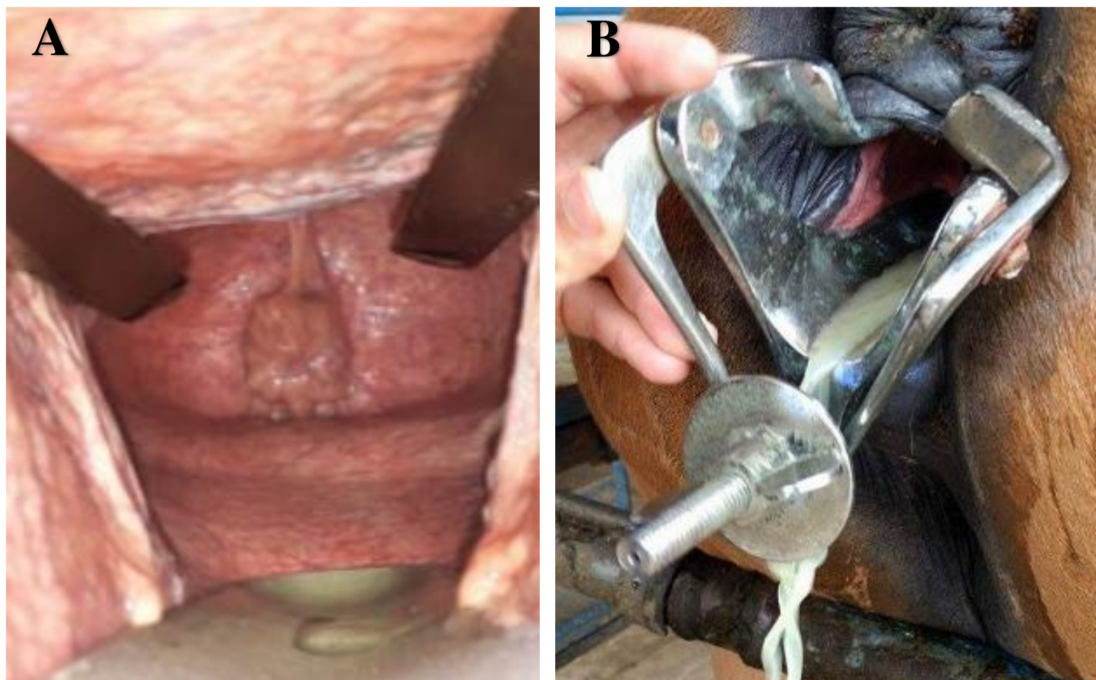


Figura 1. Vaginoscopia para avaliação clínica intravaginal com auxílio de um espéculo de Polansky. (A) Avaliação do óstio cervical externo em posição e coloração da mucosa condizente com a fase do ciclo estral de diestro. (B) Secreção purulenta originária da vagina e indicativa de vaginite. Fonte: arquivo pessoal.

A coleta de amostras uterinas para exames laboratoriais ginecológicos é realizada com diferentes técnicas transcervicais que permitem a coleta segura de material uterino (Ferris, 2016). Dentre essas técnicas, estão o uso de coletores, o lavado intrauterino de baixo volume e a biópsia endometrial (Cocchia et al., 2012). Os coletores comerciais estéreis são considerados duplamente protegidos de contaminações vaginal e ambiental, uma vez que possuem uma proteção plástica externa e um tubo plástico que contém a haste coletora (Teixeira-Soares et al., 2022). A haste coletora pode ser revestida com algodão esterilizado (tipo *swab*) ou possuir cerdas de nylon estéreis em diferentes direções (tipo escova citológica) (Kozdrowski et al., 2015). Ambos os tipos de coletores podem ser utilizados para exame citológico e cultura uterina, mas a escova citológica é preferível para o exame citológico, enquanto o *swab* é mais adequado para cultura microbiológica (Walter et al., 2012; Kozdrowski et al., 2015). Uma desvantagem desses coletores é que eles coletam amostras de uma área relativamente pequena do endométrio, aproximadamente 1 a 2 cm^2 cranial à cérvix, o que pode resultar em amostras não representativas de todo o endométrio (Leblanc, 2010; Linton & Sertich, 2016).

A técnica de lavado intrauterino de baixo volume foi descrita pela primeira vez em 1988 por Ball e colaboradores e é utilizada para coleta de amostras para exames de cultura e citologia uterina. Essa técnica envolve a infusão de um pequeno volume de solução estéril no útero, geralmente de 60 a 150 mL, através de uma sonda estéril, e após distribuição do fluido pelo útero, este é coletado por gravidade ou sucção (LeBlanc, 2008). São recuperados em média 30 mL de fluido efluente, que é então centrifugado e o sedimento resultante é submetido a exames laboratoriais (Linton & Sertich, 2016; Cocchia et al., 2012; Teixeira-Soares et al., 2022). A grande vantagem dessa técnica é que ela permite a amostragem de uma área maior do endométrio. Porém, a amostra celular pode estar diluída no conteúdo luminal, muco e/ou exsudados de uma grande superfície uterina, o que pode dificultar os exames subsequentes (Cocchia et al., 2012; Teixeira-Soares et al., 2022).

A biópsia endometrial é outra técnica considerada confiável e segura, que utiliza um pequeno fragmento de endométrio coletado para avaliação (Kenney, 1978) (Fig. 2). Existem observações contraditórias em relação à representatividade de uma única amostra de biópsia endometrial em relação ao tamanho do útero (Schoon et al., 1997; Keller et al., 2004). Alguns estudos indicaram que uma única biópsia pode não ser totalmente representativa apara avaliação de fibrose (Dybdal et al., 1991; Fiala et al., 2010), o que sugere que a coleta de múltiplas amostras pode ser vantajosa em algumas éguas (McCue, 2008). No entanto, um estudo realizado por Overbeck et al. (2013) não encontrou diferenças significativas no número de neutrófilos em amostras de biópsia coletadas *post-mortem* de diferentes locais do útero equino.

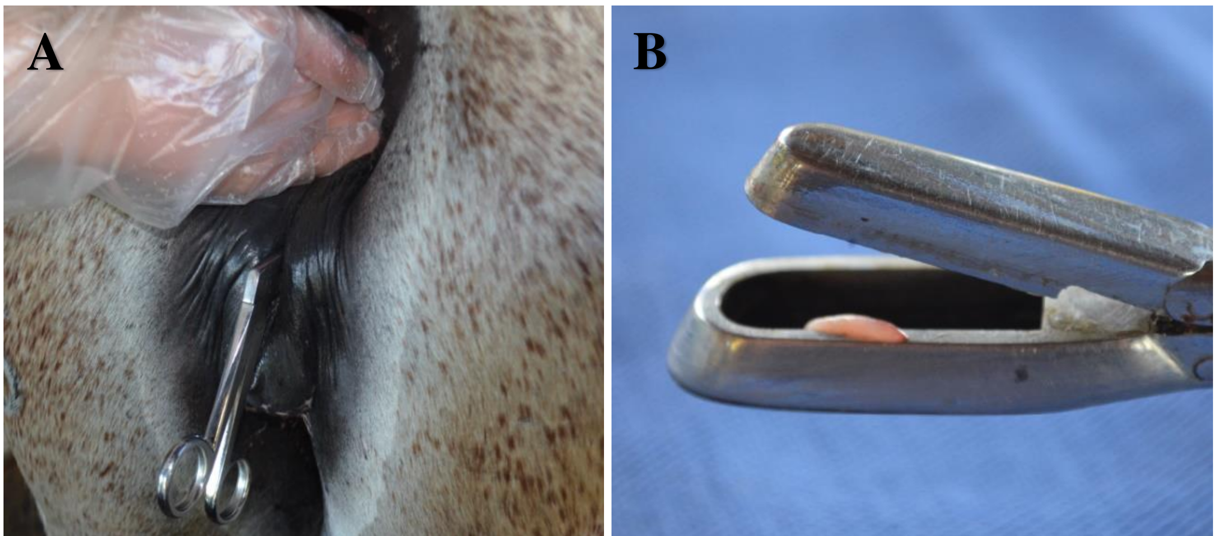


Figura 2. Coleta de amostra uterina para exames laboratoriais por biópsia transcervical utilizando uma pinça de Yoman. (A) Pinça coletora introduzida de forma transcervical no útero para biópsia endometrial. (B) Fragmento coletado após biópsia uterina transcervical. Fonte: arquivo pessoal.

Os exames laboratoriais utilizados para o diagnóstico da endometrite são considerados mais precisos e detalhados na avaliação da inflamação uterina em comparação aos exames clínicos (Kozdrowski et al., 2015; Canisso et al., 2016). Esses métodos requerem a utilização de materiais específicos e um tempo maior para obter os resultados, devido aos procedimentos

de coleta amostras e análise envolvidos (Teixeira-Soares et al., 2022). A avaliação citológica uterina é um método que envolve a coleta e interpretação das células que revestem o útero e seu lúmen (McCue, 2008). Considerada uma avaliação relativamente rápida e de fácil execução, apresenta a desvantagem de não fornecer informações suficientes sobre a causa da inflamação (Kozdrowski et al., 2015). Durante a avaliação das lâminas, é essencial realizar uma análise sistemática, minuciosa e em múltiplos campos, observando a presença de células epiteliais uterinas, polimorfonucleares, detritos, hemácias, bactérias, leveduras, organismos fúngicos e espermatozoides (Ferris, 2016; Walter et al., 2012). Existem dois métodos principais para avaliar a inflamação em tecidos citológicos: contagem do número de polimorfonucleares por campo de alta potência, realizando uma média de dez campos aleatórios em aumento de 400x, e a porcentagem de polimorfonucleares em relação às células epiteliais, contando 200 a 400 células totais (Riddle et al., 2007; LeBlanc, 2011; Cocchia et al., 2012). Estudos mostram que a identificação da porcentagem de polimorfonucleares é mais sensível no diagnóstico de endometrite (Kozdrowski et al., 2015).

A cultura microbiológica aeróbica após coleta de amostras intrauterinas com o isolamento de microrganismos é associada com a diminuição das taxas de prenhez, fazendo com que este tipo de diagnóstico seja muito importante durante o exame ginecológico para descartar ou não a endometrite infecciosa (LeBlanc, 2008). No laboratório, as amostras coletadas são aplicadas em placas contendo meio de cultura e incubadas em ambiente aeróbico a 37 ºC, uma vez que os microrganismos anaeróbios não desempenham um papel importante na endometrite equina (Ricketts & Mackintosh, 2016). O diagnóstico é baseado no tamanho da colônia, morfologia, pigmentação e hemólise, e um técnico experiente deve ser capaz de identificar a maioria dos patógenos com base em sua aparência (Ricketts, 2011; Katila, 2016). Meios de cultura especiais e testes podem ser utilizados para uma identificação mais precisa (Beehan & McKinnon, 2009; Katila, 2016). Posteriormente, a realização do teste de susceptibilidade a antimicrobianos é essencial para determinar a melhor terapia, ajustando o

tratamento de acordo com os resultados obtidos (McCue, 2008). Estudos recentes, como o realizado por Holyoak et al. (2021), demonstraram que a cultura microbiológica pode perder uma grande diversidade microbiana que está naturalmente presente no útero, e que este cultivo represente apenas uma pequena parte do microbioma uterino (Morrel & Rocha, 2022).

A avaliação histopatológica de biópsias endometriais é considerada o padrão ouro para diagnosticar a endometrite equina. Porém, sua aplicação na prática clínica é limitada a casos especiais devido à demora em obter os resultados e o método de coleta ser considerado invasivo (Overbeck et al., 2011; Nielsen et al., 2012; Morrel & Rocha, 2022). Apesar disso, o exame histológico fornece informações valiosas sobre a saúde uterina das éguas, incluindo resposta terapêutica, depuração uterina, fluxo sanguíneo e necessidade de terapia adicional (Buczkowska et al., 2014). Esta avaliação também é usada como importante indicador de prognóstico de fertilidade (LeBlanc, 2008; McCue, 2008). Alterações no tamanho e forma do epitélio, grau de edema endometrial relacionado ao estágio do ciclo, lacunas linfáticas, fibrose periglandular, figuras mitóticas no epitélio glandular ou degeneração glandular cística são achados associados a processos patológicos específicos (Kenney, 1978; LeBlanc, 2008). Um sistema de classificação dos achados histológicos baseado em escores foi proposto por Kenney e Doig (1978; 1986) e posteriormente adaptado por Schoon et al. (1992), classificando as éguas em quatro categorias com base nas alterações observadas na histopatologia do endométrio (Buczkowska et al., 2014; Canisso et al., 2016). Comparando os diferentes métodos de diagnóstico, o estudo realizado por Teixeira-Soares et al. (2022) demonstrou que, entre os exames clínicos, a palpação e a ultrassonografia obtiveram os melhores resultados (Tabela 1). No entanto, os testes laboratoriais avaliados, como a citologia uterina e a cultura microbiológica uterina, apresentaram valores ainda mais elevados de sensibilidade e especificidade em comparação aos métodos clínicos.

Tabela 1: Comparação entre os diferentes métodos de diagnóstico para endometrite em éguas utilizados na avaliação ginecológica em contraste com a avaliação histopatológica do endométrio, padrão-ouro para o diagnóstico. Adaptado de Teixeira-Soares et al., 2022.

Método de diagnóstico	Coeficiente Kappa	Força*	Sensibili-dade	Especifici-dade
Palpação e ultrassonografia transretal	0.6447	Substancial	0.76	0.92
Conformação perineal	0.2308	Leve	0.42	0.85
Vaginoscopia	0.1304	Fraca	0.30	0.85
Exame da cérvix	0.3023	Leve	0.50	0.85
Cultura microbiológica	0.7938	Substancial	0.84	1.00
Citologia endometrial	0.7000	Substancial	0.76	1.00

*Força de concordância do coeficiente Kappa: 0,00-0,19 (Fraca); 0,20-0,39 (Leve); 0,40-0,59 (Moderada); 0,60-0,79 (Substancial); 0,80-1,00 (Quase perfeito).

5. Novas abordagens de diagnóstico e potenciais biomarcadores

Pesquisas recentes têm se concentrado no desenvolvimento de biomarcadores para o diagnóstico precoce de animais com subfertilidade (Moura e Memili, 2016; Cecchini Gualandi et al., 2023). A utilização de abordagens baseadas em novas áreas de estudo, como epigenética, metagenômica e proteômica, podem fornecer uma alternativa para um diagnóstico não invasivo e confiável (Gebremedhn et al., 2021). Avanços na espectrometria de massa tornaram possível investigar o proteoma e sua relação com a fertilidade (Gibb & Aitken, 2016). O uso de ferramentas proteômicas na descoberta de biomarcadores para a fertilidade tem se mostrado promissor, especialmente na análise quantitativa em larga escala do proteoma uterino (Lawson et al., 2018; Griffin et al., 2020). Embora seja improvável que uma única proteína sirva como biomarcador, estudos futuros têm o potencial de revelar correlações confiáveis entre proteínas e funções relacionadas à reprodução equina (Andrade Souza et al., 2010; Griffin et al., 2020). Compreender o papel dessas proteínas e suas alterações na endometrite é crucial para uma melhor compreensão da etiopatogênese da doença, o diagnóstico precoce e o desenvolvimento de abordagens terapêuticas mais eficazes (Hermes et al., 2015).

A análise proteômica do fluido uterino obtido por meio de um lavado é uma técnica minimamente invasiva que permite estudar as alterações inflamatórias uterinas. Diversas proteínas foram identificadas por meio dessa abordagem (Hayes et al., 2008; Maloney et al., 2019), dentre elas destacam-se a uterocalina e o receptor de imunoglobina polimérica, as quais possuem relação com os neutrófilos e desempenham atividade imunomoduladora e anti-inflamatória (Stewart et al., 2000; Ratajczak et al., 2010; Hayes et al., 2012). Outra proteína de relevância na modulação da resposta imune inata é a vanina. Apesar do seu papel específico no útero ainda ser desconhecido, há uma possível associação dela com a inflamação e a presença de neutrófilos na citologia uterina (Diel de Amorim et al., 2020). Essa capacidade de modular a resposta imune também foi observada para as anexinas, que apresentam efeitos antimigratórios no extravasamento de leucócitos e regulam a produção de superóxido de neutrófilos (Gerke e Moss, 2002; Hayes et al., 2012). Outras proteínas ainda requerem mais estudos para estabelecer sua relação específica com a endometrite, como a uteroferrina, encontrada em grandes quantidades no fluido uterino (Ellenberger et al., 2008; Diel de Amorim et al., 2020), a secretoglobina que demonstrou um padrão secretório anormal em éguas com endometrite (Hoffmann et al., 2009; Diel de Amorim et al., 2020) e a lactoferrina que possui múltiplas funções (Levay e Viljoen, 1995; Shigeta et al., 1996).

Outra análise ômica é a metagenômica, que, ao contrário da microbiologia tradicional que depende de culturas, utiliza o sequenciamento de regiões gênicas para identificar os gêneros presentes em uma amostra (Hugenhol et al., 1998). A integração de dados proteômicos e metagenômicos pode fornecer uma visão abrangente das interações complexas entre o hospedeiro e a microbiota uterina, revelando potenciais alvos terapêuticos e biomarcadores para o diagnóstico precoce e a monitorização da doença (Koedooder et al., 2019; Lawson et al., 2021). No entanto, a variedade crescente de ferramentas, métodos analíticos e bioinformáticos usados para processar os dados de sequenciamento apresenta um desafio metodológico na comparação e interpretação dos diferentes estudos e seus resultados (Koedooder et al., 2019).

As mudanças epigenéticas também desempenham um papel significativo na endometrite, sendo potencialmente induzidas por fatores ambientais e reversíveis, o que as torna alvos promissores para diagnóstico e terapia (Alpoim-Moreira et al., 2019). O sistema imunológico, incluindo suas células de defesa, desempenha um papel importante nas modulações epigenéticas em resposta à infecção, o que impacta o proteoma uterino (Gabai et al., 2019). Tais mudanças epigenéticas afetam a produção de proteínas, especialmente nas células envolvidas nos mecanismos de modulação da resposta inflamatória uterina (Gabai et al., 2019).

É necessário investigar também a relação entre a inflamação, o estresse oxidativo e a função das proteínas, bem como sua relação com o dano tecidual (Celi & Gabai, 2015; Gabai et al., 2019). O estresse oxidativo desempenha um papel crucial na fertilidade, uma vez que agrava a inflamação e causa danos celulares, além de regular o ambiente uterino (Zhong & Zhou, 2013; Talukder et al., 2017; Gabai et al., 2019). A ativação de neutrófilos também contribui para a formação de proteínas de oxidação avançada, também consideradas biomarcadores de estresse oxidativo relacionado à atividade fagocitária (Bordignon et al., 2014; Gabai et al., 2019). Os neutrófilos produzem grandes quantidades de espécies reativas de oxigênio, levando ao estresse oxidativo e a um processo de doença conhecido como oxinflamação (Winterbourn et al., 2016; Gabai et al., 2019; Valacchi et al., 2018).

Em relação às análises sanguíneas, leucogramas e marcadores inflamatórios não são suficientemente sensíveis ou específicos para o diagnóstico preciso de endometrite (Katila, 2016). No entanto, um estudo recente demonstrou que a capacidade antioxidante de redução férrica (FRAP) apresentou diferenças significativas entre éguas positivas e negativas para endometrite, sugerindo o potencial de marcadores indiretos (Cecchini Gualandi et al., 2023). Além disso, a endometrite parece afetar não apenas as vias moleculares do endométrio, mas também o ambiente sistêmico, com citocinas inflamatórias e fragmentos de complemento atuando como mediadores do recrutamento de leucócitos durante a inflamação (Raliou et al.,

2019). Metodologias de diagnóstico mais sensíveis podem ser responsáveis por detectar genes que codificam mediadores inflamatórios envolvidos na inflamação uterina (Calder et al., 2013; Foley et al., 2015). Ainda não está claro como as mudanças no perfil gênico do endométrio se relacionam com os leucócitos circulantes em animais com endometrite (Raliou et al., 2019).

6. Tratamentos para endometrite

O primeiro tratamento para endometrite descrito foi a lavagem uterina, em 1938 por Zivotkov et al., e assim como as drogas ecbólicas e a antibioticoterapia, são considerados tratamentos tradicionais (Scoggin et al., 2016; Canisso et al., 2020). O principal objetivo dos tratamentos tradicionais é auxiliar na limpeza uterina de fluidos, detritos, microrganismos e produtos inflamatórios, reduzindo assim a inflamação endometrial (Buczkowska et al., 2015; Morrell & Rocha, 2022). A lavagem uterina com solução de Ringer Lactato ou solução salina a 0,9% apoia a estratégia de tratar e prevenir a endometrite, podendo ser realizada imediatamente antes ou quatro horas após a inseminação sem consequências prejudiciais para a fertilização (Brinsko et al., 2003; Morris et al., 2020). Além de promover a limpeza do útero, a terapia de lavagem uterina também tem o efeito de reintroduzir neutrófilos viáveis no ambiente inflamatório estagnado do útero, reativando a resposta inflamatória aguda (Canisso et al., 2020). Essa abordagem terapêutica é frequentemente combinada com outros tratamentos, pois pode servir como veículo para a administração de agentes terapêuticos em uma abordagem multimodal (Scoggin, 2016).

Os agentes ecbólicos, outra categoria de tratamento tradicional, atuam promovendo contrações miometriais para facilitar a depuração, uma vez que a baixa contratilidade é uma das principais causas do acúmulo de fluido e suscetibilidade a endometrite em éguas (Risco et al., 2009). A ocitocina é o principal agente ecbólico utilizado, apesar da sua meia-vida curta de cerca de 30 minutos (Canisso et al., 2020). Outros agentes ecbólicos como a carbetocina estão sendo testados com intuito de aumentar o período de contrações (Steckler et al., 2012; Canisso

et al., 2020). No entanto, a falta de eficácia dos tratamentos tradicionais pode também ser atribuída à fatores predisponentes a susceptibilidade a endometrite, como idade avançada, problemas anatômicos conformacionais, presença de fluido uterino e depuração uterina atrasada (Buczkowska et al., 2015).

A baixa eficácia dos tratamentos ainda é mais preocupante para a antibioticoterapia devido a resistência bacteriana, principalmente relacionada ao uso inadequado e excessivo de antibióticos, muitas vezes prescritos de forma empírica (Pyörälä et al., 2014; Díaz-Bertrana et al., 2021). A seleção do antibiótico adequado depende dos microrganismos identificados e do teste de sensibilidade, sendo que estudos anteriores apontaram diferentes antibióticos como os mais eficazes em casos específicos, como amicacina (Díaz-Bertrana et al., 2021), enrofloxacina (Teixeira-Soares et al., 2022), gentamicina (Albihn et al., 2003) e sulfonamida-trimetoprim (Mitchell et al., 2018). Além disso, a falha na antibioticoterapia também pode estar relacionada à cronicidade da infecção, à presença de crescimento misto de organismos, à natureza focal ou difusa da infecção e à formação de biofilme bacteriano, dificultando ainda mais o tratamento (Troedsson, 2011; Morrell & Rocha, 2022).

Diante desse quadro de inflamação e infecção uterina prolongada por falhas no tratamento, torna-se evidente a necessidade de abordagens alternativas (Morrell & Rocha, 2022). Diversos tratamentos não tradicionais têm sido propostos, incluindo o uso de drogas anti-inflamatórias, que tem como objetivo regular a expressão de citocinas e mediadores inflamatórios, restabelecendo o equilíbrio dos mecanismos envolvidos na endometrite (Scoggin, 2016). Porém, os anti-inflamatórios não esteroidais não seletivos, como o flunixin meglumine, agem bloqueando ciclooxigenase-1 e 2, o que pode levar à redução na produção de prostaglandinas que desempenham papéis importantes na depuração uterina e ovulação (Risco et al., 2009; Cuervo-Arango, 2011; Martínez-Boví et al., 2023). Os glicocorticoides, como a dexametasona, também possuem potenciais efeitos colaterais indesejáveis, como laminite e perda de massa muscular, que podem limitar sua utilização, apesar de não interferirem na

ovulação (Scoggin, 2016; Martínez-Boví et al., 2023). Nesse sentido, anti-inflamatórios não esteroidais seletivos para ciclooxigenase-2, como o firocoxib e o vedaprofeno, demonstram ser uma promissora alternativa para reduzir a inflamação com menor incidência de efeitos colaterais (Fitzpatrick et al., 2004; Friso et al., 2019).

As terapias com agentes biológicos são outra opção de tratamento não tradicional para endometrite devido à sua capacidade de modular e estimular o sistema imunológico, apresentando resultados promissores em casos de falhas dos tratamentos convencionais (Woodward et al., 2015; Morrell & Rocha, 2022). O extrato da parede celular de bactérias, por exemplo, é um composto biológico que regula a produção de diversas citocinas inflamatórias, reduzindo a infiltração de neutrófilos e a produção de óxido nítrico e, consequentemente, diminuindo o acúmulo de fluido uterino (Rogan et al., 2007; Woodward et al., 2013; Herrera et al., 2020). Outro importante agente testado é a infusão intrauterina de células-tronco, que podem induzir a proliferação celular e a remodelação tecidual, além de possuir funções relacionadas à redução do número de neutrófilos e ao aumento da expressão de citocinas anti-inflamatórias (Mambelli et al., 2014; Ferris et al., 2014; Rink et al., 2018). No entanto, ainda há controvérsias a respeito da migração dessas células para o endométrio e sua sobrevivência no ambiente uterino inflamado, havendo pesquisas para desenvolver novos métodos de cultivo, liofilização e a combinação com vesículas extracelulares (Perrini et al., 2016; Tongu et al., 2021; Lange-Consiglio et al., 2023).

O tratamento intrauterino com terapias de plasma sanguíneo, tais como o plasma rico e pobre em plaquetas e o lisado de plaquetas, também é promissor e tem sido investigado para tratar a endometrite em éguas, atenuando a resposta inflamatória uterina (Segabinazzi et al., 2021; Colombo et al., 2021). Essas terapias contêm o sistema complemento, que desempenha um papel importante na defesa contra agentes infecciosos, e apresentam propriedades imunomoduladoras (Schär et al., 2015). Já o lisado de plaquetas, obtido por ruptura criogênica das plaquetas do plasma rico, tem se tornado uma terapia emergente pois permite o

armazenamento a longo prazo sem comprometer as propriedades imunomoduladoras, além de uma possível maior ativação das plaquetas (Hauschild et al., 2018; Colombo et al., 2021). Outro tratamento biológico com propriedades imunomoduladoras são as proteínas do plasma seminal, que apesar de induzirem uma inflamação endometrial transitória, têm o potencial de modificar a duração e a intensidade da resposta (Woodward & Troedsson, 2013; Morelli et al., 2021). Proteínas seminais como a lactoferrina exibem diversas propriedades benéficas, tais como a supressão da expressão de citocinas inflamatórias, a promoção da ligação de neutrófilos a espermatozoides inviáveis, a atividade bactericida devido à sua capacidade de quelar o ferro livre e a ação antioxidante (Ammons & Copie, 2013; Ighodaro & Akinloye, 2018; Gabai et al., 2019).

O gás ozônio é outro potencial tratamento para a endometrite devido às suas propriedades bactericidas, fungicidas, imunoestimulantes e antioxidantes (Bocci, 2006). A infusão intrauterina de gás ozônio é segura, sem efeitos adversos na morfologia endometrial (Camargo et al., 2021; Köhne et al., 2023). No entanto, a eficácia do gás ozônio ainda é controversa, pois em estudo *in vitro* não demonstrou capacidade de eliminar completamente o biofilme bacteriano (Loncar et al., 2017) e *in vivo* não demonstrou erradicar totalmente o crescimento bacteriano (Köhne et al., 2023). Em contraste, a combinação do gás ozônio com lavagens uterinas mostrou-se eficaz no controle da infecção e inflamação uterina, havendo a necessidade de mais estudos para comprovar sua real eficácia (Ávila et al., 2022).

Diversas soluções com formulações variadas têm sido utilizadas em infusões intrauterinas para tratar a endometrite (Scoggin, 2016). Produtos químicos antissépticos ou debridantes são utilizados para combater a endometrite causada por bactérias formadoras de biofilme e agentes mucolíticos são empregados para facilitar a limpeza do útero, removendo exsudatos, muco ou biofilme (Morrell & Rocha, 2022; LeBlanc & Causey, 2009). No entanto, restrições no uso desses agentes podem ocorrer devido à falta de estudos sobre dosagem, eficácia e histórico de irritação uterina (Troedsson, 2011). O dimetilsulfóxido, por exemplo, é

um solvente químico com propriedades anti-inflamatórias e antimicrobianas que demonstrou ser capaz de romper o biofilme e reduzir a viabilidade de bactérias isoladas de úteros com endometrite (Loncar et al., 2017). O querosene, quando infundido no útero, possui alta capacidade de remover detritos, muco, material pulverizado e microorganismos por meio da curetagem do epitélio uterino, além de limpar as secreções das glândulas císticas dilatadas (Buczkowska et al., 2015; Scoggin, 2016). No entanto, essa terapia enfrenta resistência de muitos profissionais devido à promoção de uma inflamação grave (Bradecamp et al., 2014). Além do querosene, o lavado uterino com soluções diluídas de iodo e clorexidina também são utilizados, mas também com histórico de irritação uterina (Ricketts, 1999; Troedsson, 2011; Köhne et al., 2020).

O peróxido de hidrogênio é um antisséptico de amplo espectro mais seguro, que demonstra eficácia contra a maioria dos patógenos em seus estados latentes e em biofilme (Ferris et al., 2014). Soluções diluídas de peróxido de hidrogênio têm sido utilizadas no tratamento da endometrite aguda e parecem ser úteis quando há exsudatos presentes no lúmen uterino (Wolfsdorf & Caudle, 2007). No caso de endometrite fúngica, a adição de uma solução de peróxido de hidrogênio a 3% à solução salina para infusão uterina pode auxiliar na remoção de detritos contendo fungos que podem aderir ao epitélio luminal (Ricketts, 1999). Os mucolíticos, como a N-acetilcisteína, também possuem a capacidade de facilitar a eliminação da secreção e do biofilme sem causar alterações prejudiciais ao endométrio (Witte et al., 2012; Gores-Lindholm et al., 2013). Além disso, foram descritas funções anti-inflamatórias para a N-acetilcisteína ao bloquear a produção de citocinas pró-inflamatórias (Caissie et al., 2020).

Outros compostos a serem considerados são os agentes quelantes que visam melhorar a penetração de antibióticos no biofilme (Buczkowska et al., 2015). Um exemplo desses agentes é o Tris-EDTA, que, em contato direto com a parede celular bacteriana, causa a morte dos microrganismos (LeBlanc, 2010). No entanto, após a morte bacteriana, é necessário remover o acúmulo de detritos por meio de lavagem uterina, além de ser recomendada a associação com

antibióticos (LeBlanc, 2010; Buczkowska et al., 2015). Além dessa diversa gama de opções de tratamento mencionadas, outros métodos alternativos também têm sido objeto de estudo, como a acupuntura, por auxiliar as contrações uterinas e o aumento do tônus (Holyoak & Ma, 2022), a suplementação nutricional com ácidos graxos ômega-3 por reduzir da expressão de citocinas inflamatórias (Brendemuehl et al., 2014) e os exercícios por melhorar o tônus perineal e remoção de fluidos (Troedsson, 2011).

7. Referências

ABDELNABY, E. A. et al. Uterine hemodynamic patterns, oxidative stress, and chromoendoscopy in mares with endometritis. Theriogenology, v. 158, p. 112-120, 2020. Disponível em: https://doi.org/10.1016/j.theriogenology.2020.09.012.

AGUILAR, J. et al. Importance of using guarded techniques for preparation of endometrial cytology smears in mares. Theriogenology, v. 66, p. 423-430, 2006.

AKTHAR, I. et al. Sperm enter glands of preovulatory bovine endometrial explants and initiate inflammation. Reproduction, v. 159, n. 2, p. 181-192, 2020. Disponível em: https://doi.org/10.1530/REP-19-0414.

ALBIHN, A. et al. Uterine Microbiology and Antimicrobial Susceptibility in Isolated Bacteria from Mares with Fertility Problems. Acta Vet. Scand, v. 44, p. 121-129, 2003.

ALGHAMDI, A. S.; FOSTER, D. N. Seminal DNase frees spermatozoa entangled in neutrophil extracellular traps. Biology of Reproduction, v. 73, n. 6, p. 1174-1181, 2005. Disponível em: https://doi.org/10.1095/biolreprod.105.045666.

ALGHAMDI, A. S.; FOSTER, D. N.; TROEDSSON, M. H. T. Equine seminal plasma reduces sperm binding to polymorphonuclear neurophils (PMN's) and improves the fertility of fresh semen inseminated into inflamed uteri. Reproduction, v. 127, n. 5, p. 593-600, 2004. Disponível em: https://doi.org/10.1530/rep.1.00096.

ALPOIM-MOREIRA, J. et al. Collagens and DNA methyltransferases in mare endometrosis. Reproduction in Domestic Animals, v. 54, n. S3, p. 46-52, 2019. Disponível em: https://doi.org/10.1111/rda.13515.

ALVARENGA, M. A. Problems and solutions in equine embryo transfer programs in Brazil. Acta Scientiae Veterinariae, v. 38, n. 2.

AMANN, R. I.; LUDWIG, W.; SCHLEIFER, K. H. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiological Reviews, v. 59, p. 143-169, 1995. Disponível em: https://doi.org/10.1128/mmbr.59.1.143-169.1995.

AMANN, R. P.; PICKETT, B. W. Principles of cryopreservation and a review of cryopreservation of stallion spermatozoa. Journal of Equine Veterinary Science. 1987. Disponível em: https://doi.org/10.1016/S0737-0806(87)80025-4.

AMMONS, M. C.; COPIÉ, V. Mini-review: Lactoferrin: a bioinspired, anti-biofilm therapeutic. Biofouling, v. 29, n. 4, p. 443-455, 2013. Disponível em: https://doi.org/10.1080/08927014.2013.773317.

ANDRADE SOUZA, F. et al. IGF-I, leptina, insulina e proteínas associadas à qualidade do plasma seminal: ação local. Revista de Medicina Veterinaria, v. 20, p. 9-25, 2010. Disponível em: https://doi.org/10.19052/mv.578.

ÁVILA, A. C. A. et al. Effectiveness of Ozone Therapy in The Treatment of Endometritis in Mares. Journal of Equine Veterinary Science, v. 112, 2022. Disponível em: https://doi.org/10.1016/j.jevs.2022.103900.

BALL, B. A. et al. Use of a low-volume uterine flush for microbiologic and cytologic examination of the mares endometrium. Theriogenology, v. 29, p. 1269-1283, 1988.

BARBA, M. et al. Vaginal microbiota is stable throughout the estrous cycle in Arabian mares. Animals, v. 10, n. 11, p. 1-14, 2020. Disponível em: https://doi.org/10.3390/ani10112020.

BEEHAN, D. P.; MCKINNON, A. O. How to diagnose common equine reproductive tract bacterial pathogens using chromogenic agar. Proc 55th Ann Conv Am Ass Equine Practitioners, 2009.

BOCCI, V. A. Scientific and medical aspects of ozone therapy. State of the art. Rivista Italiana Di Ossigeno-Ozonoterapia, v. 5, n. 2, p. 93-104, 2006. Disponível em: https://doi.org/10.1016/j.arcmed.2005.08.006.

BRACHER, V.; NEUSCHAEFER, A.; ALLEN, W. R. The effect of intra-uterine infusion of kerosene on the endometrium of mares. J Reprod Fertil, v. 44, p. 706-707, 1991.

BRADECAMP, E. A. Pneumovagina. In: MCKINNON A. O., SQUIRES E. L., VAALA W. E., VARNER D. V., editors. Equine Reproduction, Second Edition. U.K: Wiley-Blackwell; 2011.

BRADECAMP, E. A.; AHLSCHWEDE, S. A.; COOK, J. L. The effects of intra-uterine kerosene infusion on endometrial epithelial cilia concentration. Journal of Equine Veterinary Science, v. 34, n. 1, p. 134, 2014. Disponível em: https://doi.org/10.1016/j.jevs.2013.10.092.

BRENDEMUEHL, J. P. et al. Nutritional supplementation with omega-3 fatty acids reduces endometritis severity in mares. Theriogenology, v. 81, n. 8, p. 1056-1062, 2014.

BRENDEMUEHL, J. P.; KOPP, K.; ALTMAN, J. Influence of dietary algal N-3 fatty acids on breeding induced inflammation and endometrial cytokine expression in mares bred with frozen semen. Journal of Equine Veterinary Science, v. 34, n. 1, p. 123-124, 2014. Disponível em: https://doi.org/10.1016/j.jevs.2013.10.084.

BRINSKO, S. P. et al. A practical method for recognizing mares susceptible to post-breeding endometritis. Proc Am Assoc Equine Pract, 2003. p. 363-365.

BUCZKOWSKA, J. et al. Comparison of the biopsy and cytobrush techniques for diagnosis of subclinical endometritis in mares. Reprod Biol Endocrinol, 2014.

BUCZKOWSKA, J. et al. Non-traditional treatments for endometritis in mares. Bulgarian Journal of Veterinary Medicine, Trakia University, 2015. Disponível em: https://doi.org/10.15547/bjvm.870.

CAISSIE, M. D. et al. The Effects of Treatment with N-Acetyl Cysteine on Clinical Signs in Persistent Breeding-Induced Endometritis Susceptible Mares. Journal of Equine Veterinary Science, v. 92, 2020. Disponível em: https://doi.org/10.1016/j.jevs.2020.103142.

CALDER, P. C. et al. A Consideration of Biomarkers to be Used for Evaluation of Inflammation in Human Nutritional Studies. British Journal of Nutrition, v. 109, 2013. Disponível em: https://doi.org/10.1017/S0007114512005119.

CAMARGO FERREIRA, J. et al. Endometrial angiogenesis induced by uterine insufflation with an oxygen-ozone gas mixture in mares. Reproduction in Domestic Animals, v. 56, n. 9, p. 1176-1183, 2021. Disponível em: https://doi.org/10.1111/rda.13958.

CANISSO, I. F.; SEGABINAZZI, L. G. T. M.; FEDORKA, C. E. Persistent breeding-induced endometritis in mares - a multifaceted challenge: From clinical aspects to immunopathogenesis

and pathobiology. International Journal of Molecular Sciences, v. 21, n. 4, p. 1432, 2020. Disponível em: https://doi.org/10.3390/ijms21041432.

CANISSO, I. F.; STEWART, J.; SILVA, M. A. C. Endometritis - Managing Persistent Post-Breeding Endometritis. Elsevier Inc., v. 16, p. 749-739, 2016. Disponível em: http://dx.doi.org/10.1016/j.cveq.2016.08.004.

CASLICK, E. A. The vulva and the vulvo-vaginal orifice and its relation to genital health of the thoroughbred mare. Cornell Veterinary, v. 27, p. 178, 1937.

CAUSEY, R. C. Making sense of equine uterine infections: The many faces of physical clearance. Veterinary Journal, v. 172, n. 3, p. 405-421, 2006. Disponível em: https://doi.org/10.1016/j.tvjl.2005.08.005.

CELI, P.; GABAI, G. Oxidant/antioxidant balance in animal nutrition and health: The role of protein oxidation. Frontiers in Veterinary Science, v. 2, p. 48, 2015. Disponível em: https://doi.org/10.3389/fvets.2015.00048.

CHEN, C. C., & LAU, L. F. (2009). Functions and mechanisms of action of CCN matricellular proteins. International Journal of Biochemistry & Cell Biology, 41(4), 771-783.

CHRISTOFFERSEN, M.; SÖDERLIND, M.; RUDEFALK, S. R.; PEDERSEN, H. G.; ALLEN, J.; KREKELER, N. Risk factors associated with uterine fluid after breeding caused by Streptococcus zooepidemicus. Theriogenology, v. 84, n. 8, p. 1283-1290, 2015. Disponível em: https://doi.org/10.1016/j.theriogenology.2015.07.007.

CHRISTOFFERSEN, M.; TROEDSSON, M. H. T. (Inflammation and fertility in the mare. Reproduction in Domestic Animals, 2017. Disponível em: https://doi.org/10.1111/rda.13013.

CLAES, A.; CUERVO-ARANGO, J.; VAN DEN BROEK, J.; GALLI, C.; COLLEONI, S.; LAZZARI, G.; ... STOUT, T. A. Factors affecting the likelihood of pregnancy and embryonic loss after transfer of cryopreserved in vitro produced equine embryos. Equine Veterinary Journal, v. 51, n. 4, p. 446-450, 2019. Disponível em: https://doi.org/10.1111/evj.13028.

COCCHIA, N.; PACIELLO, O.; AULETTA, L.; et al. Comparison of the cytobrush, cotton swab, and low-volume uterine flush techniques to evaluate endometrial cytology for diagnosing endometritis in chronically infertile mares. Theriogenology, 2012. v. 77. Disponível em: https://doi.org/10.1016/j.theriogenology.2011.07.018.

COLOMBO, I.; MISLEI, B.; MARI, G.; IACONO, E.; MERLO, B. Effect of platelet lysate on uterine response of mares susceptible to persistent mating-induced endometritis. Theriogenology, v. 179, p. 204-210, 2022. Disponível em: https://doi.org/10.1016/j.theriogenology.2021.12.001.

CUERVO-ARANGO, J. The effect of treatment with flunixin meglumine at different times relative to hCG administration on ovulation failure and luteal function in mares. Animal Reproduction Science, v. 127, n. 1-2, p. 84-90, 2011. Disponível em: https://doi.org/10.1016/j.anireprosci.2011.07.008.

DAKHLALLAH, D.; BATTE, K.; WANG, Y.; CANTEMIR-STONE, C. Z.; YAN, P.; NUOVO, G.; MARSH, C. B. Epigenetic regulation of mir-17~92 contributes to the pathogenesis of pulmonary fibrosis. American Journal of Respiratory and Critical Care Medicine, v. 187, n. 4, p. 397-405, 2013. Disponível em: https://doi.org/10.1164/rccm.201205-0888OC.

DASCANIO, J. J.; SCHWEIZER, C.; LEY, W. B. Equine fungal endometritis. Equine Vet. Educ., 2010. v. 13, p. 324-329. doi: 10.1111/j.2042-3292.2001.tb00122.x.

DAVIS, H. A.; STANTON, M. B.; THUNGRAT, K.; BOOTHE, D. M. Uterine bacterial isolates from mares and their resistance to antimicrobials: 8296 cases (2003–2008). J. Am. Vet. Med. Assoc., v. 242, p. 977-983, 2013.

DE HOLANDA, A. G. B.; DA SILVA LEITE, J.; CONSALTER, A.; et al. Expression of interleukins 6 and 10 and population of inflammatory cells in the equine endometrium: diagnostic implications. Mol Biol Rep, v. 46, p. 2485-2491, 2019. Disponível em: https://doi-org.ez35.periodicos.capes.gov.br/10.1007/s11033-018-04579-5.

DÍAZ-BERTRANA, M. L.; DELEUZE, S.; PITTI RIOS, L.; YESTE, M.; MORALES FARIÑA, I.; RIVERA DEL ALAMO, M. M. Microbial Prevalence and Antimicrobial Sensitivity in Equine Endometritis in Field Conditions. Animals, v. 11, p. 1476, 2021. Disponível em: https://doi.org/10.3390/ani11051476.

DIEL DE AMORIM, M.; GARTLEY, C. J.; HILL, A.; SCHOLTZ, E. L.; CHENIER, T. S.; FOSTER, R. A.; ... DIEL DE AMORIM, M. Comparison of Clinical Signs, Endometrial Culture, Endometrial Cytology, Uterine Low-Volume Lavage, and Uterine Biopsy and Combinations in the Diagnosis of Equine Endometritis. Journal of Equine Veterinary Science, v. 44, p. 54-61, 2016. Disponível em: https://doi.org/10.1016/j.jevs.2015.10.012.

DIEL DE AMORIM, M.; KHAN, F. A.; CHENIER, T. S.; SCHOLTZ, E. L.; HAYES, M. A. Analysis of the uterine flush fluid proteome of healthy mares and mares with endometritis or fibrotic endometrial degeneration. Reprod. Fertil. Dev., v. 32, p. 572-581, 2020.

DIMOCK, W. W.; SNYDER, E. B. Bacteria of the genital tract of mares and the semen of stallions and their relation to breeding efficiency. J. Am. Vet. Med. Assoc., v. 64, p. 288-297, 1924.

DONATO, G. G.; APPINO, S.; BERTERO, A.; POLETTO, M. L.; NEBBIA, P.; ROBINO, P.; ... NERVO, T. Safety and Effects of a Commercial Ozone Foam Preparation on Endometrial Environment and Fertility of Mares. Journal of Equine Veterinary Science, v. 121, 2023. Disponível em: https://doi.org/10.1016/j.jevs.2023.104222.

DOTY, A.; BUHI, W. C.; BENSON, S.; SCOGGIN, K. E.; POZOR, M.; MACPHERSON, M.; TROEDSSON, M. H. T. Equine CRISP3 modulates interaction between spermatozoa and polymorphonuclear neutrophils. Biology of Reproduction, v. 85, n. 1, p. 157-164, 2011. Disponível em: https://doi.org/10.1095/biolreprod.110.084491.

DYBDAL, N. O.; DAELS, P. F.; COUTO, M. A.; HUGHES, J. P.; KENNEDY, P. C. Investigation of the reliability of a single endometrial biopsy sample, with a note on the correlation between uterine cysts on biopsy grade. ISER Proc, v. 5, p. 697, 1991.

ELLENBERGER, C., WILSHER, S., ALLEN, W. R., HOFFMANN, C., KÖLLING, M., BAZER, F. W., KLUG, J., SCHOON, D., & SCHOON, H.-A. (2008). Immunolocalisation of the uterine secretory proteins uterocalin, uteroferrin and uteroglobin in the mare's uterus and placenta throughout pregnancy. Theriogenology, 70, 746-757.

FELIX, M. R.; TURNER, R. M.; DOBBIE, T.; HINRICHS, K. Successful in vitro fertilization in the horse: production of blastocysts and birth of foals after prolonged sperm incubation for capacitation†. Biology of Reproduction, v. 107, n. 6, p. 1551-1564, 2022. Disponível em: https://doi.org/10.1093/biolre/ioac172.

FERRIS, R. A. Bacterial endometritis: a focus on biofilms. Clinical Theriogenology, v. 6, p. 315-319, 2014. Disponível em: https://www.cabdirect.org/cabdirect/abstract/20143416808.

FERRIS, R. A. Endometritis: Diagnostic Tools for Infectious Endometritis. Veterinary Clinics of North America - Equine Practice, 2016. Disponível em: https://doi.org/10.1016/j.cveq.2016.08.001.

FIALA, R. S. Endométrio da égua pós-cobertura: ênfase na resposta inflamatória. Revista Brasileira de Reprodução Animal, v. 41, n. 1, p. 326-329, 2017.

FIALA, S. M.; ESMERALDINO, A.; JOBIM, M. I. M.; GARBADE, P.; WOLF, C. A.; RICHTER, G.; GREGORY, R. M.; MATTOS, R. C. Edmometrial fibrotic changes. Is one biopsy enough to diagnose degenerative changes? Anim. Reprod. Sci., v. 121, p. 89-90, 2010.

FITZPATRICK, F. A. Cyclooxygenase enzymes: regulation and function. Curr Pharmaceut Des, v. 10, p. 577-588, 2004.

FLO, T. H., SMITH, K. D., SATO, S., RODRIGUEZ, D. J., HOLMES, M. A., STRONG, R. K., AKIRA, S., & ADEREM, A. (2004). Lipocalin 2 mediates an innate immune response to bacterial infection by sequestering iron. Nature, 432(7019), 917-921.

FOLEY, C. et al. Integrated analysis of the local and systemic changes preceding the development of post-partum cytological endometritis. BMC Genomics, v. 16, p. 811, 2015.

FOLEY, C.; CHAPWANYA, A.; CALLANAN, J. J.; WHISTON, R.; MIRANDA-CASOLUENGO, R.; LU, J.; MEADE, K. G. Integrated analysis of the local and systemic changes preceding the development of post-partum cytological endometritis. BMC Genomics, v. 16, n. 1, p. 811, 2015. Disponível em: https://doi.org/10.1186/s12864-015-1967-5.

FRISO, A. M.; SEGABINAZZI, L. G. T. M.; CYRINO, M.; CORREAL, S. B.; FREITAS-DELL'AQUA, C. P.; TEORO DO CARMO, M.; ALVARENGA, M. A. Periovulatory administration of firocoxib did not alter ovulation rates and mitigated post-breeding inflammatory response in mares. Theriogenology, v. 138, p. 24-30, 2019. Disponível em: https://doi.org/10.1016/j.theriogenology.2019.06.045.

GABAI, G.; DE LUCA, E.; MIOTTO, G.; ZIN, G.; STEFANI, A.; DA DALT, L.; CELI, P. Relationship between protein oxidation biomarkers and uterine health in dairy cows during the postpartum period. Antioxidants, v. 8, n. 1, 2021. Disponível em: https://doi.org/10.3390/antiox8010021.

GEBREMEDHN, S.; ALI, A.; HOSSAIN, M.; HOELKER, M.; SALILEW-WONDIM, D.; ANTHONY, R. V.; TESFAYE, D. MicroRNA-mediated gene regulatory mechanisms in mammalian female reproductive health. International Journal of Molecular Sciences, 2021. Disponível em: https://doi.org/10.3390/ijms22020938.

GERKE, V., & MOSS, S. E. Annexins: From Structure to Function. Physiological Reviews, 82, 331-371, 2002.

GIBB, Z.; AITKEN, R. J. The Impact of Sperm Metabolism during in Vitro Storage: The Stallion as a Model. BioMed Research International, 2016. Disponível em: https://doi.org/10.1155/2016/9380609.

GORES-LINDHOLM, A. R.; LEBLANC, M. M.; CAUSEY, R.; HITCHBORN, A.; FAYRER-HOSKEN, R. A.; KRUGER, M.; AHLSCHWEDE, S. Relationships between intrauterine infusion of N-acetylcysteine, equine endometrial pathology, neutrophil function, post-breeding therapy, and reproductive performance. Theriogenology, v. 80, n. 3, p. 218-227, 2013. Disponível em: https://doi.org/10.1016/j.theriogenology.2013.03.026.

GUASTI, P. N.; SOUZA, F. F.; SCOTT, C.; PAPA, P. M.; CAMARGO, L. S.; SCHMITH, R. A.; PAPA, F. O. Equine seminal plasma and sperm membrane: Functional proteomic assessment. Theriogenology, v. 156, p. 70-81, 2020. Disponível em: https://doi.org/10.1016/j.theriogenology.2020.06.014.

HAAHR, T.; JENSEN, J. S.; THOMSEN, L.; DUUS, L.; RYGAARD, K.; HUMAIDAN, P. Abnormal vaginal microbiota may be associated with poor reproductive outcomes: A prospective study in IVF patients. Human Reproduction, v. 31, n. 4, p. 795-803, 2016. Disponível em: https://doi.org/10.1093/humrep/dew026.

HAHN, M. A.; HAHN, T.; LEE, D. H.; ESWORTHY, R. S.; KIM, B. W.; RIGGS, A. D.; PFEIFER, G. P. Methylation of polycomb target genes in intestinal cancer is mediated by inflammation. Cancer Research, v. 68, n. 24, p. 10280-10289, 2008. Disponível em: https://doi.org/10.1158/0008-5472.CAN-08-1957.

HANDELSMAN, J. Metagenomics: Application of Genomics to Uncultured Microorganisms. Microbiology and Molecular Biology Reviews, v. 68, n. 4, p. 669-685, 2004. Disponível em: https://doi.org/10.1128/mmbr.68.4.669-685.2004.

HAUSCHILD, G.; GEBUREK, F.; GOSHEGER, G.; EVESLAGE, M.; SERRANO, D.; STREITBÜRGER, A.; MISCHKE, R. Short term storage stability at room temperature of two different platelet-rich plasma preparations from equine donors and potential impact on growth factor concentrations. BMC Veterinary Research, v. 13, n. 1, 2017. Disponível em: https://doi.org/10.1186/s12917-016-0920-4.

HAYES, M. A., QUINN, B. A., LILLIE, B. N., CÔTÉ, O., BIENZLE, D., WAELCHLI, R. O., & BETTERIDGE, K. J. (2012). Changes in various endometrial proteins during cloprostenol-induced failure of early pregnancy in mares. Animal Reproduction, 9, 723-741.

HAYES, M. A.; QUINN, B. A.; LILLIE, B. N.; CÔTÉ, O.; BIENZLE, D.; WAELCHLI, R. O.; BETTERIDGE, K. J. Changes in various endometrial proteins during cloprostenol-induced failure of early pregnancy in mares. Anim. Reprod., v. 9, p. 723-741, 2012.

HAYES, M. A.; QUINN, B.; KEIRSTEAD, N.; KATAVOLOS, P.; WAELCHLI, R.; BETTERIDGE, K. Proteins associated with the early intrauterine equine conceptus. Reprod. Domest. Anim., v. 43, p. 232-237, 2008.

HEIL, B. A.; PACCAMONTI, D. L.; SONES, J. L. Role for the mammalian female reproductive tract microbiome in pregnancy outcomes. Physiological Genomics, 2019. Disponível em: https://doi.org/10.1152/physiolgenomics.00045.2019.

HEIL, B. A.; THOMPSON, S. K.; KEARNS, T. A.; DAVOLLI, G. M.; KING, G.; SONES, J. L. Metagenetic Characterization of the Resident Equine Uterine Microbiome Using Multiple Techniques. Journal of Equine Veterinary Science, v. 66, p. 111, 2018. Disponível em: https://doi.org/10.1016/j.jevs.2018.05.156.

HERMES, R., TÖPFER-PETERSEN, E., & AURICH, C. (2015). The challenge of endometritis in the mare - an immunological perspective. Reproductive Biology, 15(1), 1-12.

HERRERA, M. F.; OTERMIN, M.; HERRERA, J. M.; SIMOY, M. V.; BIANCHI, C. P.; AGUILAR, J. J.; FUMUSO, E. A. Effect of Mycobacterium cell wall fraction on endometrial histomorphometry of mares resistant and susceptible to persistent breeding-induced endometritis. Theriogenology, v. 156, p. 2-10, 2020. Disponível em: https://doi.org/10.1016/j.theriogenology.2020.06.034.

HOFFMANN, B., SURMANN-SCHMITT, C., WIMMER, R., ROBITZKI, A., BAUS-LONCAR, M., JOHNSON, M., MÜLLER, R., & VON DER MARK, K. (2009). Genomic and functional analysis of the mammalian heart: recent insights and clinical implications. Journal of Molecular Medicine, 87(11), 1045-1057.

HOLYOAK, G. R.; LYMAN, C. C.; WIENEKE, X. D.; DESILVA, U. The equine endometrial microbiome. A Brief Review. Am J Biomed Sci & Res, v. 11, n. 6, 2021.

HOLYOAK, G. R.; MA, C. Acupuncture treatment for equine endometritis: A review. Journal of Veterinary Internal Medicine, v. 36, n. 1, p. 12-22, 2022.

HUGENHOLTZ, P.; GOEBEL, B. M.; PACE, N. R. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. Journal of Bacteriology, v. 180, n. 18, p. 4765-4774, 1998. Disponível em: https://doi.org/10.1128/jb.180.18.4765-4774.1998.

HUGHES, J. P.; LOY, R. G. Investigations on the effect of intrauterine inoculations of Streptococcus zooepidemicus in the mare. Proc. Am. Assoc. Equine Pract., v. 15, p. 289-292, 1969.

IGHODARO, O. M.; AKINLOYE, O. A. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine, v. 54, n. 4, p. 287-293, 2018. Disponível em: https://doi.org/10.1016/j.ajme.2017.09.001.

IIJIMA, N.; THOMPSON, J. M.; IWASAKI, A. Dendritic cells and macrophages in the genitourinary tract. Mucosal Immunology, v. 1, p. 451-459, 2008. Disponível em: https://doi.org/10.1038/mi.2008.57.

KATILA, T. Evaluation of diagnostic methods in equine endometritis. Reproductive Biology, v. 16, n. 3, p. 189-196, 2016. Disponível em: https://doi.org/10.1016/j.repbio.2016.06.002.

KATILA, T.; FERREIRA-DIAS, G. Evolution of the Concepts of Endometrosis, Post Breeding Endometritis, and Susceptibility of Mares. Animals, 2022. Disponível em: https://doi.org/10.3390/ani12060779.

KELLER, A.; NEVES, A.P.; AUPPERLE, H.; STEIGER, K.; SCHOON, H. A.; KLUG, E.; GREGORY, R. M.; MATTOS, R. C. Exame histopatológico do endométrio da égua após infecções experimentais repetidas e cinco diferentes tratamentos: aspectos inflamatórios. Acta Sci. Vet., v. 32, n. 3, p. 215-223, 2004.

KENNEY, R. M. Cyclic and pathologic changes of the mare endometrium as detected by biopsy, with a note on early embryonic death. Journal of the American Veterinary Medical Association, v. 172, n. 3, p. 241-262, 1978.

KENNEY, R. M.; DOIG, P. A. Equine endometrial biopsy. Current Therapy in Theriogenology, v. 2, n. 3, p. 723-729, 1986.

KNUDSEN, L. R. V.; KARSTRUP, C. C.; PEDERSEN, H. G.; ANGEN, Ø.; AGERHOLM, J. S.; RASMUSSEN, E. L.; ... KLITGAARD, K. An investigation of the microbiota in uterine flush samples and endometrial biopsies from dairy cows during the first 7 weeks postpartum. Theriogenology, v. 86, n. 2, p. 642-650, 2015. Disponível em: https://doi.org/10.1016/j.theriogenology.2016.02.016.

KOEDOODER, R.; MACKENS, S.; BUDDING, A.; FARES, D.; BLOCKEEL, C.; LAVEN, J.; SCHOENMAKERS, S. Identification and evaluation of the microbiome in the female and male reproductive tracts. Human Reproduction Update, v. 25, n. 3, p. 298-325, 2019. Disponível em: https://doi.org/10.1093/humupd/dmy048.

KÖHNE, M.; KUHLMANN, M.; TÖNIßEN, A.; MARTINSSON, G.; SIEME, H. Diagnostic and Treatment Practices of Equine Endometritis—A Questionnaire. Frontiers in Veterinary Science, v. 7, p. 547, 2020. Disponível em: https://doi.org/10.3389/fvets.2020.00547.

KOO, H.; ALLAN, R. N.; HOWLIN, R. P.; STOODLEY, P.; HALL-STOODLEY, L. Targeting microbial biofilms: Current and prospective therapeutic strategies. Nature Reviews Microbiology, 2017. Disponível em: https://doi.org/10.1038/nrmicro.2017.99.

KOZDROWSKI, R.; SIKORA, M.; BUCZKOWSKA, J.; NOWAK, M.; RAŚ, A.; DZIECIOL, M. Effects of cycle stage and sampling procedure on interpretation of endometrial cytology in mares. Animal Reproduction Science, v. 154, p. 56-62, 2015. Disponível em: https://doi.org/10.1016/j.anireprosci.2015.01.009.

LAWSON, E. F et al. A Proteomic analysis of pregnant mare uterine fluid. Journal of Equine Veterinary Science, v. 66, n. 1, p. 171-172, 2018. Disponível em: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed19&NEWS=N&AN=623724958.

LEBLANC, M. M. Advances in the Diagnosis and Treatment of Chronic Infectious and Post-Mating-Induced Endometritis in the Mare. Reprod Dom Anim, v. 45, p. 21-27, 2010.

LEBLANC, M. M. Advances in the diagnosis and treatment of chronic infectious and post-mating-induced endometritis in the mare. Reproduction in Domestic Animals, v. 45, n. 2, p. 21-27, 2010. Disponível em: https://doi.org/10.1111/j.1439-0531.2010.01634.x.

LEBLANC, M. M. The current status of antibiotic use in equine reproduction. Equine Vet. Educ., v. 21, p. 156-167, 2009.

LEBLANC, M. M. When to refer an infertile mare to a theriogenologist. Theriogenology, v. 70, p. 421-429, 2008.

LEBLANC, M. M.; JOHNSON, R. D.; CALDERWOOD MAYS, M. B.; VALDERRAMA, C. Lymphatic Clearance of India Ink in Reproductively Normal Mares and Mares Susceptible to Endometritis. Biology of Reproduction, v. 52, n. 1, p. 501-506, 1995. Disponível em: https://doi.org/10.1093/biolreprod/52.monograph_series1.501.

LEBLANC, M. M.; MAGSIG, J.; STROMBERG, A. J. Use of a low-volume uterine flush for diagnosing endometritis in chronically infertile mares. Theriogenology, v. 68, p. 403-412, 2007.

LEBLANC, M. M.; MCKINNON, A. O. Breeding the problem mare. In: MCKINNON, A. O.; SQUIRES, E. L.; VAALA, W. E.; VARNER, D. V. (Eds.). Equine Reproduction, Second Edition. Reino Unido: Wiley-Blackwell, 2011.

LEBLANC, M.; CAUSEY, R. Clinical and subclinical endometritis in the mare: Both threats to fertility. Reproduction in Domestic Animals, v. 44, n. 3, p. 10-22, 2009. Disponível em: https://doi.org/10.1111/j.1439-0531.2009.01485.x.

LEVAY, P. F., & VILJOEN, M. (1995). Lactoferrin: a general review. Haematologica, 80, 252-267.

LEY, W. B.; BOWEN, J. M.; SPONENBERG, D. P.; LESSARD, P. N. Dimethyl sulfoxide intrauterine therapy in the mare: Effects upon endometrial histological features and biopsy classification. Theriogenology, v. 32, n. 2, p. 263-276, 1989. Disponível em: https://doi.org/10.1016/0093-691X(89)90317-8.

Li, J. et al. Comparative proteome analysis of serum uncovers differential expression of proteins in donkeys (Equus asinus) with endometritis caused by Escherichia coli. Journal of Equine Veterinary Science, v. 122, p. 104221, 2023. Disponível em: https://doi.org/10.1016/j.jevs.2023.104221.

LINTON, J. K.; SERTICH, P. L. The impact of low-volume uterine lavage on endometrial biopsy classification. Theriogenology, v. 1-4, 2016.

LIU, I. K. M.; TROEDSSON, M. H. T. The diagnosis and treatment of endometritis in the mare: Yesterday and today. Theriogenology, v. 70, n. 3, p. 415-420, 2008. Disponível em: https://doi.org/10.1016/j.theriogenology.2008.05.040.

LONCAR, K. D.; FERRIS, R. A.; MCCUE, P. M.; BORLEE, G. I.; HENNET, M. L.; BORLEE, B. R. In Vitro Biofilm Disruption and Bacterial Killing Using Nonantibiotic Compounds Against Gram-Negative Equine Uterine Pathogens. Journal of Equine Veterinary Science, v. 53, p. 94-99, 2017. Disponível em: https://doi.org/10.1016/j.jevs.2017.02.003.

MALONEY, S. E.; KHAN, F. A.; CHENIER, T. S.; DIEL DE AMORIM, M.; ANTHONY HAYES, M.; SCHOLTZ, E. L. A comparison of the uterine proteome of mares in oestrus and dioestrus. Reproduction in Domestic Animals, v. 54, n. 3, p. 473-479, 2019. Disponível em: https://doi.org/10.1111/rda.13375.

MAMBELLI, L. I. et al. Changes in expression pattern of selected endometrial proteins following mesenchymal stem cells infusion in mares with endometrosis. PLoS ONE, v. 9, n. 6, 2014. Disponível em: https://doi.org/10.1371/journal.pone.0097889.

MARCHESI, J. R. Metagenomics: Current Innovations and Future Trends. Future Microbiology, v. 7, n. 7, p. 813-814, 2012. Disponível em: https://doi.org/10.2217/fmb.12.41.

MARCHESI, J. R.; RAVEL, J. The vocabulary of microbiome research: a proposal. Microbiome, v. 3, n. 1, 2015. Disponível em: https://doi.org/10.1186/s40168-015-0094-5.

MARTH, C. D.; MACOLINO, G. P.; KREKELER, N. The Value of Innate Immune Genes as Diagnostic Markers for Endometritis in Mares. Journal of Equine Veterinary Science, v. 66, p. 112, 2018. Disponível em: https://doi.org/10.1016/j.jevs.2018.05.157.

MARTIN, F., MALERGUE, F., PITARI, G., PHILIPPE, J. M., PHILIPS, S., CHABRET, C., GRANJEAUD, S., MATTEI, M. G., MUNGALL, A. J., NAQUET, P., & GALLAND, F. (2001). Vanin genes are clustered (human 6q22-24 and mouse 10A2B1) and encode isoforms of pantetheinase ectoenzymes. Immunogenetics, 53, 296-306.

MCCUE, P. M. The Problem Mare: Management Philosophy, Diagnostic Procedures, and Therapeutic Options. Journal of Equine Veterinary Science, v. 28, n. 11, p. 619-626, 2008. Disponível em: https://doi.org/10.1016/j.jevs.2008.10.009.

MITCHELL, A.; DE AMORIM, M. D.; THACHIL, A.; ALTIER, C.; CHEONG, S. Uterine Bacterial Isolates from Mares and Their Resistance to Antimicrobials. J. Equine Vet. Sci., v. 66, p. 114, 2018.

MORELLI, M. A. C. et al. Relationships between seminal plasma metabolites, semen characteristics and sperm kinetics in donkey (Equus asinus). Animals, v. 11, n. 1, p. 1-19, 2021. Disponível em: https://doi.org/10.3390/ani11010201.

MORLEY, S. A.; MURRAY, J. A. Effects of Body Condition Score on the Reproductive Physiology of the Broodmare: A Review. Journal of equine veterinary science, v. 34, n. 7, p. 842-853, 2014.

MORRELL, J. M.; ROCHA, A. A Novel Approach to Minimising Acute Equine Endometritis That May Help to Prevent the Development of the Chronic State. Frontiers in Veterinary Science, v. 8, 2022. Disponível em: https://doi.org/10.3389/fvets.2021.799619.

MORRIS, L. H. A.; MCCUE, P. M.; AURICH, C. Equine endometritis: A review of challenges and new approaches. Reproduction, 2020. Disponível em: https://doi.org/10.1530/REP-19-0478.

MOURA, A. A.; MEMILI, E. Functional aspects of seminal plasma and sperm proteins and their potential as molecular markers of fertility. Animal Reproduction, v. 13, n. 3, p. 191-199, 2016. Disponível em: https://doi.org/10.21451/1984-3143-AR884.

NIELSEN, J. M. Endometritis in the mare: A diagnostic study comparing cultures from swab and biopsy. Theriogenology, v. 64, p. 510-518, 2005. Disponível em: https://doi.org/10.1016/j.theriogenology.2005.05.034.

NIELSEN, J. M.; NIELSEN, F. H.; PETERSEN, M. R. Diagnosis of equine endometritis microbiology, cytology and histology of endometrial biopsies and the correlation to fertility. Pferdeheilkunde, v. 1, n. 14, 2012.

OVERBECK, W. et al. Comparison of cytological and histological examinations in different locations of the equine uterus – an in vitro study. Theriogenology, v. 79, p. 1262-1268, 2013.

OVERBECK, W.; WITTE, T. S.; HEUWIESER, W. Comparison of three diagnostic methods to identify subclinical endometritis in mares. Theriogenology, v. 75, n. 7, p. 1311-1318, 2011. Disponível em: https://doi.org/10.1016/j.theriogenology.2010.12.002.

PALM, F. et al. Influence of different semen extenders and seminal plasma on PMN migration and on expression of IL-1β, IL-6, TNF-α and COX-2 mRNA in the equine endometrium. Theriogenology, v. 70, n. 5, p. 843-851, 2008. Disponível em: https://doi.org/10.1016/j.theriogenology.2008.04.054.

PALMER, E.; CHAVATTE-PALMER, P. Contribution of Reproduction Management and Technologies to Genetic Progress in Horse Breeding. Journal of Equine Veterinary Science, v. 89, 2020. Disponível em: https://doi.org/10.1016/j.jevs.2020.103016.

PARLEVLIET, J. M.; LYNN, J. W.; PACCAMONTI, D. L. The Use of Cefquinome in Equine Semen Extender. Journal of Equine Veterinary Science, v. 31, n. 3, p. 139-142, 2011. Disponível em: https://doi.org/10.1016/j.jevs.2010.12.015.

PASCOE, R. R. Observations on the length and angle of declination of the vulva and its relation to fertility in the mare. Journal of Reproduction and Fertility, Suppl., n. 27, p. 299-305, 1979.

PENNINGTON, M. P., SPLAN, R. K., JACOBS, R. D., WANG, Y., WAGNER, A. L., FREEMAN, E. W., & PUKAZHENTHI, B. S. (2021). Influence of Reproductive Status on Equine Serum Proteome: Preliminary Results. Journal of Equine Veterinary Science, 105, 103724. Disponível em: https://doi.org/10.1016/j.jevs.2021.103724.

PETERSEN, M. R. et al. High Prevalence of Subclinical Endometritis in Problem Mares – Effect of Activation and Treatment on Fertility. Journal of Equine Veterinary Science, v. 66, p. 117, 2018. Disponível em: https://doi.org/10.1016/j.jevs.2018.05.162.

PYCOCK, J. F.; ALLEN, W. E. Inflammatory components in uterine fluid from mares with experimentally induced bacterial endometritis. Equine Veterinary Journal, v. 22, n. 6, p. 422-425, 1990. Disponível em: https://doi.org/10.1111/j.2042-3306.1990.tb04309.x.

PYÖRÄLÄ, S.; TAPONEN, J.; KATILA, T. Use of Antimicrobials in the Treatment of Reproductive Diseases in Cattle and Horses. Reprod. Domest. Anim., v. 49, p. 16-26, 2014.

QUAYLE, A. J. The innate and early immune response to pathogen challenge in the female genital tract and the pivotal role of epithelial cells. Journal of Reproductive Immunology, v. 57, p. 61-79, 2002. Disponível em: https://doi.org/10.1016/S0165-0378(02)00019-0.

RALIOU, M. et al. Subclinical endometritis in dairy cattle is associated with distinct mRNA expression patterns in blood and endometrium. PLoS ONE, v. 14, n. 8, 2019. Disponível em: https://doi.org/10.1371/journal.pone.0220244.

RATAJCZAK, C. K., BOGAN, J. A., LIN, Y.-C., & OLSON, D. M. (2010). Uterine vein drug concentrations after intrauterine administration in mares: influence of inflammation and preliminary testing of an anti-inflammation therapy. Theriogenology, 74(3), 394-402.

REILAS, T.; KATILA, T. Proteins and enzymes in uterine lavage fluid of postpartum and nonparturient mares. Reprod Dom Anim, v. 37, p. 261-268, 2002.

RENNEMEIER, C. et al. Seminal plasma protects human spermatozoa and pathogenic yeasts from capture by dendritic cells. Human Reproduction, v. 26, n. 5, p. 987-999, 2011. Disponível em: https://doi.org/10.1093/humrep/der038.

RICKETTS, S. W. The treatment of equine endometritis in studfarm practice. Pferdeheilkunde, v. 15, n. 6, p. 588-593, 1999. Disponível em: https://doi.org/10.21836/pem19990619.

RICKETTS, S. W. Uterine and clitoral cultures. In: MCKINNON, A. O.; SQUIRES, E. L.; VAALA, W. E.; VARNER, D. V. (Eds.). Equine Reproduction, Second Edition. U.K: Wiley-Blackwell, 2011.

RICKETTS, S. W.; MACKINTOSH, M. E. Role of anaerobic bacteria in equine endometritis. J Reprod Fertil, v. 35, p. 343-351, 1987.

RIDDLE, W. T.; LEBLANC, M. M.; STROMBERG, A. J. Relationships between uterine culture, cytology and pregnancy rates in a thoroughbred practice. Theriogenology, v. 68, p. 395-402, 2007.

RINK, B. E. et al. The Fate of Endometrial Mesenchymal Stem Cells After Application in the Healthy Equine Uterus. Journal of Equine Veterinary Science, v. 66, p. 108, 2018. Disponível em: https://doi.org/10.1016/j.jevs.2018.05.154.

RISCO, A. M. et al. Effect of oxytocin and flunixin meglumine on uterine response to insemination in mares. Theriogenology, v. 72, n. 9, p. 1195-1201, 2009. Disponível em: https://doi.org/10.1016/j.theriogenology.2009.07.012.

ROBERTSON, S. A. Seminal plasma and male factor signalling in the female reproductive tract. Cell and Tissue Research, v. 322, p. 43-52, 2005. Disponível em: https://doi.org/10.1007/s00441-005-1127-3.

ROGAN, D. et al. Use of a mycobacterial cell wall extract (MCWE) in susceptible mares to clear experimentally induced endometritis with Streptococcus zooepidemicus. J Equine Vet Sci, v. 27, p. 112, 2007. Disponível em: https://doi.org/10.1016/j.jevs.2007.01.010.

SÁNCHEZ-RODRÍGUEZ, A. et al. Evaluation of the Effect of Seminal Plasma in the Post Insemination Uterine Inflammatory Dynamic by Power Doppler Ultrasonography and Low

Volume Lavage in the Mare. Journal of Equine Veterinary Science, v. 66, p. 134, 2018. Disponível em: https://doi.org/10.1016/j.jevs.2018.05.174.

SANTOS, T. M. A.; GILBERT, R.; BICALHO, R. C. Metagenomic analysis of the uterine bacterial microbiota in healthy and metritic postpartum dairy cows. Journal of Dairy Science, v. 94, n. 1, p. 291-302, 2011. Disponível em: https://doi.org/10.3168/jds.2010-3668.

SCHÄR, M. O. et al. Platelet-rich Concentrates Differentially Release Growth Factors and Induce Cell Migration In Vitro. Clinical Orthopaedics and Related Research, v. 473, n. 5, p. 1635-1643, 2015. Disponível em: https://doi.org/10.1007/s11999-015-4192-2.

SCHLAFER, D. H. Equine endometrial biopsy: enhancement of clinical value by more extensive histopathology and application of new diagnostic techniques? Theriogenology, v. 68, p. 413-422, 2007.

SCHOON, H. A. et al. Die endometrium biopsie bei der stute in klinisch-gynakologishen kontext. Pferdeheilkunde, v. 5, p. 453-464, 1997.

SCHOON, H. A. et al. Uterusbiopsienals Hilfsmittelfür Diagnose und Prognose von Fertilitätsstörungen der Stute. Pferdeheilkunde, v. 8, p. 355-362, 1992.

SCHUBERTH, H. J. et al. Immunological responses to semen in the female genital tract. Theriogenology, v. 70, n. 8, p. 1174-1181, 2008. Disponível em: https://doi.org/10.1016/j.theriogenology.2008.07.020.

SCOGGIN, C. F. Endometritis: Nontraditional Therapies. Veterinary Clinics of North America - Equine Practice, 2016. Disponível em: https://doi.org/10.1016/j.cveq.2016.08.002.

SEGABINAZZI, L. G. T. M. et al. Intrauterine blood plasma platelet-therapy mitigates persistent breeding-induced endometritis, reduces uterine infections, and improves embryo recovery in Mares. Antibiotics, v. 10, n. 5, 2021. Disponível em: https://doi.org/10.3390/antibiotics10050490.

SENDER, R.; FUCHS, S.; MILO, R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biology, v. 14, n. 8, 2016. Disponível em: https://doi.org/10.1371/journal.pbio.1002533.

SHIGETA, H., NEWBOLD, R. R., MCLACHLAN, J. A., & TENG, C. T. (1996). Estrogenic effect on the expression of estrogen receptor, COUP-TF, and lactoferrin mRNA in developing mouse tissues. Molecular Reproduction and Development, 45, 21-30.

SILVA, M. A. C.; ALVARENGA, M. A. Fungal endometritis. In: MCKINNON, A. O. et al. Equine Reproduction, Second Edition. U.K: Wiley-Blackwell, 2011.

SKARZYNSKI, D. J. et al. Neutrophils, monocytes and other immune components in the equine endometrium: Friends or foes? Theriogenology, v. 150, p. 150-157, 2020. Disponível em: https://doi.org/10.1016/j.theriogenology.2020.01.018.

SMITS, K. et al. Proteins involved in embryo-maternal interaction around the signalling of maternal recognition of pregnancy in the horse. Scientific Reports, v. 8, n. 1, 2018. Disponível em: https://doi.org/10.1038/s41598-018-23537-6.

SOJKA, E. J. et al. Dimethyl sulfoxide update - New applications and dosing methods. Proceed. Am. Assoc. Equine Practit., v. 36, p. 683-690, 1990.

STECKLER, D. et al. Ex vivo influence of carbetocin on equine myometrial muscles and comparison with oxytocin. Theriogenology, v. 78, n. 3, p. 502-509, 2012. Disponível em: https://doi.org/10.1016/j.theriogenology.2012.02.030.

STEWART, F., GERSTENBERG, C., SUIRE, S., & ALLEN, W. R. (2000). Immunolocalization of a novel protein (P19) in the endometrium of fertile and subfertile mares. Journal of Reproduction and Fertility. Supplement, 56, 593-599.

ZHONG, S. et al. Role of oxidant-antioxidant balance in reproduction of domestic animals. Animal Production Science, v. 57, p. 1588-1597, 2017. Disponível em: https://doi.org/10.1071/AN15619.

TEIXEIRA-SOARES, C. M. et al. Comparison Between Gynecological Examination Methods and Sample Collection Techniques for the Diagnosis of Endometritis in Subfertile Mares. Journal of Equine Veterinary Science, v. 119, 2022. Disponível em: https://doi.org/10.1016/j.jevs.2022.104147.

THURLOW, L. R. et al. Staphylococcus aureus Biofilms Prevent Macrophage Phagocytosis and Attenuate Inflammation In Vivo. The Journal of Immunology, v. 186, n. 11, p. 6585-6596, 2011. Disponível em: https://doi.org/10.4049/jimmunol.1002794.

TITHOF, P. K., ROBERTS, M. P., GUAN, W., ELGAYYAR, M., & GODKIN, J. D. (2007). Distinct phospholipase A2 enzymes regulate prostaglandin E2 and F2alpha production by bovine endometrial epithelial cells. Reproductive Biology and Endocrinology, 5, 16.

TROEDSSON, M. H. T. Breeding-Induced Endometritis in Mares. Veterinary Clinics of North America - Equine Practice, p. 705-712, 2006. Disponível em: https://doi.org/10.1016/j.cveq.2006.07.003.

TROEDSSON, M. H. T. ENDOMETRITIS. In: MCKINNON, A. O. et al. Equine Reproduction, Second Edition. U.K: Wiley-Blackwell, 2011.

TROEDSSON, M. H. T. et al. Components in seminal plasma regulating sperm transport and elimination. Animal Reproduction Science, v. 89, n. 1-4, p. 171-186, 2005. Disponível em: https://doi.org/10.1016/j.anireprosci.2005.07.005.

TROEDSSON, M. H. T. et al. Immunoglobulin (IgG and IgA) and complement (C3) concentrations in uterine secretion following an intrauterine challenge of Streptococcus zooepidemicus in mares susceptible to versus resistant to chronic uterine infection. Biology of Reproduction, v. 49, n. 3, p. 502-506, 1993. Disponível em: https://doi.org/10.1095/biolreprod49.3.502.

TROEDSSON, M. H. T. et al. Interaction between equine semen and the endometrium: The inflammatory response to semen. Animal Reproduction Science, v. 68, n. 3-4, p. 273-278, 2001. Disponível em: https://doi.org/10.1016/S0378-4320(01)00164-6.

TUPPITS, U. et al. Influence of the uterine inflammatory response after insemination with frozen-thawed semen on serum concentrations of acute phase proteins in mares. Animal Reproduction Science, v. 146, n. 3-4, p. 182-186, 2014. Disponível em: https://doi.org/10.1016/j.anireprosci.2014.02.007.

VALACCHI, G. et al. OxInflammation: From subclinical condition to pathological biomarker. Frontiers in Physiology, v. 9, p. 858, 2018. Disponível em: https://doi.org/10.3389/fphys.2018.00858.

VICK, M. M. et al. Relationships among inflammatory cytokines, obesity, and insulin sensitivity in the horse. J Anim Sci, v. 85, n. 5, p. 1144-1155, 2007.

WALTER, J. et al. Cytological diagnosis of endometritis in the mare: investigations of sampling techniques and relation to bacteriological results. Anim. Reprod. Sci., v. 132, p. 178-186, 2012.

WARD, P. P., URIBE-LUNA, S., & CONNEELY, O. M. (2002). Lactoferrin and host defense. Biochemical and Cellular Biology, 80, 95-102.

WINTERBOURN, C. C. et al. Reactive Oxygen Species and Neutrophil Function. Annual Review of Biochemistry, v. 85, p. 765-792, 2016. Disponível em: https://doi.org/10.1146/annurev-biochem-060815-014442.

WITTE, T. S. et al. Incompetent cervix and pneumouterus in a maiden mare. Equine Vet. Educ., v. 24, p. 343-346, 2012.

WOLF, C. A. et al. Effect of corticotherapy on proteomics of endometrial fluid from mares susceptible to persistent postbreeding endometritis. Theriogenology, v. 77, n. 7, p. 1351-1359, 2012. Disponível em: https://doi.org/10.1016/j.theriogenology.2011.10.042.

WOLFSDORF, K. & CAUDLE, A. B. Inflammation of the Tubular Reproductive Tract of the Mare. In: YOUNGQUIST, R. S.; THRELFALL, W. R. (Eds.). Current Therapy in Large Animal Theriogenology. 2nd ed. W.B. Saunders, 2007. p. 158-167. Disponível em: https://doi.org/10.1016/B978-072169323-1.50023-4.

WOODWARD, E. et al. Endometrial Cytokine Expression in Mares with Different Resistance to Persistent Breeding Induced Endometritis (PBIE) at Multiple Time Points after Insemination. Biology of Reproduction, v. 85, n. 1, p. 77, 2011. Disponível em: https://doi.org/10.1093/biolreprod/85.s1.77.

WOODWARD, E. M. et al. Susceptibility to persistent breeding-induced endometritis in the mare: Relationship to endometrial biopsy score and age, and variations between seasons. Theriogenology, v. 78, n. 3, p. 495-501, 2012. Disponível em: https://doi.org/10.1016/j.theriogenology.2012.02.028.

WOODWARD, E. M.; TROEDSSON, M. H. Equine Breeding-Induced Endometritis: A Review. Journal of Equine Veterinary Science, 2013. Disponível em: https://doi.org/10.1016/j.jevs.2012.11.010.

WOODWARD, E. M.; TROEDSSON, M. H. T. Inflammatory mechanisms of endometritis. Equine Veterinary Journal, 2015. Disponível em: https://doi.org/10.1111/evj.12403.

XIA, Y. W. et al. Metagenomic analysis of the equine placental microbiome. Clinical Theriogenology, v. 9, n. 3.

ZENT, W. W. et al. Postbreeding uterine fluid accumulation in a normal population of thoroughbred mares: a field study. Proc Ann Conv Am Assoc Equine Pract, 1998. p. 64-65.

ZENT, W. W. History. In: MCKINNON, A. O. et al. (Eds.). Equine Reproduction. 2nd ed. U.K: Wiley-Blackwell, 2011.

ZENT, W. W.; STEINER, J. V. Vaginal Examination. In: MCKINNON, A. O. et al. (Eds.). Equine Reproduction. 2nd ed. U.K: Wiley-Blackwell, 2011.

ZHONG, R. Z.; ZHOU, D. W. Oxidative stress and role of natural plant derived antioxidants in animal reproduction. Journal of Integrative Agriculture, v. 12, p. 1826-1838, 2013. Disponível em: https://doi.org/10.1016/S2095-3119(13)60412-8.

ZIVOTKOV H. I. Racianalizacja I perestrojka slucki iiskustvenogo osemenenija kobil na novih proizvodstvenik inaucnih obosnovanijah. Moscow: Seljhozgiz, 1938.

CAPÍTULO 2

ENDOMETRITIS PREVALENCE AND MAIN GYNECOLOGICAL FINDINGS IN SUBFERTILE EMBRYO RECIPIENT MARES

Carlos Mattos Teixeira-Soares[1*], Arabela Guedes de Azevedo Viana[1], Gilberto Guimarães Lourenço[2], Rafael Guedes Goretti[2], Yamê Fabres Robaina Sancler-Silva[3] and Mariana Machado-Neves[1,4]

[1]Post graduation program in Veterinary Medicine, Departamento of Veterinary Medicine, Universidade Federal de Viçosa, Viçosa, Brasil; [2]Autonomous veterinarian in equine reproduction, Muriaé, Brasil; [3]Departament of Zootecnia, Universidade Federal de Viçosa, Viçosa, Brasil; [4]Departament of Biology, Universidade Federal de Viçosa, Viçosa, Brasil.

*Correspondent author: Carlos Mattos Teixeira Soares

Av. Peter Henry Rolfs s/n, Campus Universitário, CEP: 36570-900. Viçosa, Minas Gerais, Brasil. E-mail: carlos.mattos@ufv.br

ABSTRACT

Background: Fertile embryo recipients are essential for equine embryo biotechnologies. Despite that, a complete gynecological examination to identify cases of subfertility is rarely performed, contributing to an inaccurate diagnosis of endometritis.

Objectives: Identifying the prevalence of endometritis and the major alterations in the gynecological examination of recipient mares removed from commercial embryo transfer programs.

Study design: Characterization of gynecological examination findings in embryo recipients with a history of subfertility.

Methods: 146 recipient mares from 15 commercial farms were evaluated from: (1) reproductive history; (2) clinical examination: evaluation of the perineum conformation, transrectal palpation and ultrasound, vaginoscopy and cervix exam; and (3) laboratory tests: cytological, microbiological and histological evaluation of the uterus, in which the last one was the "gold standard" for diagnosis.

Results: the main clinical suspicion of endometritis in the reproductive history was intrauterine fluid during the ultrasound, in addition to the fact that most mares had been submitted to antibiotic treatments for endometritis. In the clinical examinations, 59% of mares were positive in at least one of the evaluations performed, in which transrectal palpation and ultrasonography showed more positive animals for endometritis, followed by cervical changes, inadequate perineal conformations and evidence of trauma during vaginoscopy evaluation. In laboratory evaluations, 66% were positive in at least one of the methods used. Uterine microbiological culture showed 59% positive mares and the most isolated was *Escherichia coli*. Uterine cytology identifies 47% of mares with polymorphonuclear cells indicative of inflammation. The histopathological evaluation of the endometrium identified 60% of prevalence of mares diagnosed with endometritis.

Main limitations: Not having identified fungi in the microbiological culture, perhaps due to not having used a specific growth media.

Conclusions: Endometritis was the main reason for excluding recipient mares from embryo transfer programs and a complete gynecological exam is essential for the diagnosis aiming at greater reproductive biotechnologies success.

Keywords: reproductive exam, problem mare, endometrial cytology, endometrial histology.

1. INTRODUCTION

The embryo transfer and its associated technologies, such as production of embryos *in vitro* mainly using intracytoplasmic sperm injection or more recently by *in vitro* fertilization, have stood out for its scientific and commercial advances.[1,2] However, for the success of embryo biotechnologies, it is essential to use fertile recipient mares that can achieve embryonic recognition and maintenance of pregnancy.[3] The selection of recipient mares must be done carefully and it includes the evaluation of reproductive organs through gynecological exams by applying several clinical and laboratory methods, as well as the evaluation of female reproductive history.[4,5] However, the gynecological examination in equine reproduction is not performed properly very often due to lack of training, time, resources, or awareness among professionals. This can lead to inaccurate diagnoses and, consequently, a high number of recipient mares with successive embryonic losses and the inability to sustain pregnancy.[6]

Overall, problematic mares in embryo transfer programs are animals with a reproductive disorder, such as endometritis, which has been pointed out as the main cause of subfertility in mares.[7,8] The persistent inflammation of the endometrium represents a major challenge for equine reproduction as it makes the uterine environment hostile for embryo survival.[9] Consequently, managing affected animals needs intensive clinical intervention, leading to additional costs and substantial economic losses.[4] Accurate diagnosis, including identification of the cause of endometrial inflammation, is the first and main step in initiating treatment and obtaining an effective therapeutic response.[10] Hence, the early and efficient identification of endometritis in recipient mares allows their removal from the embryo transfer program, resulting in a reduction in embryonic losses and improving the effectiveness of reproductive biotechnologies. Therefore, the objective of this study was to investigate whether endometritis is the primary reason for the removal of these mares from the embryo transfer program. Additionally, we aimed to ascertain the major alterations diagnosed during the gynecological examination of embryo recipient mares with a history of subfertility.

78

79 **2. MATERIAL AND METHODS**

80 *2.1. Animals*

81 All procedures performed in this study were in accordance with the National Regulatory
82 Committee of Experimental Animal (CONCEA) and were approved by the Animal Use Ethics
83 Committee of the Federal University of Viçosa, Brazil (CEUA, protocol #14/2020). The study
84 was carried out at 15 breeding farms in Minas Gerais, Brazil, during three breeding seasons
85 (from 2020 to 2022). A total of 146 crossbred recipient mares (4-20 years old) underwent a
86 gynecological examination. During this experiment, all animals were healthy and in good body
87 condition. These mares were included in this study because they were removed from the embryo
88 transfer program due to failures to confirm successful pregnancies during the breeding season.
89 The initial suspected diagnosis of endometritis was only based on intrauterine fluid detected in
90 the transrectal ultrasound examination carried out by the responsible farm veterinarian.
91 However, none of the females were submitted to a complete gynecological examination with
92 the use of laboratory tests for accurate diagnosis.

93

94 *2.2. Survey of the reproductive history of the mares*

95 The reproductive history of the animals, including age and the number of successful
96 gestations on the farm, were collected from reproductive and health records. Also, data from
97 clinical records were considered, such as previous antibiotic treatments for endometritis,
98 number of early embryonic losses, presence of intrauterine fluid, and excessive endometrial
99 edema detected by transrectal ultrasound examination.

100

101 *2.3. Clinical reproductive exams*

102 The clinical reproductive assessment of animals encompassed procedures palpation and
103 transrectal ultrasound, assessment of perineal conformation, vaginoscopy, and evaluation of

104 cervical integrity. This examination aimed to identify indicative signs of compromised defense
105 mechanisms and, consequently, uterine inflammation. Based on the examination findings, the
106 animals were categorized as either susceptible or not susceptible to endometritis.[11] To conduct
107 these examinations, mares were individually secured in stocks, with their tails suitably
108 bandaged. The internal reproductive organs were assessed through palpation and transrectal
109 ultrasound (utilizing the Sonoscape A5Vet, Shenzhen, China). These examinations were carried
110 out by a skilled veterinarian during the estrus phase of the reproductive cycle, specifically when
111 mares exhibited at least one dominant follicle measuring 35 mm or more and lacked a corpus
112 luteum. Selecting the estrus phase facilitated the collection of uterine samples and ensured
113 homogeneity in the results. The endometrial uterine edema was graded on scores from 1 to 5
114 according to its intensity.[12] In addition, intrauterine fluid was detected and measured, if present.
115 Mares with risk factors for endometritis, such as, excessive endometrial edema (score 5) and/or
116 intrauterine fluid greater than 2 cm were classified as susceptible to endometritis and positive
117 in this exam.[13]

118 Perineal conformation assessment involved gentle washing of the perineum with soap,
119 followed by drying using paper towels. Visual analysis, as well as measurements using an
120 adapted vulvometer tool[14], were employed to evaluate perineal conformation. Mares were
121 classified as positive in this examination when physical barriers of protection were found to be
122 compromised. Conformational abnormalities, such as inadequate vulvar closure and a Caslick
123 Index exceeding 150°, were considered risk factors for endometritis.[15,16] For vaginoscopy, a
124 Polansky vaginal speculum and a flashlight were utilized to facilitate access to the internal
125 vagina and the caudal cervix ostium. Mares were categorized as positive in this examination
126 when they exhibited conformational defects suggestive of susceptibility to endometritis.
127 Indications of risk included the presence of a tortuous cervical opening, secretion during the
128 evaluation, pale or hyperemic mucosa, and abnormalities such as trauma and lacerations.[17]
129 Lastly, assessment of cervix integrity involved the insertion of a finger with a sterile palpation

130 glove into the cervix, followed by a digital examination. Mares were considered positive in this
131 examination if they displayed issues related to cervix effectiveness, which could be indicative
132 of a breakdown in natural defenses and susceptibility to endometritis. Risk factors encompassed
133 lacerations, fibrosis, adhesions, diverticulum, and/or tortuosity.[18]
134
135 *2.4. Sample collection and laboratory exams*
136 Laboratory tests encompassed uterine microbiological culture, endometrial cytology
137 and uterine biopsy for histological evaluation. Uterine samples were safely collected
138 transcervically by experienced veterinarians to prevent contamination. For microbiological
139 evaluation, a double protection swab collector (Provar Comercial Ltda, São Paulo, Brazil) was
140 carefully inserted through the cervix and into the uterus. After being in contact with the uterine
141 wall for at least 1 minute alternating gentle rotational movements, the swab was removed
142 avoiding contamination. These swab samples were cultured on plates with 5% sheep blood agar
143 and incubated at 37°C for 48 hours. Any microorganisms present were identified by a
144 specialized commercial laboratory (Ladvet, Viçosa, Brazil). Mares with the growth of at least
145 one microorganism on the culture plate were classified as positive for uterine contamination
146 and, consequently, infectious endometritis.[19]
147 Endometrial cytology was conducted using a double-guarded cytobrush collector
148 (Provar Commercial Ltda, São Paulo, Brazil) following a similar procedure to the swab for
149 uterine culture. Following sample collection, the cytology brushes were gently rotated on
150 microscopic slides to create smears. Subsequently, the smears were air-dried, stained using the
151 Diff-Quick Staining Kit (Laborclin, Pinhais, Brazil), and examined under a light microscope
152 (P207/B Coleman, São Paulo, Brazil) at a 1000x magnification, using oil immersion. This
153 facilitated the evaluation of polymorphonuclear cells in relation to endometrial cells, with a
154 total of 300 cells per sample counted. Inflammation was deemed positive when the percentage
155 of polymorphonuclear cells exceeded 2%.[7]

156 To conduct uterine histological evaluation, endometrial samples were collected
157 transcervically at the base of the right uterine horn using sterile biopsy forceps appropriate for
158 this procedure (Botupharma, Botucatu, Brazil), following the method described by Kenney in
159 1978. The biopsy samples were fixed in a 10% formalin solution and processed for histological
160 examination at the Laboratory of Veterinary Science (Universidade de Viçosa, Brazil). Routine
161 staining with hematoxylin and eosin (H.E.) was applied to these samples. Examination of the
162 samples was performed under an optical microscope (P207/B Coleman, São Paulo, Brazil) at a
163 high-power field magnification of 100x and 400x. Histological scoring was conducted based
164 on the severity, considering the quantity of inflammatory cells. The endometrial inflammation
165 was graded as either negative (grade 1) if there were no inflammatory cells in the compact
166 stratum or signs of inflammation, and positive if there were mild inflammation (grade 2A),
167 moderate inflammation (grade 2B) and severe inflammation (grade 3).[20] A comparison was
168 made between histological evaluation, considered the gold standard for the final diagnosis of
169 endometritis, and microbiological culture and uterine cytology tests based on their sensitivity,
170 specificity, and accuracy.
171
172 *2.5. Statistical Analysis*
173 The Statistical Analysis System (SAS On Demand) was used for data analysis.[21] The
174 success rates of each method were also determined by calculating sensitivity (probability of
175 positive result in diseased animals), specificity (probability of negative result in non-diseased),
176 positive predictive value (probability of disease presence when the test is positive), negative
177 predictive value (probability of disease absence when the test is negative) and accuracy
178 (probability of true positives and true negatives as a proportion of all results). The diagnostic
179 gold standard for comparisons was endometrial histological examination. The effect of
180 explanatory variables from the dataset on the probability of a positive diagnosis for endometritis

181 by endometrial histology was evaluated by univariate logistic regression, with a significance
182 level of $\alpha = 0.05$.
183

184 3. RESULTS

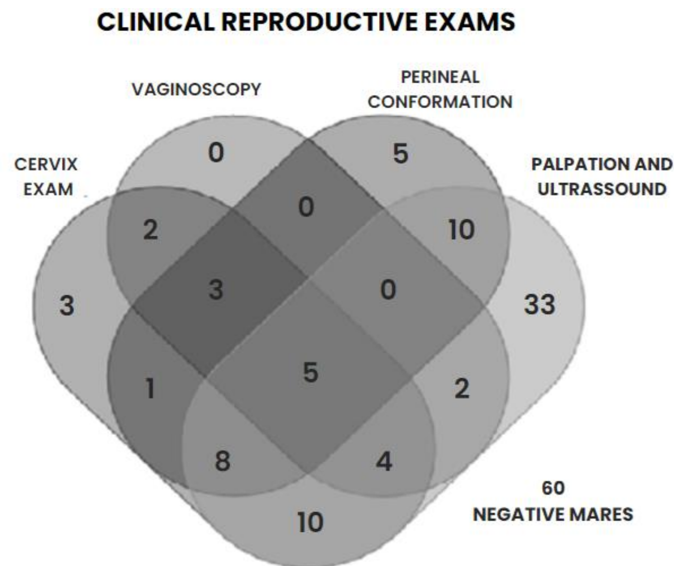
185 3.1. Reproductive history of the mares

186 The analysis of reproductive history data revealed that the average age of recipient
187 mares removed from the embryo transfer program, as assessed in this study, was 11.80 years.
188 These mares were distributed as follows: 30% were aged 10 years or less, 39% were between
189 10 and 14 years old, and 31% were 15 years or older. On average, mares had 2.62 successful
190 pregnancies before being classified as having reproductive issues. It's noteworthy that 17% of
191 these mares did not give birth on the farm.

192 Most of the mares evaluated by reproductive history presented clinical signs indicative
193 of endometritis during ultrasound examinations carried out in the reproductive routine, with
194 59% having intrauterine fluid and 16% exhibiting endometrial hyperedema. A considerable
195 proportion of the embryo recipient mares had received antibiotic treatment for endometritis
196 even if a complete gynecological examination of the animals is not carried out beforehand,
197 constituting 57% of the total mares assessed in this study for reproductive history. Additionally,
198 25% of the mares experienced early embryonic losses not maintaining the pregnancy.
199

200 3.2. Results from clinical reproductive exams

201 From 146 recipient mares evaluated by gynecological clinical examination, 86 were
202 considered positive for endometritis based on the result of at least one clinical examination.
203 However, only five mares were positive for endometritis across all clinical methods performed
204 in this study (Fig. 1).



205

206 **Figure 1.** The number of positive recipient mares diagnosed with endometritis using clinical
207 examinations out of a total of 146 animals. Clinical examinations included cervix exam,
208 vaginoscopy, perineal conformation, and transrectal palpation and ultrasonography.

209

210 The transrectal palpation and ultrasound evaluation revealed that 49% of the animals
211 tested positive during the clinical assessment for endometritis diagnosis, primarily due to the
212 identification of intrauterine fluid (Table 1). Cervical abnormalities (24%), inappropriate
213 perineal conformation (22%), and indications of trauma observed during the vaginoscopy
214 evaluation (11%) were subsequently the most common findings during the clinical examination
215 in the recipient mares (Table 1).

216

217 **Table 1.** Clinical evaluations for endometritis diagnosis of recipient mares removed from
218 embryo transfer programs (n = 146). The mares were classified as positive or negative in each
219 evaluative parameter according to the clinical findings that suggested susceptibility to
220 endometritis observed in each exam.

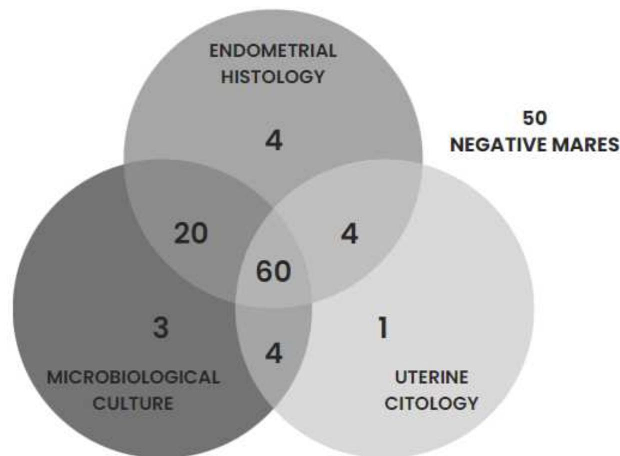
Parameters in clinical evaluations*	Diagnosis of recipient mares		
	Positive (n)	Negative (n)	Positive (%)

Palpation and ultrasound	72	74	49%
Intrauterine fluid	57	89	39%
Abnormal edema	15	131	10%
Cervix exam	36	110	24%
Tortuosity	15	131	10%
Diverticulum	9	137	6%
Adhesion	9	137	6%
Fibrosis	3	143	2%
Perineal conformation**	32	114	22%
Vaginoscopy - Evidence of trauma	16	130	11%

221 N = number of mares. *All mares were evaluated for each parameter. The same mare may be
222 classified as positive in more than one parameter. **Caslick's Index (>150°).
223

224 *3.3. Results from laboratory reproductive exams*

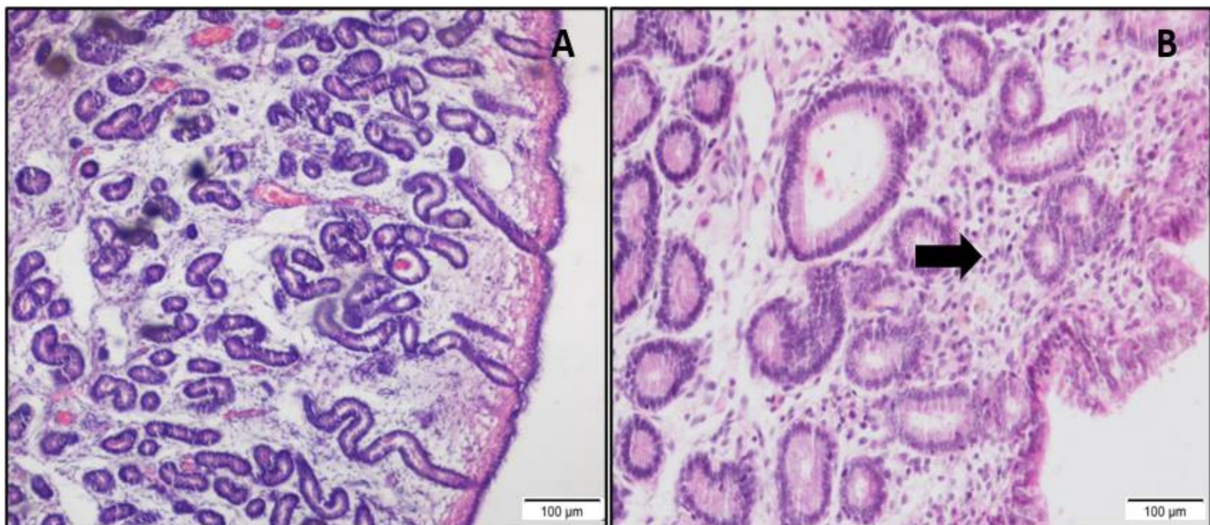
225 Regarding laboratory tests, 96 mares were considered positive for endometritis based
226 on the result of at least one examination, and 60 animals were positive in the three evaluation
227 methods used in the study (Fig. 2). The histological evaluation of the endometrium from uterine
228 biopsy detected 88 positive animals (60%) with different degrees of inflammation and
229 histopathological changes (Table 2, Fig. 3). In the uterine microbiological culture test, 59% of
230 the total number of evaluated recipient mares showed growth of, at least, one bacterium, the
231 most isolated being Escherichia coli (Table 2). Among the positive mares for microbiological
232 culture, 11% of them showed mixed growth of two different bacteria concomitantly. The uterine
233 cytology test identified 47% of recipient mares as positive for uterine inflammation (Table 2).



234

235 **Figure 2.** The number of positive recipient mares diagnosed with endometritis using laboratory
236 analyses out of a total of 146 animals. Laboratory tests included uterine microbiological culture,
237 uterine cytology and endometrial histology.

238



239

240 **Figure 3.** Histological images of the endometrium from the uterine biopsy of recipient mares
241 removed from embryo transfer programs due to subfertility. A) Endometrium without
242 histopathological alterations; B) Endometrium with mild inflammation and infiltration of
243 polymorphonuclear inflammatory cells (black arrow). Stain: H.E.

244

245 **Table 2.** Laboratory analyses for endometritis diagnosis of recipient mares removed from
246 embryo transfer programs (n = 146). The mares were classified as positive or negative in each

247 evaluative parameter according to the findings suggestive of susceptibility to endometritis
248 observed in each exam.

Parameters in laboratory evaluations*	Diagnosis of recipient mares		
	Positive (n)	Negative (n)	Positive (%)
Endometrial histology	88	58	60%
Mild inflammation - Grade 2A	50	96	34%
Moderate inflammation - Grade 2B	25	121	17%
Severe inflammation - Grade 3	13	133	9%
Uterine microbiological culture	87	59	59%
Escherichia coli	42	104	29%
Streptococcus ssp.	20	126	14%
Staphylococcus ssp.	20	126	14%
Enterobacter ssp.	11	135	7%
Citrobacter ssp.	6	140	4%
Klebsiella ssp.	2	144	1%
Mixed growth (two different bacteria)	16	130	11%
Uterine cytology	69	77	47%

249 *Each mare of the 146 was evaluated for all of the aforementioned parameters, and the same
250 mare could be positive with consistent signs of endometritis in one or more different
251 parameters.

252

253 Considering endometrial histology the gold standard for diagnosis endometritis, the
254 uterine microbiological culture had a sensibility of 90.9%, a specificity of 87.9%, and an
255 accuracy of 89.7%. The uterine cytology had a sensibility of 72.7%, specificity of 91.4%, and
256 accuracy of 80.1% (Table 4).

257 **Table 4.** Endometritis: Endometrial histology diagnosis compared with uterine microbiology
258 culture diagnosis and uterine cytology diagnosis in recipient mares removed from embryo
259 transfer programs (n = 146).

	Endometrial Histology			Sensibility	Specificity	Accuracy
	Positive	Negative	Total			
Microbial Culture*				90.9%	87.9%	89.7%
Positive	80	7	87			
Negative	8	51	59			
Total	88	58	146			
Uterine Cytology**				72.7%	91.4%	80.1%
Positive	64	5	69			
Negative	24	53	77			
Total	88	58	146			

260 *Microbial Culture: Sensibility = 80 / (80+8) = 90.9%; Specificity = 51 / (7+51) = 87.9%;
261 Accuracy = (80+51) / (80+7+8+51) = 89.7%. **Uterine Cytology: Sensibility = 64 / (64+24) =
262 72.7%; Specificity = 53 / (5+53) = 91.4%; Accuracy = (64+53) / (64+5+24+53) = 80.1%.
263

264 Univariate logistic regression analysis showed that the following explanatory variables
265 in the gynecological examination had a significant effect ($P < 0.05$) on the probability of a
266 positive diagnosis of endometritis by uterine histology: use of antibiotics as treatments for
267 endometritis, digital examination of the cervix, perineal conformation, palpation and
268 ultrasound, presence of *Escherichia coli* and presence of *Staphylococcus* ssp. The other
269 variables in the data set had no significant effect ($P > 0.05$) (Table 5).
270

271 **Table 5.** The odds ratio for each significant explanatory variable in univariate logistic
272 regression analysis from endometritis diagnosis in recipient mares removed from embryo
273 transfer programs (n = 146).

Parameter	Odds ratio
Use of antibiotics as treatments for endometritis (yes *vs* no)	3.30
Cervix exam (positive *vs* negative)	3.58
Perineal conformation (positive *vs* negative)	4.46
Palpation and ultrasound (positive *vs* negative)	45.45

274

275 **4. DISCUSSION**

276 The histological evaluation showed that 60% of embryo recipient mares with a history
277 of subfertility, tested positive for endometritis. This finding indicates that endometritis is the
278 primary factor contributing to the removal of these mares from the embryo transfer program.
279 Additionally, we identified common risk factors among the mares positive for endometritis,
280 such as advanced age, a history of intrauterine fluid, and prior use of antibiotics without
281 comprehensive diagnostic evaluation.

282 The majority of recipient mares evaluated in this study were elderly. Advanced age is a
283 risk factor for uterine inflammatory diseases, as it influences the efficiency of physical,
284 mechanical, and cellular uterine defense.[10] Overall, older mares have endometrial
285 histopathological examination results suggestive of inflammatory diseases and higher incidence
286 of endometritis.[22,23] Moreover, 17% of the recipient mares did not present a successful
287 pregnancy, probably because they had unknown reproductive problems acquired in their former
288 farms. It is possible the occurrence of endometritis in this process. The absence of a clinical
289 diagnose in these animals highlights the importance of the complete gynecological examination
290 when integrating a recipient mare into the embryo transfer program.

291 We showed evidence that the history of therapy using antibiotics for endometritis
292 increased the odds of animals being positive for endometritis by 3.30 times. The use of
293 antibiotics for endometritis treatments without previous isolation of the pathogenic
294 microorganism and susceptibility tests are usually ignored by equine practitioners, since these

295 tests demand time, financial costs, materials, and specific equipment. Thus, during the breeding
296 season, many treatments are based on pre-established protocols, showing little or no therapeutic
297 response, which leads to infection resistance and aggravation.[24]

298 Despite the main clinical finding for the diagnosis of endometritis being intrauterine
299 fluid during ultrasound evaluation,[25] our findings revealed that this clinical sign alone was not
300 able to diagnose all positive mares for endometritis. This evaluation is routinely performed in
301 recipient mares to determine whether a mare should remain in the embryo transfer program.
302 However, some mares with infectious processes may not accumulate fluid, and some mares
303 without fluid may exhibit polymorphonuclear defense cells, indicative of inflammation.[16] This
304 emphasizes the importance of the most complete gynecological examination possible, including
305 laboratory tests, for a more accurate diagnosis.

306 Evaluations of perineal conformation, vaginoscopy, and examination of the cervix
307 predict how effective anatomic barriers are and the potential risks for endometritis. Positive
308 results in these evaluated methods may suggest recurrent ascending infections and consequent
309 endometritis.[10] In the study, we showed that 24% of recipient mares had cervical alterations.
310 These clinical assessments are simple and inexpensive to perform and therefore should be
311 implemented in routine reproductive exams, mainly as one of the steps of screening for more
312 elaborate exams. In addition, although 22% of the mares presented alterations in perineal
313 conformation, none of the animals had done vulvoplasty. This is not a complex surgery and
314 could fix the anatomical problems related to poor perineal conformation, and even decrease the
315 number of animals positive for endometritis.

316 The bacteria most commonly isolated from the equine uterus are *Streptococcus ssp*. and
317 *Escherichia coli*, predominating as causes of acute and chronic endometritis, respectively.[11,26]
318 In our study, the most isolated bacteria were *Escherichia coli*, which led us to associate the
319 diagnosed cases with chronic or recurrent infections. Mares with chronic and/or subclinical
320 uterine inflammatory processes may be responsible for false negative diagnoses both in clinical

321 and laboratory tests, when compared to the histological examination in the present study. The
322 presence of a mixed population of organisms, focal infections, highly pathogenic and biofilm-
323 producing bacteria can also contribute to chronic inflammation and hinder findings during
324 diagnostic exams such as microbiological culture and, consequently, antibiotic treatments.[13,27]
325 Metagenomic studies revealed that a moderately diverse microbiome is also present in uterine
326 samples, where no significant growth was obtained by conventional culture.[28] In addition,
327 inadequate treatments and inefficient defense mechanisms leading to susceptibility can cause
328 dysbiosis.[28,29] While we recognize the value of uterine microbiological culture, we may have
329 underestimated the complexity of the uterine microbiome and the pathogenesis of
330 endometritis.[30,31]

331 Uterine infections caused by fungi are less commonly associated with endometritis (1-
332 5%) than the infections caused by bacteria.[11,32] In our study, fungi were not identified during
333 uterine microbiological culture. Specific growth media for fungi were not used, which may
334 have limited the identification of these agents. Fungal endometritis usually occurs as an
335 opportunistic infection caused by dysbiosis after repeated use of intrauterine antibiotics.[13,33]
336 This may explain the cases in which the mares were diagnosed with endometritis by the
337 diagnostic gold standard, but negative in the uterine microbiological culture, since the number
338 of animals treated with antibiotics for endometritis in the evaluation of the reproductive history
339 was high.

340 After the introduction of antigens into the uterus, the innate immune response provides
341 the first nonspecific natural defense response, consisting of polymorphonuclear leukocytes,
342 mainly neutrophils, whose function is to destroy the antigens by the phagocytosis mechanism
343 or by extracellular traps.[9,34] Through uterine cytology and the presence of polymorphonuclear
344 cells, we diagnosed 47% of mares as positive in this study. This evaluation method presented
345 an accuracy of 80.1% when compared to endometrial histology, which is a satisfactory value
346 since it is a simple and practical test to be performed. However, results for polymorphonuclear

347 cells may be influenced by individual risk factors that may predispose to failures in this defense
348 process, such as increasing age, which has been associated with changes in the systemic
349 immune response.[8,35] These factors are associated with mares considered susceptible and
350 presenting chronic endometritis, such animals are not able to efficiently eliminate
351 inflammation, which can cause persistent inflammation or late resolution, reducing the number
352 of polymorphonucleates and making diagnosis difficult.[11,36] Thus, the uterine histological
353 evaluation after collection by transcervical biopsy, together with the other findings, should be
354 considered the only reliable method and the gold standard for the diagnosis of endometritis,
355 because it gathers immunological findings (polymorphonuclear) to degeneration alterations
356 caused by chronic endometritis.
357
358 **5. CONCLUSION**
359 This study gathered a considerable number of mares and evidenced that endometritis
360 was the main reason for removing of recipient mares from commercial embryo transfer
361 programs and contributing to reports of subfertility in this category. Moreover, a comprehensive
362 gynecological examination, encompassing histological evaluation, proves crucial for
363 identifying susceptible mares and detecting chronic or subclinical endometritis. Therefore, from
364 an efficient diagnosis we can make the right decision of removing or not recipient mares from
365 the embryo transfer program or indicate the most effective treatment, considering the
366 importance of this category for the success of reproductive biotechnologies.
367
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373

374 **CONFLICT OF INTEREST STATEMENT**

375 The authors declare that they have no conflicts of interest to disclose.

376

377 **REFERENCES**

378 1. Claes A, Cuervo-Arango J, van den Broek J, Galli C, Colleoni S, Lazzari G, Stout TA.
379 Factors affecting the likelihood of pregnancy and embryonic loss after transfer of
380 cryopreserved in vitro produced equine embryos. Equine Veterinary Journal,
381 2019;51(4):446-450. https://doi.org/10.1111/evj.13028
382 2. Felix MR, Turner RM, Dobbie T, Hinrichs K. Successful in vitro fertilization in the horse:
383 production of blastocysts and birth of foals after prolonged sperm incubation for
384 capacitation. Biology of Reproduction. 2022;107(6):1551-1564.
385 https://doi.org/10.1093/biolre/ioac172
386 3. McKinnon AO, Squires EL. Embryo Transfer and Related Technologies. In Current
387 Therapy in Equine Reproduction. 2007;319-334. https://doi.org/10.1016/B978-0-7216-
388 0252-3.50055-2
389 4. Diel de Amorim M, Gartley CJ, Hill A, Scholtz EL, Chenier TS, Foster RA, Diel de
390 Amorim, M. Comparison of Clinical Signs, Endometrial Culture, Endometrial Cytology,
391 Uterine Low-Volume Lavage, and Uterine Biopsy and Combinations in the Diagnosis of
392 Equine Endometritis. Journal of Equine Veterinary Science. 2016;44:54-61.
393 https://doi.org/10.1016/j.jevs.2015.10.012
394 5. Teixeira-Soares CM, Viana AGA, Ribeiro IM, Silva KDP, Sancler-Silva YFR, Machado-
395 Neves M. Comparison Between Gynecological Examination Methods and Sample
396 Collection Techniques for the Diagnosis of Endometritis in Subfertile Mares. Journal of
397 Equine Veterinary Science. 2022;119. https://doi.org/10.1016/j.jevs.2022.104147
398 6. McCue PM. The Problem Mare: Management Philosophy, Diagnostic Procedures, and
399 Therapeutic Options. Journal of Equine Veterinary Science. 2008;28(11):619-626.
400 https://doi.org/10.1016/j.jevs.2008.10.009
401 7. Overbeck W, Witte TS, Heuwieser W. Comparison of three diagnostic methods to identify
402 subclinical endometritis in mares. Theriogenology. 2011;75(7):1311-1318.
403 https://doi.org/10.1016/j.theriogenology.2010.12.002
404 8. Woodward EM, Christoffersen M, Campos J Squires, EL, Troedsson, MHT. Susceptibility
405 to persistent breeding-induced endometritis in the mare: Relationship to endometrial

406 biopsy score and age, and variations between seasons. Theriogenology. 2012;78(3):495-
407 501. https://doi.org/10.1016/j.theriogenology.2012.02.028
408 9. Morris LHA, McCue PM, Aurich C. Equine endometritis: A review of challenges and new
409 approaches. Reproduction. 2020. https://doi.org/10.1530/REP-19-0478
410 10. Ferris RA. Endometritis: Diagnostic Tools for Infectious Endometritis. Veterinary Clinics
411 of North America - Equine Practice. 2016. https://doi.org/10.1016/j.cveq.2016.08.001
412 11. Canisso IF, Segabinazzi, LGTM, Fedorka CE. Persistent breeding-induced endometritis in
413 mares - a multifaceted challenge: From clinical aspects to immunopathogenesis and
414 pathobiology. International Journal of Molecular Sciences. 2020.
415 https://doi.org/10.3390/ijms21041432
416 12. Samper JC. A review of a practitioner's perspective on endometrial
417 edema. Pferdeheilkunde. Hippiatrika Verlagsgesellschaf. 2010.
418 https://doi.org/10.21836/PEM20100103
419 13. Morrell JM, Rocha A. A Novel Approach to Minimising Acute Equine Endometritis That
420 May Help to Prevent the Development of the Chronic State. Frontiers in Veterinary
421 Science. 2022. https://doi.org/10.3389/fvets.2021.799619
422 14. Pascoe RR. Observations on the length and angle of declination of the vulva and its relation
423 to fertility in the mare. Journal of Reproduction and Fertility, Suppl. 27, 1979;299–305.
424 15. Caslick EA. The vulva and the vulvo-vaginal orifice and its relation to genital health ofthe
425 thoroughbred mare. Cornell Veterinary. 1937;27:178.
426 16. Katila T, Ferreira-Dias G. Evolution of the Concepts of Endometrosis, Post Breeding
427 Endometritis, and Susceptibility of Mares. Animals. 2022.
428 https://doi.org/10.3390/ani12060779
429 17. Zent WW, Steiner JV. Vaginal Examination. In: McKinnon A. O., Squires E. L., Vaala
430 W. E., Varner D. V., editors. Equine Reproduction, Second Edition. U.K: Wiley-
431 Blackwell. 2011.
432 18. Sitters S. Digital Examination of the Vagina/Cervix. In Equine Reproductive Procedures.
433 2021;45-47. https://doi.org/10.1002/9781119556015.ch11
434 19. Nielsen JM. Endometritis in the mare: A diagnostic study comparing cultures from swab
435 and biopsy. Theriogenology. 2005;64:510–518.
436 https://doi.org/10.1016/j.theriogenology.2005.05.034
437 20. Schoon HA, Schoon D, Klug E. Uterusbiopsienals Hilfsmittelfür Diagnose und Prognose
438 von Fertilitätsstörungen der Stute. Pferdeheilkunde. 1992;8:355-362.
439 21. SAS Institute Inc. SAS/STAT® 9.0 User's guide. Cary, NC: SAS Institute Inc, 2002.

440 22. Pycock JF. How to maximize the chances of breeding successfully from the older maiden
441 mare. AAEP Proceedings. 2006;5:245-249.
442 http://www.ivis.org/proceedings/aaep/2006/pdf/z9100106000245.pdf
443 23. Ball BA., et al. “Survival of Equine Embryos Transferred to Normal and Subfertile
444 Mares”. Theriogenology. 1987;28(2):167-174. https://doi.org/10.1016/0093-
445 691X(87)90264-0
446 24. Cabrera T, Pastorello M, Alvarenga, MA. PREVALÊNCIA E PERFIL DE
447 SENSIBILIDADE BACTERIANA EM ÉGUAS COM ENDOMETRITE. Enciclopédia
448 Biosfera. 2016;13(23):1013-1021.
449 https://doi.org/10.18677/enciclopedia_biosfera_2016_087
450 25. Katila T. Evaluation of diagnostic methods in equine endometritis. Reproductive Biology.
451 2016;16(3):189-196. https://doi.org/10.1016/j.repbio.2016.06.002
452 26. Troedsson MHT. Endometritis. In: McKinnon AO, Squires EL, Vaala WE, Varner DV,
453 editors. Equine Reproduction, Second Edition. U.K: Wiley-Blackwell. 2011.
454 27. Buczkowska J, Kozdrowski R, Sikora M, Dzięcioł M, Matusz A. Non-traditional
455 treatments for endometritis in mares. Bulgarian Journal of Veterinary Medicine. 2015.
456 https://doi.org/10.15547/bjvm.870
457 28. Heil BA, Thompson SK, Kearns TA, Davolli GM, King G, Sones JL. Metagenetic
458 Characterization of the Resident Equine Uterine Microbiome Using Multiple
459 Techniques. Journal of Equine Veterinary Science. 2018;66:111.
460 https://doi.org/10.1016/j.jevs.2018.05.156
461 29. LeBlanc MM. Breeding the problem mare. In: McKinnon AO, Squires EL, Vaala WE,
462 Varner DV, editors. Equine Reproduction, Second Edition. U.K: Wiley-Blackwell. 2011.
463 30. Knudsen, LRV, Karstrup CC, Pedersen HG, Angen Ø, Agerholm JS, Rasmussen EL,
464 Klitgaard K. An investigation of the microbiota in uterine flush samples and endometrial
465 biopsies from dairy cows during the first 7 weeks postpartum. Theriogenology.
466 2015;86(2):642-650. https://doi.org/10.1016/j.theriogenology.2016.02.016
467 31. Amann RI. Fluorescently labelled, rRNA-targeted oligonucleotide probes in the study of
468 microbial ecology. Molecular Ecology. 1995. https://doi.org/10.1111/j.1365-
469 294X.1995.tb00255.x
470 32. Dascanio JJ, Schweizer C, Ley WB. Equine fungal endometritis. Equine Veterinary
471 Education. 2001;13(6):324-329. https://doi.org/10.1111/j.2042-3292.2001.tb00122.x
472 33. Stout TAE. Fungal endometritis in the mare. In Pferdeheilkunde. 2008;24:83-87.
473 https://doi.org/10.21836/PEM20080117

474 34. Woodward EM, Troedsson MHT. Inflammatory mechanisms of endometritis. Equine
475 Veterinary Journal. 2015. https://doi.org/10.1111/evj.12403
476 35. Christoffersen M, Söderlind M, Rudefalk SR, Pedersen HG, Allen J, Krekeler N. Risk
477 factors associated with uterine fluid after breeding caused by Streptococcus
478 zooepidemicus. Theriogenology. 2015;84(8):1283-1290.
479 https://doi.org/10.1016/j.theriogenology.2015.07.007
480 36. Christoffersen M, Troedsson MHT. Inflammation and fertility in the mare. Reproduction
481 in Domestic Animals. 2017. https://doi.org/10.1111/rda.13013

CAPÍTULO 3

UNRAVELING UTERINE FLUID PROTEOME OF MARES DIAGNOSED WITH POST-BREEDING AND INFECTIOUS ENDOMETRITIS

Carlos Mattos Teixeira-Soares[1*], Arabela Guedes de Azevedo Viana[1], Renner Philipe Rodrigues Carvalho[2], Edvaldo Barros[3], Camilo Ramirez-Lopez[1], Arlindo Moura[4], Mariana Machado-Neves[1,2*]

[1]Programa de Pós-graduação em Medicina Veterinária, Departamento de Medicina Veterinária, Universidade Federal de Viçosa, Viçosa, Brasil.

[2]Programa de Pós-graduação em Biologia Celular e Estrutural, Departamento de Biologia Geral, Universidade Federal de Viçosa, Viçosa, Brasil.

[3]Núcleo de Análise de Biomoléculas, Centro de Ciências Biológicas, Universidade Federal de Viçosa. Viçosa, Brasil.

[4]Departamento de Ciência Animal, Universidade Federal do Ceará, Fortaleza, Brasil.

*Correspondent author: Mariana Machado-Neves

Av. Peter Henry Rolfs s/n, DBG, ECS333, Campus Universitário, CEP: 36570-900.

Viçosa, Minas Gerais, Brasil. E-mail: mariana.mneves@ufv.br

ABSTRACT

Endometritis is recognized as the leading cause of subfertility in mares and a significant challenge to equine reproduction. Its multifactorial etiopathogenesis necessitates diverse diagnostic and therapeutic approaches, prompting the search for novel and more effective methods. Proteomic analysis has facilitated advancements in studying the uterine fluid proteins, offering insights into endometritis mechanisms and possible biomarkers. This study aimed to characterize and compare the proteome of the uterine fluid healthy mares and diagnosed with different types of endometritis. Twenty-four mares were selected based on gynecological exams and reproductive history, and divided into three experimental groups: healthy mares, mares with infectious endometritis, and mares with post-breeding endometritis. Uterine fluid was collected via low-volume lavage and subjected to proteomic analysis. Data analysis involved protein identification and quantitative assessment. We identified 549 proteins, revealing distinct proteomic profiles among the studied groups. Healthy mares exhibited proteins such as heat shock protein Hsp-70, cathepsin S, and inhibin beta A chain, which are associated with uterine integrity maintenance. Mares with infectious endometritis displayed proteins like glutathione S-transferase, related to inflammatory and immune responses. Conversely, mares with post-breeding endometritis showcased a proteomic profile characterized by proteins involved in innate immune response pathways, including complement activation and extracellular trap formation. Furthermore, proteins such as polymeric immunoglobulin, multimeric immunoglobulin joining chain, and vanins demonstrated abundant differences, suggesting their potential as biomarkers. Further research is warranted to validate and confirm the utility of these biomarkers, as well as to investigate their association with the severity of endometritis and treatment response.

Keywords: endometrium, equine fertility, inflammatory response, proteomic analysis.

26 **1. Introduction**

27 Endometritis is widely recognized as the primary cause of subfertility in mares
28 and poses significant challenges to equine reproduction and its associated biotechnologies
29 (Morris et al., 2020). This condition is characterized by a persistent inflammatory process
30 in the endometrium, creating an unfavorable uterine environment for embryo survival
31 (LeBlanc, 2008; Swegen, 2021). The multifactorial etiopathogenesis of different types of
32 endometritis, including persistent post-breeding endometritis caused by semen presence
33 and infectious endometritis involving microorganisms, requires distinct diagnostic and
34 therapeutic approaches (Troedsson, 2011; Scoggin, 2016; Canisso et al., 2020).
35 Therefore, gaining insight into the different mechanisms of uterine inflammation and
36 exploring new alternative diagnostic tools with potential biomarkers become crucial to
37 improving reproductive outcomes (Buczkowska et al., 2014; Morrell & Rocha, 2022).

38 Advancements in genome sequencing and proteomic-based mass spectrometry
39 have opened new prospects for investigating the equine proteome and identifying
40 potential fertility biomarkers (Milardi et al., 2012; Gibb & Aitken, 2016). Proteomic
41 analysis of uterine fluid provides a non-invasive and comprehensive approach to
42 understanding the mechanisms related to equine fertility and the processes included in
43 pregnancy establishment and maintenance (Khan et al., 2020). Proteins regulate various
44 physiological processes and are directly involved in immune and inflammatory responses
45 (Milardi et al., 2012; Gibb & Aitken, 2016). The uterine fluid proteome landscape
46 enhances the knowledge of the complex interactions between inflammatory mediators,
47 immune cells, and the uterine environment, investigating the mechanisms involved in
48 endometritis.

49 Several studies have used proteomic analysis to evaluate and compare treatments
50 for endometritis (Wolf et al., 2012; Arlas et al., 2014; 2015), to compare healthy mares

51 with degenerative endometritis (Diel de Amorim et al., 2020), to investigate differences
52 across estrous cycle phases (Maloney et al., 2019; Khan et al., 2020), and to explore early
53 pregnancy (Hayes et al., 2008; 2012; Swegen et al., 2017; Smits et al., 2018; Lawson et
54 al., 2018; Waugh et al., 2023). Despite these recent efforts to uncover uterine proteins and
55 their potential implications for equine fertility, a comprehensive description of the uterine
56 protein profile concerning different types of endometritis remains elusive. Therefore, this
57 study characterized and compared the uterine fluid proteome of healthy mares with post-
58 breeding and infectious endometritis. Our results may provide valuable insights into the
59 molecular alterations associated with the establishment and progression of endometritis,
60 contributing to further identification of potential biomarkers and therapeutic targets.
61
62 **2. Material and methods**
63 *2.1. Animal selection*
64 Twenty-four Mangalarga Marchador breed mares, aged between 5 and 12 y, were
65 selected for this study from six distinct horse breeding farms in Muriaé (Latitude: 21° 7'
66 49'' South, Longitude: 42° 22' 3'' West), Brazil, during the 2020/2021 reproductive
67 season. The mares have regular health monitoring and were kept in paddocks with grass
68 pastures and free access to water and mineral salt, in addition to being fed hay and
69 concentrated grains. The animals underwent a screening process that involved a
70 comprehensive review of their reproductive history and gynecological examinations for
71 selection and inclusion in the experimental groups. Mares' welfare and all procedures
72 adhered to the guidelines established by the National Committee for the Regulation of
73 Animal Experimentation (CONCEA) and were approved by the Ethics Committee for
74 Animal Use of the Federal University of Viçosa, Brazil (CEUA, protocol no. 14/2020).

75 Mares were subjected to daily transrectal ultrasonography examinations until the
76 detection of estrus, characterized by a follicle size ≥ 35 mm and endometrial edema grade
77 3 (Samper, 2010). Subsequently, ovulation was induced using 250 µg of histrelin acetate
78 (Strelin - Botupharma, São Paulo, Brazil), and insemination with 1 billion of fresh and
79 viable spermatozoa. After 48 h, mares underwent to transrectal ultrasonography, uterine
80 cytology, uterine microbiological culture, and endometrial histopathology to categorize
81 and diagnose the endometritis.

82 Transrectal ultrasonography was assessed to detect intrauterine fluid, in which
83 amounts greater than 2 cm indicated a positive diagnosis for endometritis susceptibility
84 (Brinsko et al., 2003; Katila & Ferreira-Dias, 2022). A low-volume uterine lavage was
85 conducted to collect samples for endometrial cytology and uterine microbiological culture
86 (Ball et al., 1988). Mares with over 2% polymorphonuclear cells in the cytological
87 evaluation (Kozdrowski et al., 2015) and isolation of microorganism in the
88 microbiological culture were considered positive for endometritis. Subsequently, a
89 transcervical biopsy was performed to evaluate endometrial histopathology, graded
90 adapted from Schoon et al. (1992), in which mares with grades II or III were classified as
91 positive for endometritis. Complete reproductive data and diagnostic results for each mare
92 are provided in Supplementary Table S1. The mares were then divided into three
93 experimental groups: (1) healthy mares without any evidence of endometritis (control
94 group, n = 8); (2) mares diagnosed with infectious endometritis (n = 8); and (3) mares
95 diagnosed with post-breeding endometritis (n = 8), according to the findings of the
96 examinations (Table 1).

97

98

99 **Table 1**. Selection criteria for categorization of mares in experimental groups based on
100 evaluative diagnostic parameters to endometritis.

Parameters	Healthy mares (Control group)	Infectious endometritis group	Post-breeding endometritis group
Intrauterine fluid in ultrassonography	Absence	Absence or Presence	Presence (≥ 2 cm)
Endometrial cytology	≤ 2% PMNs	> 2% PMNs	> 2% PMNs
Microbiological culture	No microorganism growth	Microorganism growth	No microorganism growth
Histopathological evaluation	Grade I	Grade II or III	Grade II or III

101 PMNs: polymorphonuclear cells. Grade I: Negative for endometritis; Grade II or III:
102 Positive for endometritis.

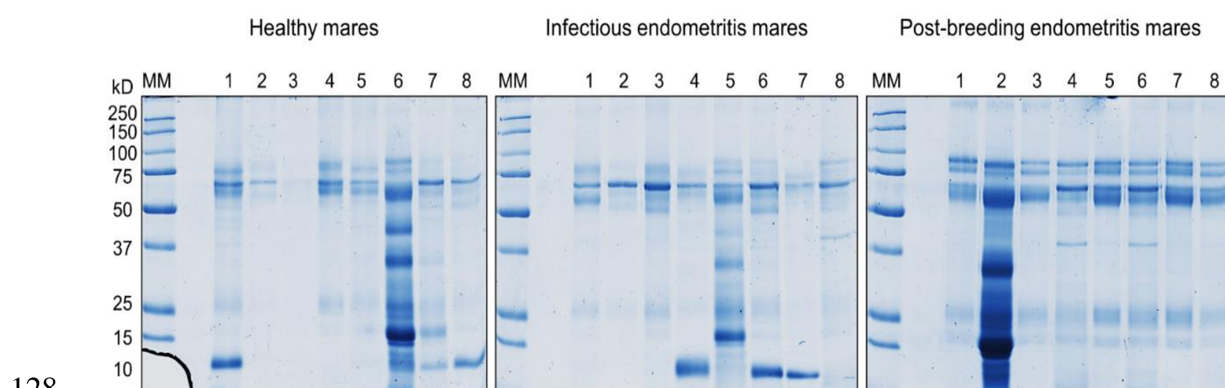
103

104 *2.2. Collection and processing of uterine fluid*

105 For proteomic analysis, a volume of 60 mL of 0.9% saline solution (Fresenius
106 Kabi, Bad Homburg, Germany) was transcervical infused into the uterus using a
107 disposable sterile catheter (Embramed, São Paulo, Brazil). After 2 min, the fluid was
108 recovered into two sterile 15 mL plastic tubes (Corning Incorporated Life Science,
109 Tewksbury, England) by gravity and with the assistance of intravenous administration of
110 20 IU of oxytocin (Ocitocina Forte, UCBVET, São Paulo, Brazil) to promote uterine
111 contractions.

112 Subsequently, the uterine fluid was centrifuged at 400 x *g* for 10 min. While the
113 sediment was used for screening animal diagnosis, the supernatant was separated for
114 proteomic analysis. Thus, this fluid was recovered and transferred to cryogenic tubes (2

115 mL volume; Corning Incorporated Life Science, Tewksbury, England) containing
116 protease inhibitor cocktail 1:1000 (Viana et al., 2018) and stored at -80 °C.
117
118 *2.3. Proteomic analysis*
119 Equine endometrial fluids were resuspended in a solution containing urea 8 M,
120 NaCl 75 mM, and Tris 50 mM and centrifuged at 14.000 × g for 10 min at 4 °C. The
121 supernatant was subjected to protein precipitation by adding cold acetone (1:4) and placed
122 at –20 °C overnight. Then, three sequential centrifugations (14.000 × *g* at 4 °C for 10
123 min) were applied, always discarding the supernatant. The dried pellets were resuspended
124 in ammonium bicarbonate 50 mM, and the proteins were quantified using the Bradford
125 method (Bradford, 1976; Couto-Santos et al., 2019). An aliquot of 20 µg of proteins from
126 each sample was used to perform one-dimensional electrophoresis SDS-PAGE (Figure
127 1).



128
129 **Figure 1.** SDS-PAGE analysis of endometrial fluids of healthy mares, infectious
130 endometritis mares and post-breeding endometritis mares. MM = Molecular marker; kDa
131 = kilodalton.
132
133 For protein digestion, an aliquot of 50 µg of proteins from each sample was
134 resuspended in ammonium bicarbonate 50 mM with the addition of 500 mM DTT
135 (dithiothreitol), followed by incubation at 55 °C for 25 min. Further, iodoacetamide (IAA)

136 was added to reach a final concentration of 0.014 M, and the solution was kept at 21 °C
137 in the dark for 40 min. Further, 0.001 M $CaCl_2$ was added before digestion of proteins
138 with trypsin (Promega, Fitchburg, WI, USA), with a 1/50 (w/w) enzyme/substrate. The
139 solution was incubated at 37-°C for 18 h. Later, trifluoroacetic acid (TFA) was added to
140 a final concentration of 1% to stop tryptic activity (Viana et al., 2018). The peptides were
141 desalted using stage tip C18 columns manually packed with Empore TM SPE disks
142 (Sigma-Aldrich, Darmstadt, Germany; Viana et al., 2018) and lyophilized.

143 The lyophilized peptides were resuspended in 20 µL of water, containing formic
144 acid 0.1% and acetonitrile 2%. One microliter of each sample was injected in the maXis
145 3G QTOF orthogonal mass spectrometer (Bruker Daltonics, Billerica, MA, USA)
146 coupled with an ultra-performance liquid chromatography system (nanoACQUITY,
147 Waters, Milford, MA, USA), using trapping column (nanoAcquity UPLC® 2G-V/MTrap
148 5 µm Symmetry® C18 180 µm x 20 mm) at 7 µL/min for 3 min. The analytical column,
149 nanoAcquity UPLC® 1.8 µm HSS T3 75 µm x 200 mm, was used at 0.2 µl/min flow for
150 240 min in a gradient concentration of acetonitrile 0-85%. The mass spectrometer was
151 operated in data-dependent mode (DDA), and each MS full scan was acquired with
152 resolving power. The most abundant ions were selected for fragmentation via collision-
153 induced dissociation.

154

155 *2.4. Data analysis*

156 The raw data were converted into mzXML files using the CompassXport software,
157 version 3.0 (Bruker Daltonics, Billerica, MA, USA). The protein identification was
158 performed using PEAKS software 7.0 (Bioinformatics Solutions Inc., Canada) and the
159 PEAKS DB method (Zhang et al., 2012). Data were searched at 20 ppm and 0.6 Da mass
160 tolerance for precursor and fragmentation ions, respectively. Carbamidomethylation of

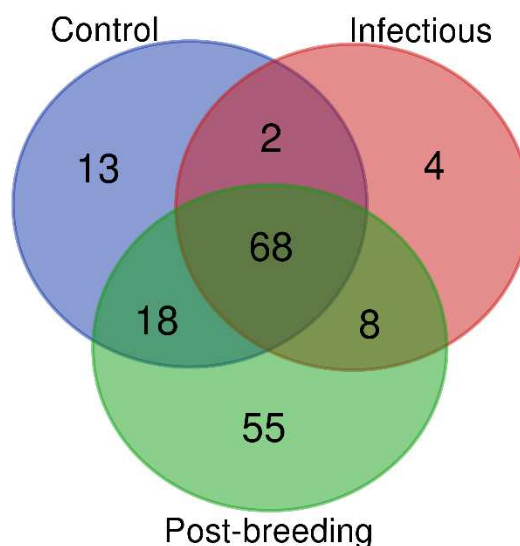
161 cysteine and oxidation of methionine were settled as static and variable modifications. A
162 minimum of two peptides per protein and a protein false discovery rate (FDR) < 1% were
163 applied for protein identification (Couto-Santos et al., 2021). Using the strict criteria
164 mentioned above, we considered the proteins identified in at least one sample from each
165 group. However, for the qualitative and quantitative analysis of potential biomarkers, we
166 considered proteins present in at least five mares from each group (n = 8). Proteins were
167 analyzed through metabolic pathways annotated in the KEGG (Kyoto Encyclopedia of
168 Genes and Genomes) database, biological processes, and molecular functions. Further,
169 they were clustered and defined according to enrichment scores and p-values.
170 Quantification of identified proteins was performed using the Label-Free method
171 available in the PEAKS Q module. Initially, a comparison analysis between all samples
172 was performed. Pearson correlation values between samples within each group and
173 between groups. The three samples of each treatment that showed the best correlation in
174 the cluster were selected (Ramírez-López et al., 2023).

175

176 **6. Results**

177 Our results showed that 549 proteins were identified, and more than half of them
178 (n = 279) common in the uterine fluid of mares from the three experimental groups
179 (Supplementary Table S2). However, for analysis of potential biomarkers, only 168
180 proteins were considered when used the criterion of protein presence in at least five out
181 of eight mares per group, being 68 expressed in the uterine fluid of mare from all
182 experimental groups. Thirteen proteins were detected in control animals, while four
183 proteins were expressed in mares with infectious endometritis, and 55 were found in
184 mares with post-breeding endometritis (Fig. 2).

185



186

187 **Fig. 2.** A Venn diagram to represent proteins present* in uterine fluid samples of mares
188 with post-breeding endometritis (green), infectious endometritis (red) and control group
189 (healthy mares, without endometritis – blue). *Proteins detected in at least five uterine
190 fluid samples of mares from each group.

191

192 The proteins found in the uterine fluid of control mares were membrane
193 metalloendopeptidase, follistatin-like 3, cadherin 1, inhibin beta A chain, Ig-like domain-
194 containing protein, heat shock 70kDa protein 1A, coagulation factor V,
195 phosphatidylethanolamine-binding protein 1, beta-hexosaminidase, junctional adhesion
196 molecule A, cutA divalent cation tolerance homolog, mesothelin, and cathepsin S. In
197 contrast, mares with infectious endometritis expressed four proteins: annexin, glutathione
198 S-transferase, cellular communication network factor 2, and malate dehydrogenase. The
199 55 proteins detected in at least five animals of the post-breeding endometritis group are
200 listed in Table 2. From those proteins, two were exclusively identified in most of mares
201 with post-breeding endometritis only, complement C3 and ADAM metallopeptidase with
202 thrombospondin type 1 motif 1. Three were detected in animals from this group rather
203 than healthy mares, azurocidin 1, Lipocalin/cytosolic fatty-acid binding domain-

204 containing protein, and olfactomedin 4 (Tab. 2). ADP-ribosylarginine hydrolase and t-
205 plasminogen activator were detected in mares with post-breeding endometritis but not in
206 mares with infectious endometritis (Tab. 2).

207

208 **Table 2.** Proteins (n = 55) expressed in the uterine fluid of at least five mares with post-
209 breeding endometritis in comparison with their expression in healthy mares (control) and
210 mares with infectious endometritis (infectious group).

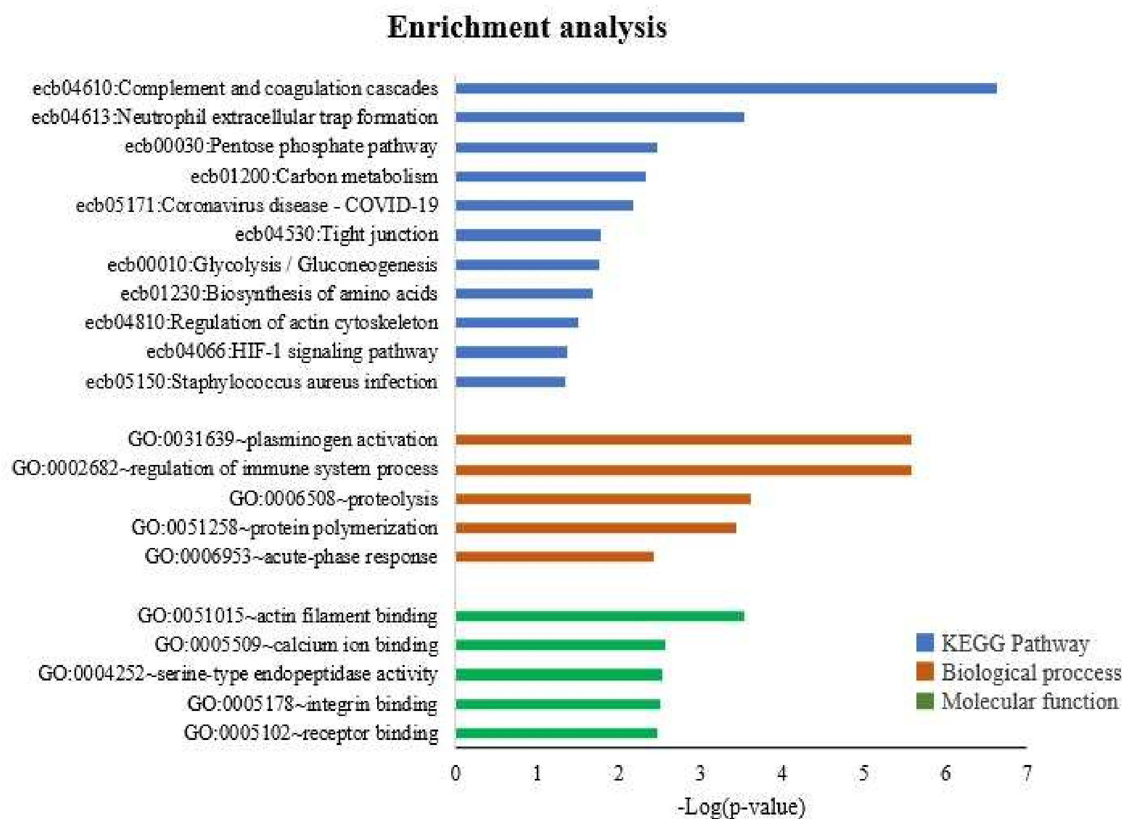
211

Proteins	Number of animals per group		
	Control	Infectious	Post-breeding
Proteins expressed only in mares with post-breeding endometritis			
Complement C3	0	0	6
ADAM metallopeptidase with thrombospondin type 1 motif 1	0	0	5
Proteins expressed in all mares (n=8) with post-breeding endometritis in comparison with their expression in other groups			
Complement component 4 binding protein alpha	3	1	8
Alpha-2-macroglobulin like 1	4	2	8
Dystroglycan 1	4	3	8
Proteins expressed in mares (n=7, n = 6, or n=5) with post-breeding endometritis in comparison with their expression in other groups			
Midkine	2	1	7
Lysozyme	2	4	7
Mucin-5B	3	1	7
Secretory leukocyte peptidase inhibitor	3	2	7
Neuropilin	3	3	7
Polymeric immunoglobulin receptor	4	1	7
Fibrinogen gamma chain	4	4	7
Azurocidin 1	0	2	6
t-plasminogen activator	1	0	6

ADP-ribosylarginine hydrolase	2	0	6
Cathepsin H	1	1	6
Spondin 2	1	1	6
IF rod domain-containing protein	1	3	6
Actin-related protein 2/3 complex subunit 4	2	2	6
Myeloperoxidase	2	2	6
Prolactin-inducible protein homolog	2	2	6
Phosphoglycerate kinase	2	3	6
Transketolase	2	3	6
Moesin	3	4	6
Uteroglobin	3	4	6
Myosin heavy chain 9	4	3	6
WD repeat domain 1	4	3	6
Lymphocyte cytosolic protein 1	4	4	6
Peroxidasin	4	4	6
Lipocalin/cytosolic fatty-acid binding domain-containing protein	0	2	5
Olfactomedin 4	0	3	5
Calreticulin	1	1	5
Lipocalin/cytosolic fatty-acid binding domain-containing protein	1	1	5
45 kDa calcium-binding protein	1	1	5
Proteinase 3	1	1	5
Sorcin	1	1	5
Resistin	1	3	5
Glutathione S-transferase	2	2	5
Actin related protein 3	2	2	5
Rho GDP-dissociation inhibitor 2-like protein	2	2	5
Syndecan binding protein	2	2	5
Fibrinogen alpha chain	2	3	5
L-lactate dehydrogenase	2	4	5
6-phosphogluconate dehydrogenase, decarboxylating	2	4	5

Abhydrolase domain containing 14B	3	3	5
Actinin alpha 1	3	3	5
Uncharacterized protein	3	3	5
Fibrinogen beta chain	3	4	5
Heat shock protein HSP 90-beta	3	4	5
AE binding protein 1	4	1	5
Desmocollin 2	4	1	5
Nucleoside diphosphate kinase	4	2	5
Rab GDP dissociation inhibitor	4	2	5
Fructose-bisphosphate aldolase	4	3	5
Transgelin	4	4	5

212
213
214 Functional clustering analyses were performed to evaluate the insight into
215 biological processes, molecular functions, and metabolic pathways. The proteins
216 identified in the control and infectious endometritis groups did not contribute to the
217 enrichment of functions analysis. On the other hand, the figure 3 shows the results of
218 KEGG pathway analysis demonstrated enrichment of eleven terms in mares with post-
219 breeding endometritis, including terms related to complement and coagulation cascades,
220 neutrophil extracellular trap formation, pentose phosphate pathway, and carbon
221 metabolism. Biological processes involved in the plasminogen activation, regulation of
222 immune system process, proteolysis, protein polymerization, and acute phase-response
223 were enriched in mares from this group (Fig. 3). The enriched terms related to molecular
224 functions were actin filament binding, followed by endopeptidase activity and integrin,
225 receptor, and calcium ion binding (Fig. 3).
226



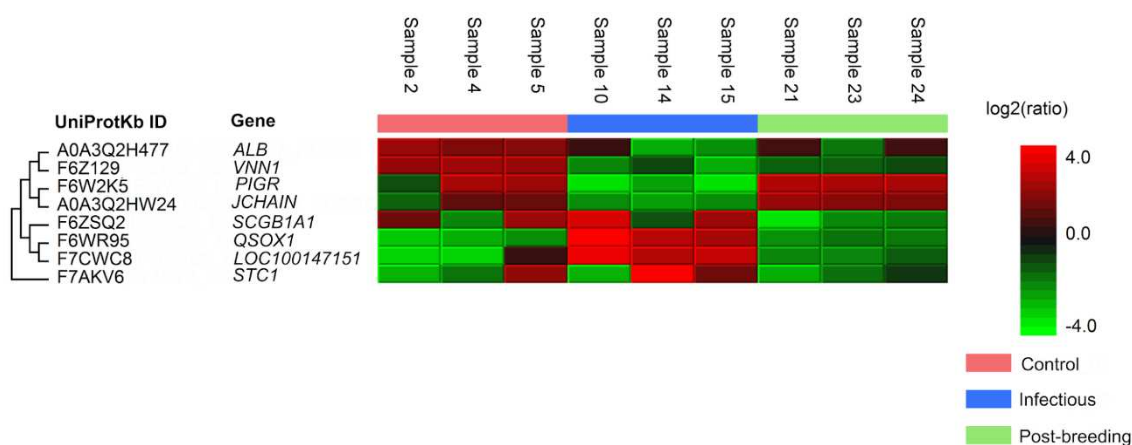
227

228 **Fig. 3.** Functional annotations [-log (p-value)] of proteins' clusters identified in the
229 uterine fluid of at least five mares with post-mating endometritis included in the post-
230 breeding experimental group, according to metabolic pathways annotated in the KEGG
231 (Kyoto Encyclopedia of Genes and Genomes) database, biological process, and molecular
232 functions.

233

234 Pearson's correlation was calculated within each group and between groups. The
235 three samples from each treatment that exhibited the highest correlation within their
236 respective groups were selected for further quantitative analysis. Subsequently, a
237 comparative analysis of the three selected samples from each group revealed differences
238 in the abundance of eight proteins: albumin, vanin 1, polymeric immunoglobulin receptor,
239 joining the chain of multimeric IgA and IgM, uteroglobin, sulfhydryl oxidase, amine
240 oxidase, and stanniocalcin 1 (Fig. 4).

241



242

243 **Fig. 4.** Heatmap of the differentially available proteins found by comparative analysis of
244 the three selected samples from each group that exhibited the highest correlation. Control
245 group, infectious endometritis, and post-coverage endometritis. A0A3Q2H477-Albumin,
246 F6Z129-Vanin 1, F6W2K5-Polymeric immunoglobulin receptor, A0A3Q2HW24-
247 Joining chain of multimeric IgA and IgM, F6ZSQ2-Uteroglobin, F6WR95-Sulfhydryl
248 oxidase, F7CWC8-Amine oxidase, and F7AKV6-Stanniocalcin 1.

249

250 **7. Discussion**

251 We conducted a comprehensive proteome analysis of uterine fluid obtained
252 through low-volume lavage from 24 mares resulting in 549 proteins that displayed
253 significant differences between different types of endometritis, including infectious and
254 post-breeding, and healthy individuals. This approach aimed to enhance our
255 understanding of the endometritis etiopathogeneses and indicate potential biomarkers
256 according to the animal phenotype. Thirteen proteins were found in the uterine fluid of
257 most of the healthy mares included in this study, exhibiting diverse functions primarily
258 related to cellular protection and adhesion, among them heat shock 70kDa protein (Hsp-
259 70), cathepsin S and inhibin beta A chain. These findings highlight the significance of
260 these proteins in the normal functioning of the equine uterus. However, it is important to

261 note that due to their abundance in uterine fluid and their nonspecific nature, further in-
262 depth investigations are warranted to establish their precise roles and assess their potential
263 as markers of uterine integrity.

264 Our results revealed that only four proteins were expressed in the uterine fluid of
265 at least five mares with infectious endometritis. Annexins deserve attention because they
266 are associated with the inflammatory state of the endometrium and were previously
267 çponted out as potential biomarkers (Hayes et al., 2012; Diel de Amorim et al., 2020).
268 Annexins are detected in uterine fluid, and their genes are locally expressed in the equine
269 endometrium (Hayes et al., 2012). These proteins are involved in anti-inflammatory
270 mechanisms, exerting antimigratory effects on neutrophil extravasation and regulating
271 the production of superoxide by these cells (Gerke and Moss, 2002). Similar to our study,
272 Diel de Amorim (2020) found a relationship between annexins and endometritis, as they
273 detected an increase in the levels of annexin A2 in mares with endometritis but in the
274 degenerative form of the disease. These findings prove the biomarker potential of this
275 protein for endometritis. Another protein identified in mares with infectious endometritis
276 was glutathione S-transferase. It is an enzyme with antioxidant function, playing a role in
277 protecting against damage caused by oxidative stress (Pelin et al., 2015; Markovic et al.,
278 2009). Oxidative stress plays a crucial role in several steps involved in the fertility process
279 as it exacerbates inflammation, causes cell damage, and influences the uterine
280 environment (Zhong & Zhou, 2013; Talukder et al., 2017; Gabai et al., 2019). Neutrophils
281 are responsible for reactive oxygen species production, leading to oxidative stress and
282 contributing to a disease process known as oxinflammation (Winterbourn et al., 2016;
283 Gabai et al., 2019; Valacchi et al., 2018). It is relevant to investigate the relationship
284 between oxinflammation, the presence of pathogenic microorganisms, the abundance of
285 neutrophils, and the role of glutathione S-transferase in infectious endometritis. The other

286 two proteins found in the majority of mares with infective endometritis were cellular
287 communication network factor 2 (CCN2) and malate dehydrogenase. They are not
288 directly associated with an immune or inflammatory response. Instead, they are possibly
289 related to a possible progression of the disease. The CCN2 is responsible for different
290 signaling activities in various pathways and is mainly associated with fibrosis (Leguit et
291 al., 2021). Malate dehydrogenase, in turn, is associated with tissue damage and is
292 considered an exploratory biomarker enzyme that can indicate cellular dysfunctions
293 (Marrer & Dieterle, 2010). Therefore, the presence of both in infective endometritis
294 suggests a higher progression of endometrial cell damage and perhaps a more rapid
295 evolution to fibrous tissue formation in this condition.

296 Several proteins were identified in most mares with post-breeding endometritis.
297 The enrichment analysis revealed that signaling pathways and biological processes were
298 predominantly associated with the innate immune response. The pathway of complement
299 and coagulation cascades plays a crucial role in neutrophil recruitment and activation of
300 the complement system, an important defense mechanism (Markiewski et al., 2007;
301 Amaral et al., 2010). Another pathway involved with neutrophils is the formation of
302 extracellular traps composed of proteins that play a significant role in the innate defense
303 against microorganisms (Rebordão et al., 2019). These findings indicate that post-
304 breeding endometritis increases the innate immune response compared to infectious
305 endometritis, suggesting a defense mechanism involving neutrophils mainly and a
306 predominantly acute inflammatory response.

307 The quantitative analysis observed differences in the abundance of eight proteins,
308 such as albumin, which was more abundant in the control group and act in oxidative stress
309 by neutralizing free radicals and transporting antioxidants (Plantier et al., 2016). Its higher
310 abundance in in healthy mares group suggests that its function may be compromised

311 during inflammation and your absence may play a important roles in the inflammatory
312 process. Like albumins, the vanins were observed in a significantly lower abundance in
313 the endometritis groups compared to the healthy mares group. These results are in
314 agreement with the findings by Diel de Amorim et al. (2020), who reported a significant
315 decrease in vanins in mares with degenerative endometritis. Vanins play an important role
316 in modulating the uterine innate immune response, as they are negatively correlated with
317 the presence of neutrophils in the endometrium (Diel de Amorim et al., 2020). These
318 proteins are highly expressed and abundant in uterine fluid, although their specific role is
319 not yet fully understood (Hayes et al., 2012). It is believed that they may be involved in
320 oxidative stress and inflammatory signaling pathways, affecting the signaling molecules
321 of this process and contributing to the regulation of the inflammatory response at the
322 molecular level (Ferreira et al., 2015). Therefore, vanins are potential biomarkers of
323 uterine inflammation suggesting a possible fundamental role of these proteins in
324 maintaining inflammatory balance.

325 In turn, the polymeric immunoglobulin receptor (PIgR) and joining chain of
326 multimeric immunoglobulin (J chain) demonstrated differences in concentrations
327 between the two examined groups of endometritis, with low abundance detected in mares
328 with infectious endometritis and higher with post-breeding endometritis. Both proteins
329 play a relevant role in the immune response by mediating the transport of immunoglobulin
330 A from the mucosa to the uterine lumen during inflammatory processes (Braathen et al.,
331 2007; Wei et al., 2021). The difference in PIgR and J chain levels between the groups
332 with endometritis could be attributed to a greater influx of immunoglobulin A in post-
333 breeding endometritis compared to infectious endometritis. However, Woodward &
334 Troedsson (2013) observed no differences in the pattern of immunoglobulin A response
335 between these types of endometritis, as both showed an increase in this immunoglobulin.

336 Therefore, it is necessary to clarify the secretion and the relationship of immunoglobulin
337 A in the different types of endometritis, as these proteins may be potential biomarkers of
338 the uterine humoral immune response in mares.

339 In our study, there were a lower abundance of uteroglobin and stanniocalcin in
340 mares with post-breeding endometritis. The uteroglobin, or secretoglobin, also plays a
341 significant role in regulating the immune response and possess anti-inflammatory
342 properties (Müller-Schöttle et al., 2002; Hoffmann et al., 2009). This may indicate a more
343 pronounced immunosuppressive effect in this type of endometritis, as a decrease in
344 uteroglobin expression has been suggested to increase susceptibility to endometritis
345 (Hoffmann et al., 2009). Another important aspect is that uteroglobin interacts with
346 hydrophobic ligands such as progesterone, suggesting its role in supporting pregnancy
347 (Ellenberger et al., 2008; Hayes et al., 2012). Similar to uteroglobulin, the stanniocalcin
348 contributes to endometrial preparation for embryo implantation and has anti-
349 inflammatory functions by inhibiting the production of pro-inflammatory cytokines and
350 modulating the immune response (Bishop et al., 2021). This protein is involved in calcium
351 metabolism and too has antioxidant properties, protecting organs and tissues from
352 inflammation-induced damage. Thus, we hypothesize that the reduced levels of
353 uteroglobin and stanniocalcin found in post-breeding endometritis may be related to
354 lower chances of establishing pregnancy in this condition.

355 The other two enzymes differentially abundant, the sulfhydryl oxidase and amine
356 oxidase, exhibited similar abundance patterns with higher levels in infectious
357 endometritis compared to the other groups. The sulfhydryl oxidase catalyzes the oxidation
358 of sulfhydryl groups in proteins, converting them into disulfide bonds. These bonds are
359 essential for the stability and structure of proteins and play a role in cellular repair and
360 maintenance (Farkye & Bansal, 2011). Additionally, this enzyme is known for assisting

361 bacteria in adapting to the host environment, facilitating their ability to colonize and infect
362 (Faccio et al., 2011). Therefore, the high abundance of this enzyme in mares with
363 infectious endometritis may indicate a defense process through the repair of proteins
364 essential for proper endometrial function and suggest a mechanism employed by
365 infectious agents to colonize the uterine microenvironment. Amine oxidases are enzymes
366 involved in the oxidation of amines, resulting in the formation of oxidized products, along
367 with the release of secondary products such as aldehydes, ammonia, and hydrogen
368 peroxide, which can influence cells and tissues (Floris & Finazzi Agrò, 2013). Some
369 amine oxidases, when present in specific tissues or cells, can directly influence the
370 modulation of the inflammatory response. However, the exact mechanisms and clinical
371 implications of these effects can vary considerably (Thézénas et al., 2020). Further
372 research is needed to clarify the exact mechanisms and clinical implications of these
373 effects in the mare's uterus and their potential relationship with endometritis.

374 In conclusion, our results revealed differences in the proteomic profiles between
375 groups and provided a comprehensive view of the changes associated with uterine
376 inflammation in mares. Healthy animals exhibited a proteomic profile characterized by
377 proteins involved in maintaining uterine integrity, mares with infectious endometritis
378 exhibited a distinct proteomic profile with proteins associated with inflammatory and
379 immune responses, while mares with post-reproductive endometritis presented proteins
380 mainly related to the innate immune response. Our unprecedented qualitative and
381 quantitative findings will contribute to future validation studies of proteins as potential
382 biomarkers for diagnosis, as well as to elucidate the course of the disease and its most
383 effective treatment.

384

385 **Declaration of competing interest**

386 The authors declare no competing interests.

387

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393

394 **8. References**

395 Amaral U, Flierl MA, Rittirsch D, Klos A, Chen H, Acker B, et al. Molecular
396 intercommunication between the complement and coagulation systems. Journal of
397 Immunology. 2010;185(9):5628-5636.

398 Arlas TR, Wolf CA, Petrucci BPL, Estanislau JF, Gregory RM, Jobim MIM, et al. Effect
399 of dexamethasone on proteomics of endometrial fluid from mares susceptible to
400 endometritis. Journal of Equine Veterinary Science. 2014;34(1):125-126.
401 doi:10.1016/j.jevs.2013.10.085.

402 Arlas TR, Wolf CA, Petrucci BPL, Estanislau JF, Gregory RM, Jobim MIM, et al.
403 Proteomics of endometrial fluid after dexamethasone treatment in mares susceptible to
404 endometritis. Theriogenology. 2015;84(4):617-623.
405 doi:10.1016/j.theriogenology.2015.04.019.

406 Ball BA, Shin SJ, Patten VH, Lein DH, Woods GL. Use of a low-volume uterine flush
407 for microbiologic and cytologic examination of the mare's endometrium. Theriogenology.
408 1988;29(6):1269-1283. doi:10.1016/0093691X(88)90007-6.

409 Bishop, A., Cartwright, J. E., & Whitley, G. S. (2021). Stanniocalcin-1 in the female
410 reproductive system and pregnancy. Human Reproduction Update, 27(6), 1098–1114.
411 https://doi.org/10.1093/humupd/dmab028

412 Braathen R, Hohman VS, Brandtzaeg P, Johansen FE. Secretory antibody formation:
413 conserved binding interactions between J chain and polymeric Ig receptor from humans
414 and amphibians. Journal of Immunology. 2007;178(3):1589-1597.
415 doi:10.4049/jimmunol.178.3.1589.

416 Brinsko SP, Rigby SL, Varner DD. A practical method for recognizing mares susceptible
417 to post-breeding endometritis. In Proceedings of the American Association Equine
418 Practitioners. 2003;49:363-365.

419 Buczkowska J, et al. Comparison of the biopsy and cytobrush techniques for diagnosis of
420 subclinical endometritis in mares. Reproductive Biology and Endocrinology.
421 2014;12:113. doi:10.1186/1477-7827-12-113.

422 Canisso IF, Kenney RM, Swerczek TW. Equine endometritis: An updated review. Journal
423 of Equine Veterinary Science. 2020;85:102920.

424 Couto-Santos F, Viana AG de A, Souza AC F, Dutra AA de A, Mendes TA de O, Ferreira
425 AT da S, Machado-Neves M. Prepubertal arsenic exposure alters phosphoproteins profile,
426 quality, and fertility of epididymal spermatozoa in sexually mature rats. Toxicology.
427 2021;460. doi:10.1016/j.tox.2021.152886.

428 Diel de Amorim M, Khan FA, Chenier TS, Scholtz EL, Hayes MA. Analysis of the
429 uterine flush fluid proteome of healthy mares and mares with endometritis or fibrotic
430 endometrial degeneration. Reproduction in Domestic Animals. 2020;55(6):572-581.

431 Ellenberger C, Wilsher S, Allen WR, Hoffmann C, Kölling M, Bazer FW, Klug J, Schoon
432 D, Schoon H-A. Immunolocalization of the uterine secretory proteins uterocalin,
433 uteroferrin, and uteroglobin in the mare's uterus and placenta throughout pregnancy.
434 Theriogenology. 2008;70:746-757.

435 Faccio, G., Nivala, O., Kruus, K., Buchert, J., & Saloheimo, M. (2011, August).
436 Sulfhydryl oxidases: Sources, properties, production and applications. Applied
437 Microbiology and Biotechnology. https://doi.org/10.1007/s00253-011-3440-y

438 Farkye NY, Bansal N. Enzymes Indigenous to Milk | Other Enzymes. In J. W. Fuquay
439 (Ed.), Encyclopedia of Dairy Sciences (Second Edition). 2011;pp. 327-334. Academic
440 Press. DOI: 10.1016/B978-0-12-374407-4.00162-X.

441 Ferreira, D., Naquet, P., & Manautou, J. (2015). Influence of Vanin-1 and Catalytic
442 Products in Liver During Normal and Oxidative Stress Conditions. Current Medicinal
443 Chemistry, 22(20), 2407–2416. https://doi.org/10.2174/0929867322220150722124307

444 Floris G, Finazzi Agrò A. Amine Oxidases. In W. J. Lennarz & M. D. Lane (Eds.),
445 Encyclopedia of Biological Chemistry (Second Edition). 2013;pp. 87-90. Academic
446 Press. DOI: 10.1016/B978-0-12-378630-2.00182-1.

447 Gabai G, De Luca E, Miotto G, Zin G, Stefani A, Da Dalt L, Barberio A, Celi P.
448 Relationship between protein oxidation biomarkers and uterine health in dairy cows
449 during the postpartum period. Antioxidants. 2019;8(1). doi:10.3390/antiox8010021.

450 Gerke V, Moss SE. Annexins: From Structure to Function. Physiological Reviews.
451 2002;82:331-371.

452 Gibb Z, Aitken RJ. The Impact of Sperm Metabolism during in Vitro Storage: The
453 Stallion as a Model. BioMed Research International. 2016;2016:9380609.
454 doi:10.1155/2016/9380609.

455 Hayes MA, et al. Proteins associated with the early intrauterine equine conceptus.
456 Reproduction in Domestic Animals. 2008;43(Suppl. 2):232-237.

457 Hayes T, et al. Transcriptional and proteomic analysis of endometrial and conceptus
458 responses to cloprostenol administration in the third week of pregnancy. Journal of
459 Equine Veterinary Science. 2012;32(7):407-408. doi:10.1016/j.jevs.2012.05.028.

460 Hoffmann C, Bazer FW, Klug J, Aupperle H, Ellenberger C, Schoon HA.
461 Immunohistochemical and histochemical identification of proteins and carbohydrates in
462 the equine endometrium: Expression patterns for mares suffering from endometrosis.
463 Theriogenology. 2009;71:264-274.

464 Katila T, Ferreira-Dias G. Evolution of the concepts of endometrosis, post breeding
465 endometritis, and susceptibility of mares. Animals. 2022;12:779.

466 Kozdrowski R, et al. Diagnostic methods and endometritis treatment efficiency in mares.
467 Bulletin of the Veterinary Institute in Pulawy. 2015;59:171-177.

468 Lawson EF, Gibb Z, de Ruijter-Villani M, Smith ND, Stout TA, Clutton-Brock A,
469 Swegen A. Proteomic analysis of pregnant mare uterine fluid. Journal of Equine
470 Veterinary Science. 2018;66:171-172.

471 LeBlanc MM. Advances in the diagnosis and treatment of chronic infectious and post-
472 mating-induced endometritis in the mare. Reproduction in Domestic Animals.
473 2008;43:64-74.

474 Leguit RJ, Raymakers RAP, Hebeda KM, Goldschmeding R. CCN2 (Cellular
475 Communication Network factor 2) in the bone marrow microenvironment, normal and
476 malignant hematopoiesis. Journal of Cell Communication and Signaling. 2021;15(1):25-
477 56. doi:10.1007/s12079-020-00602-2.

478 Maloney SE, et al. A comparison of the uterine proteome of mares in estrus and diestrus.
479 Reproduction in Domestic Animals. 2019;54(3):473-479. doi:10.1111/rda.13375.

480 Markiewski MM, Nilsson B, Ekdahl KN, Mollnes TE, Lambris JD. Complement and
481 coagulation: strangers or partners in crime? Trends in Immunology. 2007;28(4):184-192.

482 Markovic J, Mora NJ, Broseta AM, Gimeno A, de-la-Concepcion N, Vina J, Pallardo FV.
483 The depletion of nuclear glutathione impairs cell proliferation in 3T3 fibroblasts. PLoS
484 One. 2009;4:e6413.

485 Marrer E, Dieterle F. Impact of biomarker development on drug safety assessment.
486 Toxicology and Applied Pharmacology. 2010;243(2):167-179.
487 doi:10.1016/j.taap.2009.12.015.

488 Milardi D, et al. Proteomic approach in the identification of fertility pattern in seminal
489 plasma of fertile men. Fertility and Sterility. 2012;97(1):67-73.
490 doi:10.1016/j.fertnstert.2011.10.013.

491 Morrell JM, Rocha A. A Novel Approach to Minimising Acute Equine Endometritis That
492 May Help to Prevent the Development of the Chronic State. Frontiers in Veterinary
493 Science. 2022;8:799619. doi:10.3389/fvets.2021.799619.

494 Morris AR, Reichenbach L, Soares BP, Gionbelli MP, Freitas-Dell'Aqua CP, Barros VR,
495 Dell'Aqua JA. Endometritis in the mare: Challenges and future perspectives. Veterinary
496 Medicine: Research and Reports. 2020;11:179-189.

497 Müller-Schöttle F, Bogusz A, Grötzinger J, Herrler A, Krusche CA, Beier-Hellwig K,
498 Beier HM. Full-length complementary DNA and the derived amino acid sequence of
499 horse uteroglobin. Biology of Reproduction. 2002;66:1723-1728.

500 Plantier, J. L., Duretz, V., Devos, V., Urbain, R., & Jorieux, S. (2016). Comparison of
501 antioxidant properties of different therapeutic albumin preparations. Biologicals, 44(4),
502 226–233. https://doi.org/10.1016/j.biologicals.2016.04.002

503 Pelin M, et al. Role of Oxidative Stress Mediated by Glutathione-S-transferase in
504 Thiopurines' Toxic Effects. Chemical Research in Toxicology. 2015;28(6):1186-1195.
505 doi:10.1021/acs.chemrestox.5b00019.

506 Ramírez-López CJ, Barros E, Vidigal PM, Silva DO, Gomes LL, Carvalho RPR, Castro
507 AG, Baracat-Pereira MC, Guimarães SEF, Guimarães JD. Oxidative stress associated
508 with proteomic and fatty acid profiles of sperm from Nellore bulls at rest. *Biology of*
509 *Reproduction*. 2023; ioad121. doi.org/10.1093/biolre/ioad121

510 Rebordão MR, Amaral A, Lukasik K, Szóstek-Mioduchowska A, Pinto-Bravo P, Galvão
511 A, Skarzynski DJ, Ferreira-Dias G. Impairment of the antifibrotic prostaglandin E2
512 pathway may influence neutrophil extracellular traps-induced fibrosis in the mare
513 endometrium. Domestic Animal Endocrinology. 2019;67:1-10.
514 doi:10.1016/j.domaniend.2018.10.004.

515 Samper JC. A review of a practitioner's perspective on endometrial edema.
516 Pferdeheilkunde. 2010;26:14-18.

517 Schoon HA, Schoon D, Klug E. Uterusbiopsien als Hilfsmittel für Diagnose und
518 Prognose von Fertilitätsstörungen der Stute. Pferdeheilkunde. 1992;8:355-362.

519 Scoggin CF. Endometritis: Nontraditional Therapies. Veterinary Clinics of North
520 America - Equine Practice. 2016. doi:10.1016/j.cveq.2016.08.002.

521 Smits K, et al. Proteins involved in embryo-maternal interaction around the signaling of
522 maternal recognition of pregnancy in the horse. Scientific Reports. 2018;8(1):11546.
523 doi:10.1038/s41598-018-23537-6.

524 Swegen A. Current understanding and management of endometritis in mares. Animals.
525 2021;11(1):182.

526 Swegen A, Grupen CG, Gibb Z, Baker MA, de Ruijter-Villani M, Smith ND, Aitken RJ.
527 From Peptide Masses to Pregnancy Maintenance: A Comprehensive Proteomic Analysis
528 of The Early Equine Embryo Secretome, Blastocoel Fluid, and Capsule. Proteomics.
529 2017;17(17–18). doi:10.1002/pmic.201600433.

530 Talukder S, Kerrisk KL, Gabai G, Celi P. Role of oxidant-antioxidant balance in
531 reproduction of domestic animals. Animal Production Science. 2017;57:1588-1597.
532 doi:10.1071/AN15619.

533 Teixeira-Soares CM, et al. Comparison Between Gynecological Examination Methods
534 and Sample Collection Techniques for the Diagnosis of Endometritis in Subfertile Mares.
535 Journal of Equine Veterinary Science. 2022;119:104147.
536 doi:10.1016/j.jevs.2022.104147.

537 Thézénas, M. L., De Leo, B., Laux-Biehlmann, A., Bafligil, C., Elger, B., Tapmeier, T.,
538 … Oppermann, U. (2020). Amine oxidase 3 is a novel pro-inflammatory marker of
539 oxidative stress in peritoneal endometriosis lesions. *Scientific Reports*, *10*(1).
540 https://doi.org/10.1038/s41598-020-58362-3

541 Troedsson MHT. Endometritis. In: McKinnon AO, Squires EL, Vaala WE, Varner DD,
542 editors. Equine Reproduction. Second Edition. U.K: Wiley-Blackwell; 2011. p. 421-429.

543 Valacchi G, Virgili F, Cervellati C, Pecorelli A. OxInflammation: From subclinical
544 condition to pathological biomarker. Frontiers in Physiology. 2018;9:858.
545 doi:10.3389/fphys.2018.00858.

546 Viana AGA, Martins AMA, Pontes AH, Fontes W, Castro MS, Ricart CAO, Moura AA.
547 Proteomic landscape of seminal plasma associated with dairy bull fertility. Scientific
548 Reports. 2018;8(1). doi:10.1038/s41598-018-34152-w.

549 Waugh SJ, Lawson EF, Gibb Z, Skerrett-Byrne DA, Swegen A. Embryo-maternal
550 interactions revealed by phosphoproteomic analysis of endometrial organoids exposed to
551 embryo secretome. Journal of Equine Veterinary Science. 2023;125:104794.
552 doi:10.1016/j.jevs.2023.104794.

553 Wei H, Wang JY. Role of Polymeric Immunoglobulin Receptor in IgA and IgM
554 Transcytosis. International Journal of Molecular Sciences. 2021;22(5):2284.
555 doi:10.3390/ijms22052284.

556 Winterbourn CC, Kettle AJ, Hampton MB. Reactive Oxygen Species and Neutrophil
557 Function. Annual Review of Biochemistry. 2016;85:765–792. doi:10.1146/annurev-
558 biochem-060815-014442.

559 Wolf CA, et al. Effect of corticotherapy on proteomics of endometrial fluid from mares
560 susceptible to persistent post-breeding endometritis. Theriogenology. 2012;77(7):1351-
561 1359. doi:10.1016/j.theriogenology.2011.10.042.

562 Woodward EM, Troedsson MHT. Equine Breeding-Induced Endometritis: A Review.
563 Journal of Equine Veterinary Science. 2013. doi:10.1016/j.jevs.2012.11.010.

564 Woodward EM, Troedsson MHT. Inflammatory mechanisms of endometritis. Equine
565 Veterinary Journal. 2015;47:20-30. doi:10.1111/evj.12403.

566 Zhang J, Xin L, Shan B, Chen W, Xie M, Yuen D, Ma B. PEAKS DB: De novo
567 sequencing assisted database search for sensitive and accurate peptide identification.
568 Molecular and Cellular Proteomics. 2012;11(4). doi:10.1074/mcp.M111.010587.

569 Zhong RZ, Zhou DW. Oxidative stress and role of natural plant derived antioxidants in
570 animal reproduction. Journal of Integrative Agriculture. 2013;12:1826-1838.
571 doi:10.1016/S2095-3119(13)60412-8.

Supplementary Table S1. Reproductive data and test results for the diagnosis of endometritis for subdivisions in experimental groups: control group of mares without endometritis or healthy, group of mares with infectious endometritis and group of mares with post mating endometritis. N = 24.

Mare	Age (years)	Reproductive history	IUF diameter (mm)	Cytology (% of neutrophil)	Microbiological culture	Histopathological grade	Diagnosis group
01	05	absence of IUF and normal embryonic recovery*	0	1%	no growth	I**	Control group
02	09	absence of IUF and normal embryonic recovery	0	0%	no growth	I	Control group
03	07	absence of IUF and normal embryonic recovery	0	0%	no growth	I	Control group
04	10	absence of IUF and normal embryonic recovery	0	1%	no growth	I	Control group
05	08	absence of IUF and normal embryonic recovery	0	0%	no growth	I	Control group
06	06	absence of IUF and normal embryonic recovery	0	0%	no growth	I	Control group
07	06	absence of IUF and normal embryonic recovery	0	0%	no growth	I	Control group
08	05	absence of IUF and normal embryonic recovery	0	0%	no growth	I	Control group
09	11	absence of embryonic recovery in 3 last cycles	12	8%	*Escherichia coli*	II**	Infectious endometritis
10	12	absence of embryonic recovery in 3 last cycles	14	4%	*Streptococcus* spp	II	Infectious endometritis
11	08	absence of embryonic recovery in 3 last cycles	0	6%	*Escherichia coli*	II	Infectious endometritis
12	07	IUF accumulation during estrus	10	4%	*Escherichia coli*	II	Infectious endometritis
13	11	absence of embryonic recovery in 3 last cycles	0	5%	*Staphylococcus* spp	II	Infectious endometritis
14	06	IUF accumulation during estrus	15	5%	*Streptococcus* spp	II	Infectious endometritis
15	08	IUF accumulation during estrus	22	3%	*Staphylococcus* spp	II	Infectious endometritis
16	12	absence of embryonic recovery in 3 last cycles	15	6%	*Escherichia coli*	II	Infectious endometritis
17	11	IUF accumulation after AI and poor embryonic recovery*	23	10%	no growth	II	Post-breeding endometritis
18	11	IUF accumulation after AI and poor embryonic recovery	30	8%	no growth	II	Post-breeding endometritis
19	09	IUF accumulation after AI	24	7%	no growth	II	Post-breeding endometritis
20	06	IUF accumulation after AI	32	10%	no growth	II	Post-breeding endometritis
21	10	IUF accumulation after AI and poor embryonic recovery	28	10%	no growth	II	Post-breeding endometritis
22	09	IUF accumulation after AI and poor embryonic recovery	22	12%	no growth	II	Post-breeding endometritis
23	10	IUF accumulation after AI and poor embryonic recovery	26	8%	no growth	II	Post-breeding endometritis
24	07	IUF accumulation after AI	24	6%	no growth	II	Post-breeding endometritis

*Normal embryonic recovery rate (>60%) and poor embryonic recovery (<20%). **Histopathological grade I = negative endometritis and grade II = positive endometritis.

Supplementary table S2. Endometrial fluid proteins from control (healthy) mares and mares with infectious or post-breeding endometritis

Accession	Protein name	Detected in:			Number of samples		
		Control (healthy)	Infectious endometritis	Post-breeding endometritis	Control (healthy)	Infectious endometritis	Post-breeding endometritis
A0A3Q2I8H8	Galectin-3-binding protein	Yes	Yes	Yes	8	8	8
A0A3Q2IDD2	Complement C2	Yes	Yes	Yes	8	8	8
A0A5F5PJL2	Mucin 4, cell surface associated	Yes	Yes	Yes	8	8	8
F6PQ46	Ceruloplasmin	Yes	Yes	Yes	8	8	8
F6VAS5	Vanin 2	Yes	Yes	Yes	8	8	8
F6VJR6	Alpha-1-B glycoprotein	Yes	Yes	Yes	8	8	8
F6W2K5	Polymeric immunoglobulin receptor	Yes	Yes	Yes	8	8	8
H9GZQ9	Immunoglobulin heavy constant mu	Yes	Yes	Yes	8	8	8
Q29482	Clusterin	Yes	Yes	Yes	8	8	8
Q95M34	Immunoglobulin gamma 1 heavy chain constant region (Fragment)	Yes	Yes	Yes	7	8	8
A0A3Q2HWQ6	Complement C3	Yes	Yes	Yes	8	7	8
A0A3Q2I427	Leucine rich alpha-2-glycoprotein 1	Yes	Yes	Yes	8	7	8
F6WFF6	Tartrate-resistant acid phosphatase type 5	Yes	Yes	Yes	8	7	8
F6YV40	Glyceraldehyde-3-phosphate dehydrogenase	Yes	Yes	Yes	8	7	8
F6Z129	Vanin 1	Yes	Yes	Yes	8	7	8
F7CZW9	Serpin family G member 1	Yes	Yes	Yes	8	7	8
A0A3Q2HW24	Joining chain of multimeric IgA and IgM	Yes	Yes	Yes	7	7	8
A0A5K1UTF7	Phospholipase A2	Yes	Yes	Yes	7	7	8
B7S833	chitinase	Yes	Yes	Yes	7	7	8
F6WR95	Sulfhydryl oxidase	Yes	Yes	Yes	7	7	8

F6YQM5	Prosaposin	Yes	Yes	Yes	7	7	8
F7APU2	Complement factor I	Yes	Yes	Yes	7	7	8
H9GZV1	Ig-like domain-containing protein	Yes	Yes	Yes	7	7	8
F6WDW3	Keratin 10A	Yes	Yes	Yes	6	7	8
F7AKV6	Stanniocalcin 1	Yes	Yes	Yes	6	7	8
A0A3Q2I1X0	CN hydrolase domain-containing protein	Yes	Yes	Yes	8	6	8
A2Q0Z1	Heat shock cognate 71 kDa protein	Yes	Yes	Yes	8	6	8
F6QP34	WAP four-disulfide core domain 2	Yes	Yes	Yes	8	6	8
F6WFS0	Ephrin A1	Yes	Yes	Yes	8	6	8
F7BPT4	Ezrin	Yes	Yes	Yes	8	6	8
F6YNT8	Phosphatidylethanolamine binding protein 4	Yes	Yes	Yes	7	6	8
P0DUB3	Immunoglobulin heavy constant alpha (Fragment)	Yes	Yes	Yes	7	6	8
F6X6A6	Peptidyl-prolyl cis-trans isomerase	Yes	Yes	Yes	6	6	8
F6ZC75	Thy-1 cell surface antigen	Yes	Yes	Yes	6	6	8
F7BC37	Serine protease 8	Yes	Yes	Yes	6	6	8
F6XRY7	Cystatin E/M	Yes	Yes	Yes	4	6	8
A0A3Q2LTF6	Mucin 6, oligomeric mucus/gel-forming	Yes	Yes	Yes	8	5	8
F6ZHQ5	Chloride intracellular channel protein	Yes	Yes	Yes	8	5	8
F6R6Z6	Epithelial cell adhesion molecule	Yes	Yes	Yes	7	5	8
F6TJX5	Tripeptidyl-peptidase 1	Yes	Yes	Yes	7	5	8
A0A5F5PQU7	Peptidylglycine alpha-amidating monooxygenase	Yes	Yes	Yes	6	5	8
A0A3Q2I1S9	Matrix metallopeptidase 26	Yes	Yes	Yes	5	5	8
A0A3Q2IAY9	Histone H4	Yes	Yes	Yes	4	5	8

F6T7K7	Transcobalamin 1	Yes	Yes	Yes	4	5	8
F7AB03	Granulin	Yes	Yes	Yes	4	5	8
A0A5F5PYS7	Keratin, type II cytoskeletal 1	Yes	Yes	Yes	8	4	8
F6USP9	Plasminogen	Yes	Yes	Yes	7	4	8
A0A3Q2HD15	Cathepsin B	Yes	Yes	Yes	5	4	8
F6X9U4	Dystroglycan 1	Yes	Yes	Yes	4	3	8
F6YA47	Alpha-2-macroglobulin like 1	Yes	Yes	Yes	4	2	8
A0A3Q2HVB6	Complement component 4 binding protein alpha	Yes	Yes	Yes	3	1	8
Q8HZM6	Annexin A1	Yes	Yes	Yes	8	8	7
F6XWM5	Haptoglobin	Yes	Yes	Yes	7	8	7
A0A3Q2H477	Albumin	Yes	Yes	Yes	7	8	7
F6Z2L5	Apolipoprotein E	Yes	Yes	Yes	7	7	7
A0A5F5PWY1	Fetuin B	Yes	Yes	Yes	6	7	7
F7CWC8	Amine oxidase	Yes	Yes	Yes	6	7	7
F6ZI35	Histidine rich glycoprotein	Yes	Yes	Yes	7	6	7
A0A3Q2GYK6	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta	Yes	Yes	Yes	6	6	7
F6X1X3	Vitelline membrane outer layer 1 homolog	Yes	Yes	Yes	6	6	7
F6XZQ1	Calcyphosine	Yes	Yes	Yes	5	6	7
F6YG82	78 kDa glucose-regulated protein	Yes	Yes	Yes	5	6	7
F7DXG8	Cofilin 1	Yes	Yes	Yes	5	6	7
F7BL38	Mucin 1, cell surface associated	Yes	Yes	Yes	7	5	7
F7CYR1	Antithrombin-III	Yes	Yes	Yes	7	5	7
F6XBM5	Golgi apparatus protein 1	Yes	Yes	Yes	6	5	7

A0A061DBP6	von Willebrand factor	Yes	Yes	Yes	5	5	7
F6W2Y1	Fibrinogen gamma chain	Yes	Yes	Yes	4	4	7
F7CU94	Lysozyme	Yes	Yes	Yes	2	4	7
F6UJ33	Profilin	Yes	Yes	Yes	5	3	7
O97508	Thioredoxin	Yes	Yes	Yes	5	3	7
A0A3Q2HQD7	Neuropilin	Yes	Yes	Yes	3	3	7
A2Q0Z0	Elongation factor 1-alpha 1	Yes	Yes	Yes	6	2	7
A0A3Q2HSY2	Secretory leukocyte peptidase inhibitor	Yes	Yes	Yes	3	2	7
P0DUB1	Polymeric immunoglobulin receptor	Yes	Yes	Yes	4	1	7
A0A3Q2HF40	Mucin-5B	Yes	Yes	Yes	3	1	7
A0A3Q2KXH7	Midkine	Yes	Yes	Yes	2	1	7
P30441	Beta-2-microglobulin	Yes	No	Yes	5	0	7
F7C0V8	Serpin family B member 7	Yes	Yes	Yes	7	7	6
A0A3Q2HNV2	Insulin-like growth factor-binding protein 2	Yes	Yes	Yes	6	6	6
F6S6J4	Peroxiredoxin-1	Yes	Yes	Yes	6	6	6
D5SHI5	Serine peptidase inhibitor clade A (alpha-1 antiproteinase, antitrypsin) member 14	Yes	Yes	Yes	4	6	6
F6ZSQ2	Uteroglobin	Yes	Yes	Yes	7	5	6
A0A3Q2IC05	Serpin family B member 1	Yes	Yes	Yes	5	5	6
Q28377	Fibronectin	Yes	Yes	Yes	5	5	6
F6SRP7	Adenylyl cyclase-associated protein	Yes	Yes	Yes	4	5	6
F6RM73	Apolipoprotein A-II	Yes	Yes	Yes	5	4	6
A0A3Q2GX59	Lymphocyte cytosolic protein 1	Yes	Yes	Yes	4	4	6
F7BYG0	Peroxidasin	Yes	Yes	Yes	4	4	6
F6PUX2	Moesin	Yes	Yes	Yes	3	4	6

I1VZ96	Uteroglobin	Yes	Yes	Yes	3	4	6
A0A5F5Q0V8	Alpha-2-HS-glycoprotein	Yes	Yes	Yes	5	3	6
F6X485	Myosin heavy chain 9	Yes	Yes	Yes	4	3	6
A0A3Q2I4F4	WD repeat domain 1	Yes	Yes	Yes	2	3	6
F7D1R1	Phosphoglycerate kinase	Yes	Yes	Yes	2	3	6
F7D9J2	Transketolase	Yes	Yes	Yes	2	3	6
A0A3Q2L5E0	IF rod domain-containing protein	Yes	Yes	Yes	1	3	6
F6TZS9	Triosephosphate isomerase	Yes	Yes	Yes	7	2	6
A0A5F5PR55	Actin-related protein 2/3 complex subunit 4	Yes	Yes	Yes	2	2	6
F6V6R7	Prolactin-inducible protein homolog	Yes	Yes	Yes	2	2	6
F7BZ88	Myeloperoxidase	Yes	Yes	Yes	2	2	6
A0A3Q2HZX9	Azurocidin 1	No	Yes	Yes	0	2	6
F6Y4K8	Spondin 2	Yes	Yes	Yes	1	1	6
F7BJD8	Cathepsin H	Yes	Yes	Yes	1	1	6
A0A5F5PLP9	ADP-ribosylarginine hydrolase	Yes	No	Yes	2	0	6
A0A3Q2IDK5	t-plasminogen activator	Yes	No	Yes	1	0	6
F7BTW7	Complement C3	No	No	Yes	0	0	6
A0A3Q2I334	Superoxide dismutase [Cu-Zn]	Yes	Yes	Yes	8	6	5
A0A3Q2LPE6	Annexin	Yes	Yes	Yes	7	6	5
F6TIR2	Lipocalin 2	Yes	Yes	Yes	7	6	5
F7CWT0	P19 lipocalin	Yes	Yes	Yes	7	6	5
F7CKH3	Aldehyde dehydrogenase 1 family member A1	Yes	Yes	Yes	4	6	5
A0A3Q2GXQ7	Galectin	Yes	Yes	Yes	6	5	5
F7BKE1	Serpin family F member 1	Yes	Yes	Yes	4	5	5
F6W3I4	Otoancorin	Yes	Yes	Yes	6	4	5
F6X1I8	Hemopexin	Yes	Yes	Yes	6	4	5

A0A5F5PQC9	G protein-coupled receptor class C group 5 member C	Yes	Yes	Yes	5	4	5
F6QXN5	Transgelin	Yes	Yes	Yes	4	4	5
F6PH38	Fibrinogen beta chain	Yes	Yes	Yes	3	4	5
Q9GKX8	Heat shock protein HSP 90-beta	Yes	Yes	Yes	3	4	5
A0A3Q2LKR5	L-lactate dehydrogenase	Yes	Yes	Yes	2	4	5
F7D917	6-phosphogluconate dehydrogenase, decarboxylating	Yes	Yes	Yes	2	4	5
A0A3Q2HAN3	Protein S	Yes	Yes	Yes	5	3	5
A5YBL8	Peptidyl-prolyl cis-trans isomerase	Yes	Yes	Yes	5	3	5
A0A3Q2HN78	Fructose-bisphosphate aldolase	Yes	Yes	Yes	4	3	5
F6XSF7	Uncharacterized protein	Yes	Yes	Yes	3	3	5
F7BK45	Abhydrolase domain containing 14B	Yes	Yes	Yes	3	3	5
A0A3Q2HG20	Actinin alpha 1	Yes	Yes	Yes	2	3	5
A0A3Q2HTG2	Fibrinogen alpha chain	Yes	Yes	Yes	2	3	5
F6W1S0	Resistin	Yes	Yes	Yes	1	3	5
A0A3Q2I574	Olfactomedin 4	No	Yes	Yes	0	3	5
F7AEK5	Alkaline phosphatase	Yes	Yes	Yes	5	2	5
F7DQS6	Phosphoglycerate mutase	Yes	Yes	Yes	5	2	5
F6YY66	Nucleoside diphosphate kinase	Yes	Yes	Yes	4	2	5
F6Z4J4	Rab GDP dissociation inhibitor	Yes	Yes	Yes	4	2	5
A0A3Q2GT03	Syndecan binding protein	Yes	Yes	Yes	2	2	5
F7BU09	Glutathione S-transferase	Yes	Yes	Yes	2	2	5
K9KB03	Rho GDP-dissociation inhibitor 2-like protein	Yes	Yes	Yes	2	2	5
F7AED2	Lipocalin/cytosolic fatty-acid binding domain-containing protein	No	Yes	Yes	0	2	5
F7BND9	Protein S100	Yes	Yes	Yes	5	1	5

A0A3Q2KJH8	Desmocollin 2	Yes	Yes	Yes	4	1	5
F7C2Y5	AE binding protein 1	Yes	Yes	Yes	4	1	5
F6W354	Actin related protein 3	Yes	Yes	Yes	2	1	5
A0A3Q2HN65	Proteinase 3	Yes	Yes	Yes	1	1	5
A0A3Q2IEQ4	Sorcin	Yes	Yes	Yes	1	1	5
F6PNM1	45 kDa calcium-binding protein	Yes	Yes	Yes	1	1	5
F6TQR2	Calreticulin	Yes	Yes	Yes	1	1	5
F7CQ86	Lipocalin/cytosolic fatty-acid binding domain-containing protein	Yes	Yes	Yes	1	1	5
F6YLN3	ADAM metallopeptidase with thrombospondin type 1 motif 1	No	No	Yes	0	0	5
A0A5F5PGW4	Ig-like domain-containing protein	Yes	Yes	Yes	7	6	4
F6S6M5	Carboxypeptidase Q	Yes	Yes	Yes	6	5	4
F6VSN2	Glutathione S-transferase	Yes	Yes	Yes	4	5	4
F7DW69	Heat shock 70kDa protein 1A	Yes	Yes	Yes	7	4	4
F6VUW2	Cathepsin S	Yes	Yes	Yes	5	4	4
F6R1I4	Uteroglobin	Yes	Yes	Yes	4	4	4
F6X8Q2	Phosphoglucomutase 1	Yes	Yes	Yes	3	4	4
F6YAV2	Serpin family B member 11	Yes	Yes	Yes	3	4	4
F6ZE54	Glucose-6-phosphate isomerase	Yes	Yes	Yes	3	4	4
F6QXW2	Phosphatidylethanolamine binding protein 1	Yes	Yes	Yes	5	3	4
F6Q8R8	Divergent protein kinase domain 2A	Yes	Yes	Yes	4	3	4
A0A5F5PF23	Transaldolase	Yes	Yes	Yes	3	3	4
F6T3Y8	Actin beta	Yes	Yes	Yes	3	3	4
F6R5B2	High mobility group box 2	No	Yes	Yes	0	3	4
F7B6C3	Junctional adhesion molecule A	Yes	Yes	Yes	6	2	4
F6Y0D9	Cadherin 1	Yes	Yes	Yes	5	2	4

A0A3Q2L4I6	Alpha-mannosidase	Yes	Yes	Yes	3	2	4
Q3S4D6	GM2 activator protein	Yes	Yes	Yes	2	2	4
A0A3Q2HI93	Phospholipid transfer protein	Yes	Yes	Yes	1	2	4
F6RCA8	Peroxiredoxin-5	Yes	Yes	Yes	1	2	4
F7C456	UPAR/Ly6 domain-containing protein	Yes	Yes	Yes	1	2	4
A0A5F5PZU4	Matrix metallopeptidase 9	No	Yes	Yes	0	2	4
F7B5C4	Vimentin	No	Yes	Yes	0	2	4
A0A3Q2HRV7	Profilin	Yes	Yes	Yes	1	1	4
F7D216	Kazal-like domain-containing protein	Yes	Yes	Yes	1	1	4
A0A3Q2I063	Fibronectin type-II domain-containing protein	No	Yes	Yes	0	1	4
A0A3Q2KYS2	Bactericidal permeability-increasing protein	No	Yes	Yes	0	1	4
F6RP73	Cathelicidin antimicrobial peptide	No	Yes	Yes	0	1	4
F7D2U1	Phospholipase B-like	No	Yes	Yes	0	1	4
O19010	Cysteine-rich secretory protein 3	No	Yes	Yes	0	1	4
F6TA04	Out at first protein homolog	Yes	No	Yes	1	0	4
F7B5T3	Hyaluronidase	Yes	No	Yes	1	0	4
F7DJE3	Surfactant protein D	Yes	No	Yes	1	0	4
F7DZV9	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein beta	Yes	No	Yes	1	0	4
K9K2B0	Tetraspanin	Yes	No	Yes	1	0	4
A0A3Q2HEG1	Fibronectin type-II domain-containing protein	No	No	Yes	0	0	4
F6QB61	Peptidoglycan-recognition protein	No	No	Yes	0	0	4
F6Z563	S100 calcium binding protein P	No	No	Yes	0	0	4

A0A5F5PR26	Malate dehydrogenase	Yes	Yes	Yes	4	6	3
F6S9R7	Annexin	Yes	Yes	Yes	4	5	3
A0A3Q2L2A7	Membrane metalloendopeptidase	Yes	Yes	Yes	5	4	3
P55102	Inhibin beta A chain	Yes	Yes	Yes	5	4	3
A0A3Q2HC96	Carboxypeptidase	Yes	Yes	Yes	4	4	3
F6VSN9	Protein disulfide-isomerase	Yes	Yes	Yes	3	4	3
F7B821	Protein disulfide-isomerase	Yes	Yes	Yes	2	4	3
F6VE62	Beta-hexosaminidase	Yes	Yes	Yes	5	3	3
A0A3Q2HRJ9	Retinoic acid receptor responder 1	Yes	Yes	Yes	4	3	3
A0A3Q2I5T2	Cystatin C	Yes	Yes	Yes	4	3	3
F7AXI9	Peroxiredoxin-6	Yes	Yes	Yes	4	3	3
F6W039	Rho GDP dissociation inhibitor alpha	Yes	Yes	Yes	3	3	3
F6XA04	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein epsilon	Yes	Yes	Yes	2	3	3
F6ZAK1	Annexin	Yes	Yes	Yes	4	2	3
F6YW90	Plasminogen activator, urokinase receptor	Yes	Yes	Yes	3	2	3
F7E3Y7	Heat shock protein beta-1	Yes	Yes	Yes	3	2	3
A0A5F5PFG3	Adenosylhomocysteinase	Yes	Yes	Yes	2	2	3
F6SP74	Complement factor properdin	Yes	Yes	Yes	2	2	3
A0A3Q2HRN8	Rac family small GTPase 2	Yes	Yes	Yes	1	2	3
F6YRE7	Leukotriene A(4) hydrolase	Yes	Yes	Yes	1	2	3
A0A3Q2HMR5	Actin related protein 2	No	Yes	Yes	0	2	3
A0A3Q2IBE3	UTP--glucose-1-phosphate uridylyltransferase	No	Yes	Yes	0	2	3
A0A5F5PKL6	Cathepsin G	No	Yes	Yes	0	2	3

F6QIZ4	Talin 1	No	Yes	Yes	0	2	3
F6VP61	Serpin family B member 10	No	Yes	Yes	0	2	3
F6W6E0	Galectin	No	Yes	Yes	0	2	3
F6Y014	F-actin-capping protein subunit beta	No	Yes	Yes	0	2	3
F7CSL8	Alpha-1-antitrypsin	Yes	Yes	Yes	4	1	3
A0A3Q2H4E3	Solute carrier family 34 member 2	Yes	Yes	Yes	3	1	3
F6WWW7	F-actin-capping protein subunit alpha	Yes	Yes	Yes	2	1	3
H9GZM1	Growth arrest specific 6	Yes	Yes	Yes	2	1	3
K9K2L1	Aldo-keto reductase family 1 member A1	Yes	Yes	Yes	2	1	3
Q8MKD1	Polyubiquitin-B	Yes	Yes	Yes	2	1	3
F6WFZ5	Serpin domain-containing protein	Yes	Yes	Yes	1	1	3
F6ZSZ5	Vinculin	Yes	Yes	Yes	1	1	3
F7DEB1	Insulin like growth factor binding protein 6	Yes	Yes	Yes	1	1	3
K9K202	Cell division control protein 42 homolog	Yes	Yes	Yes	1	1	3
Q95182	Major allergen Equ c 1	Yes	Yes	Yes	1	1	3
A0A3Q2HBY9	Fibronectin type-II domain-containing protein	No	Yes	Yes	0	1	3
A0A3Q2HNU3	Fibronectin type-II domain-containing protein	No	Yes	Yes	0	1	3
A0A3Q2HUK6	H1.5 linker histone, cluster member	No	Yes	Yes	0	1	3
A0A3Q2I5V1	Copine 3	No	Yes	Yes	0	1	3
A0A3Q2KWM2	WAP domain-containing protein	No	Yes	Yes	0	1	3
F6Q6X3	Coronin	No	Yes	Yes	0	1	3
F6S9Z1	C-X-C motif chemokine	No	Yes	Yes	0	1	3
F6T8Y6	Neutrophil cytosolic factor 2	No	Yes	Yes	0	1	3

F6XC16	Vesicle amine transport 1	No	Yes	Yes	0	1	3
F7DMG5	Glucose-6-phosphate 1-dehydrogenase	No	Yes	Yes	0	1	3
Q6H321	Kallikrein-1E2	No	Yes	Yes	0	1	3
Q712U3	Seminal plasma protein-1 (Fragment)	No	Yes	Yes	0	1	3
F7DXY0	Glutathione hydrolase	Yes	No	Yes	3	0	3
F6RMM1	SH3 domain-binding glutamic acid-rich-like protein	Yes	No	Yes	1	0	3
F6WGX6	Keratin 77	No	No	Yes	0	0	3
F7D301	RAB11B, member RAS onco family	No	No	Yes	0	0	3
H9GZQ2	Ig-like domain-containing protein	No	No	Yes	0	0	3
I3VZ33	Uteroglobin	No	No	Yes	0	0	3
F7DZ01	Coagulation factor V	Yes	Yes	Yes	6	4	2
H9GZT5	Ig-like domain-containing protein	Yes	Yes	Yes	6	4	2
F7DRS2	Serpin family A member 6	Yes	Yes	Yes	4	4	2
F6TVZ7	CutA divalent cation tolerance homolog	Yes	Yes	Yes	6	3	2
A0A3Q2I189	N-acetylglucosamine-6-sulfatase	Yes	Yes	Yes	2	3	2
F7C6F2	E1 ubiquitin-activating enzyme	Yes	Yes	Yes	2	3	2
A0A5F5PPC4	Beta-hexosaminidase	Yes	Yes	Yes	3	2	2
F7C1X7	Na(+)/H(+) exchange regulatory cofactor NHE-RF	Yes	Yes	Yes	3	2	2
A0A3Q2IF61	Calcium activated nucleotidase 1	Yes	Yes	Yes	2	2	2
F6W9B1	ST13 Hsp70 interacting protein	Yes	Yes	Yes	2	2	2
F6R5V4	Carboxypeptidase vitellogenic like	Yes	Yes	Yes	1	2	2
F6SN37	Protein S100	No	Yes	Yes	0	2	2
A0A3Q2HWP9	Transitional endoplasmic reticulum ATPase	Yes	Yes	Yes	3	1	2

F6R7J2	isocitrate dehydrogenase (NADP(+))	Yes	Yes	Yes	3	1	2
F6WFS9	Abhydrolase domain containing 14A	Yes	Yes	Yes	3	1	2
F7AZ73	Syntaxin binding protein 2	Yes	Yes	Yes	2	1	2
F6PH25	Sphingomyelin phosphodiesterase 1	Yes	Yes	Yes	1	1	2
F6Q4Q1	Heterogeneous nuclear ribonucleoprotein K	Yes	Yes	Yes	1	1	2
F7B3U1	G protein-coupled receptor class C group 5 member B	Yes	Yes	Yes	1	1	2
A0A3Q2GT23	Alpha-1,4 glucan phosphorylase	No	Yes	Yes	0	1	2
A0A3Q2HL41	Annexin	No	Yes	Yes	0	1	2
A0A3Q2HLN1	Beta-1,4-galactosyltransferase	No	Yes	Yes	0	1	2
A0A3Q2HQE1	RNA binding motif protein 12	No	Yes	Yes	0	1	2
A0A3Q2I4V9	Myosin light chain 12B	No	Yes	Yes	0	1	2
A0A3Q2LAX6	Kazal-like domain-containing protein	No	Yes	Yes	0	1	2
A0A3Q2LJK9	L-lactate dehydrogenase	No	Yes	Yes	0	1	2
A0A5F5PT34	Glycogenin 1	No	Yes	Yes	0	1	2
F6Q841	C-X-C motif chemokine	No	Yes	Yes	0	1	2
F6VZY5	Vasodilator stimulated phosphoprotein	No	Yes	Yes	0	1	2
F6YBT5	H(+)-transporting two-sector ATPase	No	Yes	Yes	0	1	2
F6YMM5	Transglutaminase 3	No	Yes	Yes	0	1	2
F7BHB6	Protein-arginine deiminase	No	Yes	Yes	0	1	2
F7DA17	Transglutaminase 2	No	Yes	Yes	0	1	2
F7DE06	Annexin	No	Yes	Yes	0	1	2
M1ZMK5	aldehyde oxidase	No	Yes	Yes	0	1	2
O02722	Metalloproteinase inhibitor 1	No	Yes	Yes	0	1	2
F7E419	Annexin	Yes	No	Yes	3	0	2
A0A3Q2I2H1	Ig-like domain-containing protein	Yes	No	Yes	1	0	2

A0A5F5PF14	Brain abundant membrane attached signal protein 1	Yes	No	Yes	1	0	2
F6PVG3	EGF containing fibulin extracellular matrix protein 1	Yes	No	Yes	1	0	2
A0A3Q2GVZ5	Jacalin-type lectin domain-containing protein	No	No	Yes	0	0	2
A0A3Q2HLK8	Secretoglobin family 1A member 1	No	No	Yes	0	0	2
A0A3Q2HU56	UPAR/Ly6 domain-containing protein	No	No	Yes	0	0	2
A0A3Q2I560	Sodium/nucleoside cotransporter	No	No	Yes	0	0	2
A0A3Q2LG11	Chitinase 3 like 1	No	No	Yes	0	0	2
A0A5F5PMH2	Ectonucleotide pyrophosphatase/phosphodiesterase 3	No	No	Yes	0	0	2
A0A5F5PST6	Arrestin domain containing 1	No	No	Yes	0	0	2
F6UCN5	Uridine phosphorylase	No	No	Yes	0	0	2
F6WE31	Actin-related protein 2/3 complex subunit 3	No	No	Yes	0	0	2
F6WER3	protein-tyrosine-phosphatase	No	No	Yes	0	0	2
F6ZL17	Serum amyloid A protein	No	No	Yes	0	0	2
F7E4G6	Heteroous nuclear ribonucleoprotein U	No	No	Yes	0	0	2
O62841	Myeloid cathelicidin 2	No	No	Yes	0	0	2
F6X0K3	Cellular communication network factor 2	Yes	Yes	Yes	4	7	1
F6QWH2	PKHD1 like 1	Yes	Yes	Yes	4	3	1
F6XNT7	Ribonuclease T2	Yes	Yes	Yes	4	3	1
F7BLN9	Hexosyltransferase	Yes	Yes	Yes	4	3	1
F6U954	Netrin 4	Yes	Yes	Yes	3	3	1

F6YX31	Transforming growth factor beta	Yes	Yes	Yes	2	3	1
A0A3Q2KV79	Rac family small GTPase 1	Yes	Yes	Yes	1	3	1
F7D6J7	Mesothelin	Yes	Yes	Yes	5	2	1
A0A3Q2HCD2	Aldo-keto reductase family 1 member B	Yes	Yes	Yes	4	2	1
B8Y403	Macrophage migration inhibitory factor	Yes	Yes	Yes	4	2	1
F6W5W1	Alpha glucosidase	Yes	Yes	Yes	4	2	1
F7B5P1	Carnosine dipeptidase 2	Yes	Yes	Yes	4	2	1
A0A3Q2HGX2	Palmitoyl-protein thioesterase 1	Yes	Yes	Yes	3	2	1
Q9XS63	Chromogranin-A	Yes	Yes	Yes	3	2	1
A0A3Q2HLX2	Choloylglycine hydrolase/NAAA C-terminal domain-containing protein	Yes	Yes	Yes	2	2	1
A0A3Q2I943	Iduronate 2-sulfatase	Yes	Yes	Yes	2	2	1
A0A3Q2LNN5	Glia maturation factor	No	Yes	Yes	0	2	1
F6QX36	Inter-alpha-trypsin inhibitor heavy chain 1	No	Yes	Yes	0	2	1
F6QSX9	Legumain	Yes	Yes	Yes	4	1	1
A0A3Q2HAG4	Phospholipase B-like	Yes	Yes	Yes	3	1	1
A0A3Q2LQH7	G protein subunit beta 1	Yes	Yes	Yes	3	1	1
E0A923	Transforming growth factor beta	Yes	Yes	Yes	3	1	1
F6SS69	Alpha-L-fucosidase	Yes	Yes	Yes	3	1	1
F6X2I3	Biotinidase	Yes	Yes	Yes	3	1	1
A0A3Q2H1S8	Filamin B	Yes	Yes	Yes	2	1	1
A0A3Q2HXM5	Alpha-galactosidase	Yes	Yes	Yes	2	1	1
F6PVN3	CD200 molecule	Yes	Yes	Yes	2	1	1
F6RRL9	Insulin-like growth factor-binding protein 3	Yes	Yes	Yes	2	1	1

F6T8U8	Semaphorin-3C	Yes	Yes	Yes	2	1	1
F6TLF7	Fibroblast growth factor	Yes	Yes	Yes	2	1	1
F6V812	ATPase H+ transporting V0 subunit a1	Yes	Yes	Yes	2	1	1
F6Z105	receptor protein-tyrosine kinase	Yes	Yes	Yes	2	1	1
F7B762	Pro-epidermal growth factor	Yes	Yes	Yes	2	1	1
F7BLK5	Aminopeptidase	Yes	Yes	Yes	2	1	1
A0A3Q2GYT0	Platelet derived growth factor D	Yes	Yes	Yes	1	1	1
A0A3Q2HC68	Granulysin	Yes	Yes	Yes	1	1	1
A0A3Q2HH68	Agrin	Yes	Yes	Yes	1	1	1
A0A3Q2HIQ0	Integrin subunit alpha M	Yes	Yes	Yes	1	1	1
A0A3Q2HYP3	Secreted phosphoprotein 1	Yes	Yes	Yes	1	1	1
A0A3Q2HZW1	Heat shock protein family A (Hsp70) member 2	Yes	Yes	Yes	1	1	1
A0A3Q2I1E2	Epiplakin 1	Yes	Yes	Yes	1	1	1
A0A3Q2ICB7	Desmoglein 2	Yes	Yes	Yes	1	1	1
A0A3Q2IDE0	Complement C2	Yes	Yes	Yes	1	1	1
A0A3Q2IJP6	porphobilinogen synthase	Yes	Yes	Yes	1	1	1
A0A3Q2L8V5	SEA domain-containing protein	Yes	Yes	Yes	1	1	1
A0A5F5PGX7	Ig-like domain-containing protein	Yes	Yes	Yes	1	1	1
A0A5F5PIE1	Attractin	Yes	Yes	Yes	1	1	1
A0A5F5PKC5	Titin	Yes	Yes	Yes	1	1	1
A0A5F5PKF4	ArfGAP with SH3 domain, ankyrin repeat and PH domain 2	Yes	Yes	Yes	1	1	1
A0A5F5PLA5	Amine oxidase	Yes	Yes	Yes	1	1	1
A0A5F5PLF6	CXADR Ig-like cell adhesion molecule	Yes	Yes	Yes	1	1	1
A0A5F5Q196	Prostaglandin E synthase 3	Yes	Yes	Yes	1	1	1
F6PVK7	Cadherin EGF LAG seven-pass G-type receptor 2	Yes	Yes	Yes	1	1	1

F6R8T8	N-acyl-aliphatic-L-amino acid amidohydrolase	Yes	Yes	Yes	1	1	1
F6RI26	Aldehyde dehydrogenase 9 family member A1	Yes	Yes	Yes	1	1	1
F6TNX4	Triokinase/FMN cyclase	Yes	Yes	Yes	1	1	1
F6VZN7	Ras homolog family member G	Yes	Yes	Yes	1	1	1
F6W3D6	Acrosin-binding protein	Yes	Yes	Yes	1	1	1
F6WDY8	Glucosylceramidase	Yes	Yes	Yes	1	1	1
F6YUR2	Cathepsin F	Yes	Yes	Yes	1	1	1
F6YZJ4	Heat shock 70 kDa protein 4	Yes	Yes	Yes	1	1	1
F6Z4B4	Heat shock 70 kDa protein 13	Yes	Yes	Yes	1	1	1
F6Z688	Proteasome subunit alpha type	Yes	Yes	Yes	1	1	1
F6ZFH9	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein gamma	Yes	Yes	Yes	1	1	1
F7BFJ1	Prothrombin	Yes	Yes	Yes	1	1	1
F7BR32	Galactocerebrosidase	Yes	Yes	Yes	1	1	1
F7BXA6	Aminopeptidase	Yes	Yes	Yes	1	1	1
F7CFF4	ABO, alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase	Yes	Yes	Yes	1	1	1
F7CJW5	Arylsulfatase A	Yes	Yes	Yes	1	1	1
A0A3Q2GW64	Heterogeneous nuclear ribonucleoproteins A2/B1	No	Yes	Yes	0	1	1
A0A5F5PG05	Integrin beta	No	Yes	Yes	0	1	1
A0A5F5PT22	Integrin beta	No	Yes	Yes	0	1	1
A0A5F5PTW4	FERM domain containing kindlin 3	No	Yes	Yes	0	1	1

F6RAT7	peptidylprolyl isomerase	No	Yes	Yes	0	1	1
F6YIU8	C-1-tetrahydrofolate synthase, cytoplasmic	No	Yes	Yes	0	1	1
F7B570	Actin-related protein 2/3 complex subunit 5	No	Yes	Yes	0	1	1
K9K2F2	Proteasome subunit alpha type (Fragment)	No	Yes	Yes	0	1	1
H9GZQ6	Follistatin like 3	Yes	No	Yes	5	0	1
F6VKZ3	Ig-like domain-containing protein	Yes	No	Yes	2	0	1
A0A3Q2HLF7	protein-tyrosine-phosphatase	Yes	No	Yes	1	0	1
A0A3Q2HR98	Ig-like domain-containing protein	Yes	No	Yes	1	0	1
A0A3Q2KTL8	Tryptophan--tRNA ligase, cytoplasmic	Yes	No	Yes	1	0	1
A0A3Q2LAH2	Multiple inositol polyphosphate phosphatase 1	Yes	No	Yes	1	0	1
A0A5F5PHD4	Fibulin-1	Yes	No	Yes	1	0	1
A0A5F5PSL8	Heteroous nuclear ribonucleoprotein D like	Yes	No	Yes	1	0	1
F6Q0M8	Collagen type IV alpha 2 chain	Yes	No	Yes	1	0	1
F6T4E1	Laminin subunit beta 3	Yes	No	Yes	1	0	1
F7ACR2	alpha-L-fucosidase	Yes	No	Yes	1	0	1
F7B8Z3	Beta-1,4-galactosyltransferase	Yes	No	Yes	1	0	1
F7D0B5	Beta-1,4-glucuronyltransferase 1	Yes	No	Yes	1	0	1
A0A3Q2GSZ5	Jacalin-type lectin domain-containing protein	No	No	Yes	0	0	1
A0A3Q2GTT9	Capping actin protein, gelsolin like	No	No	Yes	0	0	1
A0A3Q2GXY7	Cellular repressor of E1A stimulateds 1	No	No	Yes	0	0	1
A0A3Q2HL47	Lipase	No	No	Yes	0	0	1

A0A3Q2HUS7	Lipocalin/cytosolic fatty-acid binding domain-containing protein	No	No	Yes	0	0	1
A0A3Q2I4G7	protein-tyrosine-phosphatase	No	No	Yes	0	0	1
A0A3Q2KN19	Nucleobindin 1	No	No	Yes	0	0	1
A0A3Q2LD29	Fructose-bisphosphate aldolase	No	No	Yes	0	0	1
A0A5F5PLP2	Nectin cell adhesion molecule 2	No	No	Yes	0	0	1
A0A5F5PZS2	Potassium channel tetramerization domain containing 12	No	No	Yes	0	0	1
A0A5F5Q258	C-type lectin domain-containing protein	No	No	Yes	0	0	1
A0A5F5Q2T7	Peptidoglycan-recognition protein	No	No	Yes	0	0	1
A0A5F5Q379	Alpha-L-iduronidase	No	No	Yes	0	0	1
B7UBX3	C-X-C motif chemokine	No	No	Yes	0	0	1
F6PZY1	Tropomodulin 3	No	No	Yes	0	0	1
F6SIE2	Tyrosine kinase 2	No	No	Yes	0	0	1
F6TYW4	Malate dehydrogenase	No	No	Yes	0	0	1
F6V2X7	procollagen-lysine 5-dioxygenase	No	No	Yes	0	0	1
F6VHV0	Alpha-mannosidase	No	No	Yes	0	0	1
F6YK46	Phosphoglucomutase 2	No	No	Yes	0	0	1
F6Z5L1	Complement C1r	No	No	Yes	0	0	1
F6ZRN7	Peptidase inhibitor 3	No	No	Yes	0	0	1
F7BK61	Grancalcin	No	No	Yes	0	0	1
F7CK20	Tyrosine-protein kinase	No	No	Yes	0	0	1
F7DMZ7	Glycosyltransferase 8 domain containing 1	No	No	Yes	0	0	1
F7DWN5	G protein subunit alpha i2	No	No	Yes	0	0	1

K9KAI3	Erythrocyte band 7 integral membrane protein-like protein (Fragment)	No	No	Yes	0	0	1
S5M630	Uteroglobin (Fragment)	No	No	Yes	0	0	1
F6U5V3	Transcobalamin 2	Yes	Yes	No	2	3	0
F6W4R2	Angiotensinogen	Yes	Yes	No	2	3	0
F7B3I5	Afamin	Yes	Yes	No	2	3	0
F7ASE1	Interleukin 1 receptor accessory protein	Yes	Yes	No	1	3	0
A0A5F5PGY6	Complement component C6	Yes	Yes	No	4	2	0
F6Z8Y3	Basal cell adhesion molecule	Yes	Yes	No	4	2	0
A0A3Q2H8Y8	5'-nucleotidase	Yes	Yes	No	3	2	0
F6SKV7	Latent transforming growth factor beta binding protein 4	Yes	Yes	No	3	2	0
F6XQM8	Uroplakin 3B like 1	Yes	Yes	No	3	2	0
A0A3Q2LAN4	Aminopeptidase	Yes	Yes	No	2	2	0
F6Z2I7	Glutathione S-transferase	Yes	Yes	No	2	2	0
F7ALR7	Ectonucleotide pyrophosphatase/phosphodiesterase 1	Yes	Yes	No	2	2	0
F6ZUJ2	Endoplasmin-like protein	Yes	Yes	No	1	2	0
F7CV04	Laminin subunit gamma 1	Yes	Yes	No	1	2	0
A0A3Q2GTT0	Ig-like domain-containing protein	No	Yes	No	0	2	0
A0A3Q2H3G9	Lipocalin 15	No	Yes	No	0	2	0
A0A3Q2LC76	IST1 homolog	No	Yes	No	0	2	0
A0A5F5PM55	Inter-alpha-trypsin inhibitor heavy chain 2	No	Yes	No	0	2	0
F6RMQ1	GLI pathosis related 2	No	Yes	No	0	2	0

F6ZU48	Rac GTPase activating protein 1	No	Yes	No	0	2	0
F7A1L2	Complement C1r subcomponent like	No	Yes	No	0	2	0
F6WPB4	protein deglycase	Yes	Yes	No	3	1	0
F6Y2Q4	creatine kinase	Yes	Yes	No	3	1	0
F6Z041	Beta-2-glycoprotein 1	Yes	Yes	No	3	1	0
A0A3Q2IFY3	Adenylate kinase isoenzyme 1	Yes	Yes	No	2	1	0
A0A5F5PS32	Peptidase S1 domain-containing protein	Yes	Yes	No	2	1	0
F6Q892	Serpin family B member 9	Yes	Yes	No	2	1	0
F6TBN7	peptidylprolyl isomerase	Yes	Yes	No	2	1	0
F6TED1	Ig-like domain-containing protein	Yes	Yes	No	2	1	0
F6TZ61	Aldehyde dehydrogenase	Yes	Yes	No	2	1	0
F6XS73	Chromosome 13 C16orf89 homolog	Yes	Yes	No	2	1	0
F6ZCA4	Protein-arginine deiminase	Yes	Yes	No	2	1	0
F7CHI7	Lactoylglutathione lyase	Yes	Yes	No	2	1	0
K9KCF1	Glyoxalase domain-containing protein 4-like protein	Yes	Yes	No	2	1	0
A0A3Q2GXZ5	Cystatin domain-containing protein	Yes	Yes	No	1	1	0
A0A3Q2HHB0	Inter-alpha-trypsin inhibitor heavy chain 5	Yes	Yes	No	1	1	0
A0A3Q2ICA0	Plastin 3	Yes	Yes	No	1	1	0
A0A3Q2ICG4	Alpha 1,4-galactosyltransferase (P blood group)	Yes	Yes	No	1	1	0
A0A3Q2LL09	Slit guidance ligand 2	Yes	Yes	No	1	1	0
A0A5F5PLI4	Aspartylglucosaminidase	Yes	Yes	No	1	1	0
A0A5F5Q0C3	MANSC domain containing 1	Yes	Yes	No	1	1	0
F6PQA7	Arylsulfatase family member K	Yes	Yes	No	1	1	0
F6PSH3	Carboxypeptidase B2	Yes	Yes	No	1	1	0

F6QYS3	Extracellular matrix protein 1	Yes	Yes	No	1	1	0
F6SHJ8	IF rod domain-containing protein	Yes	Yes	No	1	1	0
F6V2B6	Charged multivesicular body protein 4B	Yes	Yes	No	1	1	0
F6X3N4	Peptidase S1 domain-containing protein	Yes	Yes	No	1	1	0
F6ZCL2	Semaphorin 3G	Yes	Yes	No	1	1	0
F7BK76	Macrophage stimulating 1	Yes	Yes	No	1	1	0
F7E1X2	Stress induced phosphoprotein 1	Yes	Yes	No	1	1	0
P01220	Glycoprotein hormones alpha chain	Yes	Yes	No	1	1	0
P08751	Lutropin/choriogonadotropin subunit beta	Yes	Yes	No	1	1	0
A0A3Q2GT15	Dipeptidyl peptidase 9	No	Yes	No	0	1	0
A0A3Q2H1C7	Serine peptidase inhibitor Kazal type 7	No	Yes	No	0	1	0
A0A3Q2H536	CD44 antigen	No	Yes	No	0	1	0
A0A3Q2HC57	GTP-binding nuclear protein Ran	No	Yes	No	0	1	0
A0A3Q2HHA5	Adenosine kinase	No	Yes	No	0	1	0
A0A3Q2HJ90	FAM3 metabolism regulating signaling molecule D	No	Yes	No	0	1	0
A0A3Q2HN68	PEAK family member 3	No	Yes	No	0	1	0
A0A3Q2HT63	Serpin domain-containing protein	No	Yes	No	0	1	0
A0A3Q2I386	Thymidine phosphorylase	No	Yes	No	0	1	0
A0A3Q2I4S3	Radixin	No	Yes	No	0	1	0
A0A3Q2I6Q2	Rho GTPase activating protein 25	No	Yes	No	0	1	0
A0A3Q2IAJ9	GNAS complex locus	No	Yes	No	0	1	0
A0A3Q2KSQ5	Cysteine rich protein 1	No	Yes	No	0	1	0

A0A3Q2KZQ1	ST3 beta-galactoside alpha-2,3-sialyltransferase 6	No	Yes	No	0	1	0
A0A3Q2L6Y5	WAP domain-containing protein	No	Yes	No	0	1	0
A0A3Q2L7X0	Proteasome subunit alpha type	No	Yes	No	0	1	0
A0A3Q2LB81	Myeloid cathelicidin 2	No	Yes	No	0	1	0
A0A5F5PEF5	Diazepam binding inhibitor, acyl-CoA binding protein	No	Yes	No	0	1	0
A0A5F5PPN1	peptide-methionine (S)-S-oxide reductase	No	Yes	No	0	1	0
A0A5F5PS90	40S ribosomal protein S3	No	Yes	No	0	1	0
A2Q127	Elongation factor 1-gamma	No	Yes	No	0	1	0
B8K1X7	40S ribosomal protein SA	No	Yes	No	0	1	0
F6RIZ2	Insulin like growth factor binding protein 7	No	Yes	No	0	1	0
F6RL46	6-phosphogluconolactonase	No	Yes	No	0	1	0
F6UB77	Protein kinase domain containing, cytoplasmic	No	Yes	No	0	1	0
F6UCU8	Lamin B1	No	Yes	No	0	1	0
F6Y4J0	Vacuolar proton pump subunit B	No	Yes	No	0	1	0
F7A6K3	Glucosaminyl (N-acetyl) transferase 3, mucin type	No	Yes	No	0	1	0
F7ANR3	Proteasome subunit alpha type	No	Yes	No	0	1	0
F7B1S5	BRO1 domain-containing protein BROX	No	Yes	No	0	1	0
F7C555	Bifunctional purine biosynthesis protein ATIC	No	Yes	No	0	1	0
F7D3E3	UMP-CMP kinase	No	Yes	No	0	1	0
F7DYV0	Galectin	No	Yes	No	0	1	0

K9K236	T-complex protein 1 subunit theta	No	Yes	No	0	1	0
P82615	Latherin	No	Yes	No	0	1	0
F6Z9H2	Prostate stem cell antigen	Yes	No	No	4	0	0
A0A5F5PZL7	protein-tyrosine-phosphatase	Yes	No	No	3	0	0
F6PWC8	Prostaglandin reductase 1	Yes	No	No	3	0	0
A0A3Q2H5U7	Immunoglobulin superfamily member 8	Yes	No	No	2	0	0
A0A3Q2I6F9	Keratin 82	Yes	No	No	2	0	0
A0A5F5PMV8	Lipocalin/cytosolic fatty-acid binding domain-containing protein	Yes	No	No	2	0	0
F6PQH3	Choline transporter-like protein	Yes	No	No	2	0	0
F6U065	Prominin 2	Yes	No	No	2	0	0
F6ZV64	Desmoplakin	Yes	No	No	2	0	0
F7AGY4	Keratin 73	Yes	No	No	2	0	0
F7BV85	Clathrin heavy chain	Yes	No	No	2	0	0
H9GZV0	Immunoglobulin heavy constant epsilon	Yes	No	No	2	0	0
Q8HZI9	Laminin subunit gamma-2	Yes	No	No	2	0	0
A0A3Q2GT53	C-type lectin domain family 3 member B	Yes	No	No	1	0	0
A0A3Q2GX86	Furin, paired basic amino acid cleaving enzyme	Yes	No	No	1	0	0
A0A3Q2H337	Polypeptide N-acetylgalactosaminyltransferase	Yes	No	No	1	0	0
A0A3Q2HYF9	Lymphocyte antigen 6 family member G6C	Yes	No	No	1	0	0
A0A3Q2I175	Aconitase 1	Yes	No	No	1	0	0
A0A3Q2I3I7	Dipeptidyl peptidase 4	Yes	No	No	1	0	0

A0A3Q2IEQ6	Notum, palmitoleoyl-protein carboxylesterase	Yes	No	No	1	0	0
A0A3Q2KUD5	Myosin VIIA	Yes	No	No	1	0	0
A0A3Q2LBP6	Complement factor D	Yes	No	No	1	0	0
A0A5F5PL54	EGF containing fibulin extracellular matrix protein 2	Yes	No	No	1	0	0
A0A5F5PMY8	P19 lipocalin	Yes	No	No	1	0	0
A0A5F5PVD1	Heparin binding growth factor	Yes	No	No	1	0	0
A0A5F5PZI1	Golgi membrane protein 2	Yes	No	No	1	0	0
F6PZ21	Multivesicular body subunit 12A	Yes	No	No	1	0	0
F6S5E7	threonine--tRNA ligase	Yes	No	No	1	0	0
F6T201	Chondroitin sulfate proteoglycan 4	Yes	No	No	1	0	0
F6V1T9	Junction plakoglobin	Yes	No	No	1	0	0
F6W8C8	Serpin family B member 6	Yes	No	No	1	0	0
F6XCQ3	Dynein cytoplasmic 2 heavy chain 1	Yes	No	No	1	0	0
F6XNR9	Collagen type XVIII alpha 1 chain	Yes	No	No	1	0	0
F6XVP2	SPARC related modular calcium binding 1	Yes	No	No	1	0	0
F6ZLR1	Serpin family A member 3	Yes	No	No	1	0	0
F7ACF3	Carboxypeptidase	Yes	No	No	1	0	0
F7APH4	SPARC	Yes	No	No	1	0	0
F7BKK5	Glutathione S-transferase	Yes	No	No	1	0	0
F7BKQ6	Alpha-mannosidase	Yes	No	No	1	0	0
F7BQD6	Complement C1s	Yes	No	No	1	0	0
F7C0E6	Plastin 1	Yes	No	No	1	0	0
F7C603	Acid sphingomyelinase-like phosphodiesterase	Yes	No	No	1	0	0
F7CJ82	Arginase	Yes	No	No	1	0	0

F7D2D9	Biliverdin reductase B	Yes	No	No	1	0	0
F7DMX6	Insulin-like growth factor-binding protein 1	Yes	No	No	1	0	0
H9GZS4	Reticulon 4 receptor	Yes	No	No	1	0	0
K9K1Z1	Stathmin	Yes	No	No	1	0	0
K9KEL6	Proteasome subunit alpha type (Fragment)	Yes	No	No	1	0	0

CAPÍTULO 4

A SYSTEMATIC REVIEW AND META-ANALYSIS OF THE EFFICACY OF TREATMENTS FOR POST-BREEDING INDUCED ENDOMETRITIS ON THE UTERINE RESPONSE IN MARES

Carlos Mattos Teixeira Soares[1*], Kamilla Dias Paes Silva[1], Iara Magalhães Ribeiro[1], Arabela Guedes de Azevedo Viana[1], Mariana Machado-Neves[1,2*]

[1]Programa de Pós-graduação em Medicina Veterinária, Departamento de Medicina Veterinária, Universidade Federal de Viçosa, Viçosa, Brasil.

[2]Departamento de Biologia Geral, Universidade Federal de Viçosa, Viçosa, Brasil.

*Correspondent author: Mariana Machado-Neves

Av. Peter Henry Rolfs s/n, DBG, ECS333, Campus Universitário, CEP: 36570-900. Viçosa, Minas Gerais, Brasil. E-mail: mariana.mneves@ufv.br

ABSTRACT

The post-breeding induced endometritis (PBIE) is the main type of endometritis that negatively impact mare fertility. Its diagnosis and treatments are routine procedures in equine reproductive management. However, the low effectiveness of traditional treatments raises the interest for developing non-traditional therapies. Therefore, we conducted a meta-analysis to determine the effect of PIBE treatments on uterine parameters of mares. We extracted 227 independent comparisons from 17 published articles selected from Pubmed, Web of Science, and Scopus. Our findings evidenced a moderate effect of PBIE treatments by reducing the proportion of inflammatory cells (d_{++} = -0.43, df 61, CI -0.43 to -0.19), clinical ultrasonographic signs (d_{++} = -0.42, df 47, CI -0.55 to -0.27), and concentration of inflammatory markers (d_{++} = -0.28, df 90, CI -0.47 to -0.09) in the mare's uterus. Treatments did not affect the endometrial histomorphometry and mare fertility ($p > 0.05$). Considering treatment categories, biological immunomodulatory therapies exerted a significant reduction in uterine parameters observed through ultrasound (d_{++} = -0.58, df 25, CI -0.77 to -0.39) and in the proportion of inflammatory cells (d_{++} = -0.64, df 34, CI -0.89 to -0.4) rather than anti-inflammatories and support in uterine clearance. In the individual assessment of treatments, mesenchymal stem cell was the main substance influencing the inflammatory cells results, followed by firocoxib and blood plasma. Despite continuous advances in the development of new treatments, it is essential to use as many evaluative parameters as possible, including fertility as an indicator of treatment success. Furthermore, the exploration of combination therapies emerges as a promising strategy to enhance the effectiveness of treatments and restore the delicate balance of endometrial inflammation.

Keywords: non-traditional therapies, biological immunomodulatory, uterine inflammation.

26 **1. Introduction**

27 Endometritis has been cited as the main pathology affecting mare fertility, being
28 a relevant challenge to equine reproduction (Troedsson, 1999; Morris et al., 2020). The
29 most common type of endometritis in mares is post-breeding induced endometritis
30 (PBIE), which treatment and diagnostic are routine in reproductive management (Canisso
31 et al., 2020). The physiological and transient inflammation of the endometrium ceases
32 within 48 h after natural mating or artificial insemination for uterine preparation and
33 embryo arrival (Christoffersson and Troedsson, 2017). Once this multifactorial
34 endometrial inflammation persists intensely, mares are classified as susceptible to PBIE
35 (Brinsko et al., 2003; Morrell & Rocha, 2022). The persistence of this prolonged
36 inflammation creates an unfavorable uterine environment for embryo survival and
37 interferes with the maternal recognition of pregnancy signals, resulting in subfertility and
38 lower reproductive rates (Davies Morel, 2015; Swegen, 2021).

39 The susceptibility to PBIE primarily arises from compromised uterine clearance
40 mechanisms and dysregulated uterine immune response, resulting in an imbalance
41 between pro-inflammatory and anti-inflammatory factors (Woodward & Troedsson,
42 2015). Susceptible mares not properly treated can develop more severe forms of the
43 disease, such as infectious endometritis and chronic degenerative endometritis,
44 culminating in infertility (Morris et al., 2020). The diagnostic methods for PBIE
45 encompass a combination of techniques for gynecological evaluation, including
46 assessment of inflammatory signs, such as an excessive presence of polymorphonuclear
47 cells in the endometrium, and ultrasound detection of uterine fluid accumulation
48 exceeding 2 cm at estrus and/or 36 hours post-reproduction (LeBlanc, 2010; Cuervo-
49 Arango & Newcombe, 2010).

50 The inability to establish or develop a standard treatment for PBIE raises great
51 scientific interest in the equine reproductive area since many treatments are empirical and
52 controversial (Liu and Troedsson, 2008). Overall, PBIE treatment uses anti-
53 inflammatories, uterine lavage, and ecbolic agents for supporting uterine clearance. Over
54 the last decades, the low effectiveness of traditional therapeutic modalities has been
55 described for susceptible elderly mares and intensively manipulated reproductively
56 embryo donors (LeBlanc, 2012; Buczkowska et al., 2015). Nevertheless, the lack of
57 effectiveness of traditional treatments for PBIE triggers efforts of developing new non-
58 traditional therapies (Scoggin, 2016).
59 Although PBIE treatments have been widely studied over the past 40 years, much
60 progress has been made in the last decade toward understanding the molecular aspects
61 and regulatory pathways in response to new therapies in development (Canisso et al.,
62 2020). Indeed, the application of non-traditional therapies has been growing, mainly
63 biological immunomodulators with a regulatory effect on inflammation in the uterus
64 (Segabinazzi et al., 2021). Therefore, we conducted a meta-analysis to evidence the effect
65 of treatments for PBIE on the uterine parameters of mares. Also, this review establishes
66 a critical appraisal of the knowledge state of the effectiveness of several treatments on
67 mares with PBIE and indicates research directions.
68

69 **2. Material and methods**

70 ***2.1. Search strategy***

71 This review followed the updated Preferred Reporting Items for Systematic
72 Reviews and Meta-Analysis (PRISMA) statement (Liberati et al., 2009; Page et al.,
73 2021). This study involved an extensive review to address the following focus question:
74 What are the effects of treatments for PBIE on the uterine response of mares? To this end,

75 a systematic search was conducted using the Web of Science, Scopus, and PubMed
76 databases. We included original papers published up to June 2023. The electronic
77 searches used 'mare' or 'equine' or 'horse', 'endometritis', and 'treatment' or 'therapy' or
78 'therapeutics' as search terms. Reference lists of included studies were manually searched
79 to identify any additional eligible studies.
80
81 ***2.2. Studies reviewed***
82 The following criteria were previously established for the inclusion of studies in
83 this review: i) published in a peer-reviewed journal; ii) published in the English language;
84 iii) presented control (solutions or vehicle) and treatment (therapeutic agents) groups for
85 experimental studies; iv) mare was used as the animal model; v) experimental studies
86 with susceptible mares for PBIE; vi) analyses of endometrial responses in mares after
87 breeding or artificial insemination; vii) reported means, sample size, and a measure of
88 variance (standard deviation, standard error and/or confidence intervals [CIs]) for both
89 the control and treatment groups. Studies in which sample sizes for experimental groups
90 either were not clear or were lower than four biological repeats were excluded from this
91 meta-analytical review. Other reasons for study exclusion were as follows: i) studies
92 using other species than equine as animal models; ii) there was no evaluation of any type
93 of endometritis treatment; iii) there were no analyses of uterine response after PBIE
94 treatment; and iv) review studies and conference articles. Two independent reviewers
95 screened the search results to assess whether they met the selection criteria, with
96 disagreement resolved with a third reviewer. Response means values ($X_{control}$ and
97 $X_{treatment}$), standard deviations ($S_{control}$ and $S_{treatment}$), and sample size ($N_{control}$ and $N_{treatment}$)
98 were retrieved from the text, tables, and/or figures from each of the studies included in
99 this review. Data available in the figures were digitized and means and variances were

100 obtained using ImageJ software after calibrating each picture to the nearest 0.01 mm
101 (Schneider et al., 2012). Measurements of variance were converted to standard deviations
102 of the mean using MetaWin Statistical Calculator (Rosenberg et al., 2000; Machado-
103 Neves, 2022).

104 Firstly, we performed a random-effects model of meta-analysis for each one of
105 the uterine variables evaluated in the eligible studies: i) proportion of inflammatory cells,
106 ii) ultrasound clinical signal, iii) concentrations of inflammatory markers, iv) endometrial
107 histomorphometry, and v) fertility. Data on mare fertility were extracted because it may
108 reflect the success or not of applying PBIE treatment. These variables were established
109 considering the outcomes extracted from the eligible studies. The parameters included in
110 each variable are shown in Table 1. Later on, a mixed-effects model of meta-analysis was
111 used by arranging the studies according to PBIE treatment, based on the information
112 provided by the authors. Thereby, the treatments used in the eligible studies were
113 categorized into i) support in uterine clearance: oxytocin, intrauterine lavage, and N-
114 acetyl cysteine; ii) anti-inflammatory drugs: firocoxib, dexamethasone, vedaprofen and
115 flunixin meglumine; and iii) biological immunomodulatory therapies: seminal plasma
116 proteins (lactoferrin and cysteine-rich secretory protein *3*), blood plasma (plasma rich and
117 poor platelet, platelet lysate), mycobacterial cell wall extract, ozone, and mesenchymal
118 stem cells.

119 Additionally, we recovered qualitative data from the studies to better characterize
120 them, including authors, year, country of study, mare breed, mare age, number of mares
121 susceptible and resistant to endometritis, semen storage conditions (fresh, cooled, or
122 freeze), sperm concentrations used at the artificial insemination. Moreover, we extracted
123 information regarding the treatments administered, including treatment category,
124 administration route, dose, and timing of application.

125

126 **Table 1.** Parameters collected from the eligible studies and grouped into five variables

127 analyzed under meta-analytical approach.

Variables	Parameters
Proportion of inflammatory cells	Percentage of polymorphonuclear cells in the uterine lumen and endometrium.
Ultrasound clinical signals	Endometrial edema score, intrauterine fluid retention, and uterine contractions.
Concentrations of inflammatory markers	Measurement of interleukins, nitric oxide, and cytokines.
Endometrial histomorphometry	Morphometry of endometrial glands, stroma and epithelium.
Fertility	Conception rate and diameter of the embryonic vesicle.

128

129

130 ***2.3. Meta-analysis***

131 The standardized difference between control and treatment groups was used to

132 interpret and summarize the effects of treatment in PBIE. For each study, the magnitude

133 of effect (*d*) was calculated as follows: d = (X*t* - X*c*/SD) × J, where Xt and, X*c* are the

134 responses of the treatment and control group, respectively, SD is the pooled standard

135 deviation, and J is a correction term to remove small sample size bias (Rosenberg et al.,

136 2000). After Hedge's d was calculated for each independent comparison, the cumulative

137 effect (d_{++}) of each variable surveyed was calculated using a random-effect model. This

138 review also used a mixed-effects model for the analysis of methodological moderators

139 (Carvalho et al., 2021; de Azevedo Viana et al., 2022), which assumes that differences

140 between studies within a class are determined by sampling errors and random variation.

141 Upper and lower CIs were calculated according to the average cumulative effect, and

142 intervals that did not overlap with zero, with n-1 degrees of freedom (df), were considered
143 significant. Conventionally, d_{++} values around 0.2, 0.5, and 0.8 are considered to indicate
144 weak, moderate, and strong effects, respectively (Cohen, 1992). Moreover, positives and
145 negatives d_{++} values indicate that the treatment effect increased and decreased the
146 measured variable values, respectively.

147 Heterogeneity analyses (Q statistic) were used to test whether categorical groups
148 in mixed models were homogeneous concerning the calculated effect sizes. The total
149 heterogeneity (Q_t) for all effects tested and the heterogeneity within (Q_w) and between
150 groups (Q_B) was calculated. The significance of these statistics was evaluated according
151 to a chi-square distribution with n - 1 df. Because the analyzes were based on only
152 published studies, and studies showing large and significant effects may be more likely
153 to be published than studies that show weak or no effects (the 'file-drawer problem';
154 Rosenthal, 1979), fail-safe numbers were calculated for each effect tested. Fail-safe
155 numbers indicate the number of non-significant, unpublished, or missing studies that
156 would need to be added to the sample to change its results from significant to non-
157 significant (Rosenberg et al., 2000). As a rule of thumb, fail-safe results are considered
158 robust if the fail-safe number exceeds $5k + 10$, where k is the number of comparisons in
159 the analysis. All analyzes were conducted using MetaWin 2.1 (Rosenberg et al., 2000),
160 and figures were made using Sigma Plot 10.0 software.

161

162 ***2.4. Risk of bias analysis***

163 The risk of bias analysis is crucial to evaluate the factors that may affect the
164 outcomes and conclusions of a study. In systematic reviews and meta-analyses, in which
165 the eligible studies are the primary studies, the analysis of bias is crucial to assess their
166 transparency and accuracy. Here we followed the ARRIVE (Animal Research: Reporting

167 In Vivo Experiments) guidelines 2.0 with few modifications (du Sert et al., 2020). We
168 evaluated 18 items from each of the 17 included studies regarding studies methodologies
169 and reporting, such as abstract, objectives, study design, statistical analysis, ethical
170 statement, experimental animals, outcome measures, interpretation, presentation of
171 results, and scientific implications.

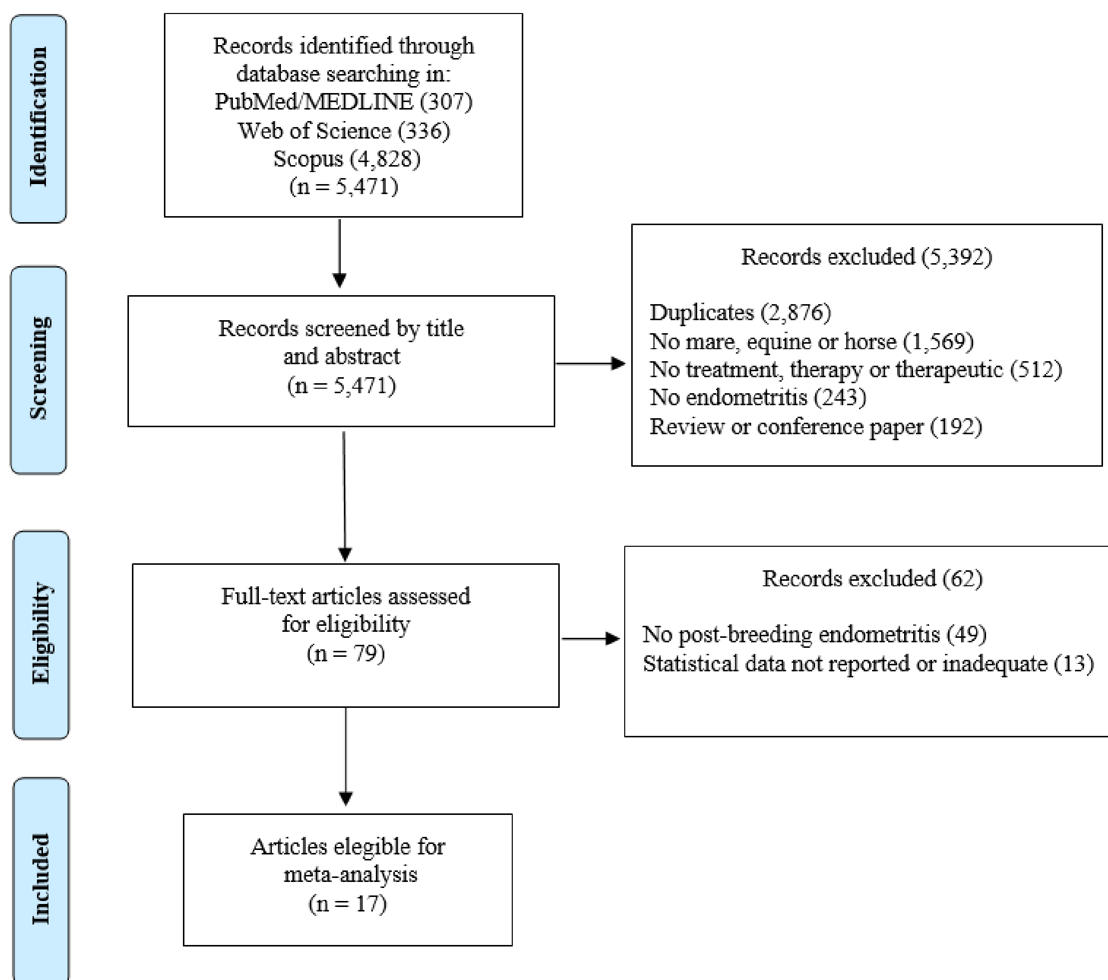
172

173 **3. Results**

174 ***3.1. Search results and study characteristics***

175 The initial search resulted in 5,471 records identified through database searches
176 in Medline/PubMed, Web of Science, and Scopus. Following the removal of duplicates
177 and exclusions according to the criteria previously mentioned, 17 studies published from
178 2003 to 2023 were eligible for this meta-analysis study. The flow diagram summarizing
179 the literature search process is shown in Figure 1. Most of the selected articles were from
180 the United States of America (n = 6), Brazil (n = 3), and Italy (n = 3). Argentina, Austria,
181 Canada, Chile, and Finland contributed to one study each (Supplementary Table S1). The
182 number of animals evaluated in each study varied between six and 32 mares. Overall,
183 insemination procedures used live and dead sperm from fresh, cooled, or frozen semen
184 samples at concentrations ranging from 0.5 to 2.0 x 10^9 (Supplementary Table S1). The
185 PBIE treatment was mostly performed using biological immunomodulators therapies
186 (Table 2). Aspects of treatment via, dose, and time of application is shown in
187 Supplementary Table S2.

188



189

190 **Figure 1.** Flow diagram of search results to define the articles to be included in the meta-
191 analysis, according to PRISMA (Preferred Reporting Items for Systematic Review and
192 Meta-Analysis).

193

194 **Table 2.** Characterization of treatments used for post-breeding induced endometritis in
195 mares according to the studies included in the meta-analysis.

Authors	Sample size	Treatment categories	Treatment agents
Vanderwall & Woods, 2003	20	SUC	Uterine lavage
Risco et al., 2009	31	SUC AID	Oxytocin Flunixin meglumine
Rojer & Aurich, 2010	17	AID	Vedaprofen

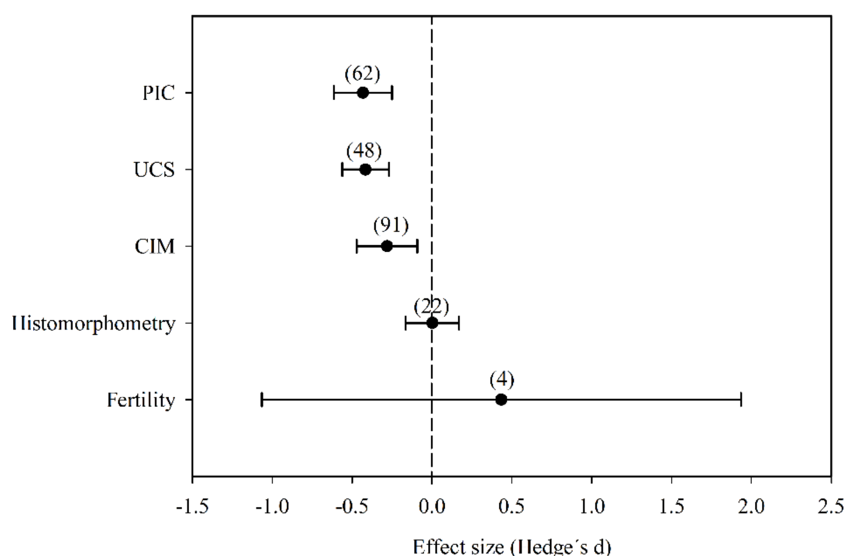
Woodward et al., 2013	6	AID BIT	Dexamethasone Mycobacterial cell wall extract
Woodward et al., 2015	6	AID BIT	Dexamethasone Mycobacterial cell wall extract
Fedorka et al., 2018	6	BIT	Seminal plasma proteins
Segabinazzi et al., 2017	13	BIT	Blood plasma
Fedorka et al., 2018	6	BIT	Seminal plasma proteins
Friso et al., 2019	9	AID	Firocoxib
Herrera et al., 2020	19	BIT	Mycobacterial cell wall extract
Felipe Navarrete et al., 2020	9	BIT	Mesenchymal stem cells
Caissie et al., 2020	9	SUC	N-acetil cystein
Tongu et al., 2021	20	BIT	Mesenchymal stem cells
Segabinazzi et al., 2021	12	BIT	Blood plasma
Colombo et al., 2022	14	BIT	Blood plasma
Donato et al., 2023	24	BIT	Ozone
Lange-Consiglio et al., 2023	16	BIT	Mesenchymal stem cells

196 SUC: support in uterine clearance; AID: anti-inflammatory drugs; BIT: biological
197 immunomodulatory therapies.
198

199 Samples and data used to evaluate treatments were collected by ultrasonography
200 analysis, low-volume intra-uterine lavage, endometrial cytology, and endometrial biopsy.
201 For a better understanding of the extracted data from each study, we categorized the
202 variables: ultrasound clinical signs, histomorphometry, the proportion of inflammatory
203 cells, the concentration of inflammatory markers, and fertility. The time of treatment
204 application showed great variation among studies, ranging from 48 hours before artificial
205 insemination to 14 days after ovulation (Supplementary Table S3).
206

207 ***3.2. Effect of PBIE treatments on uterine variables in mares***

208 The 17 eligible studies generated 227 independent comparisons of the effects of
209 treatments on uterine variables from PBIE mares. The random-effects model showed that
210 PBIE treatments elicited moderate effects by reducing the proportion of inflammatory
211 cells, ultrasound clinical signs, and concentration of inflammatory markers in the mare's
212 uterus (Fig. 2). Rosenthal's fail-safe numbers for these effects were fairly high relative to
213 the number of independent comparisons included in the meta-analysis (Supplementary
214 Table S4), indicating the strength of the results. Moreover, PBIE treatments exerted a
215 non-significant effect on endometrial histomorphometry and mare fertility (Fig. 2).

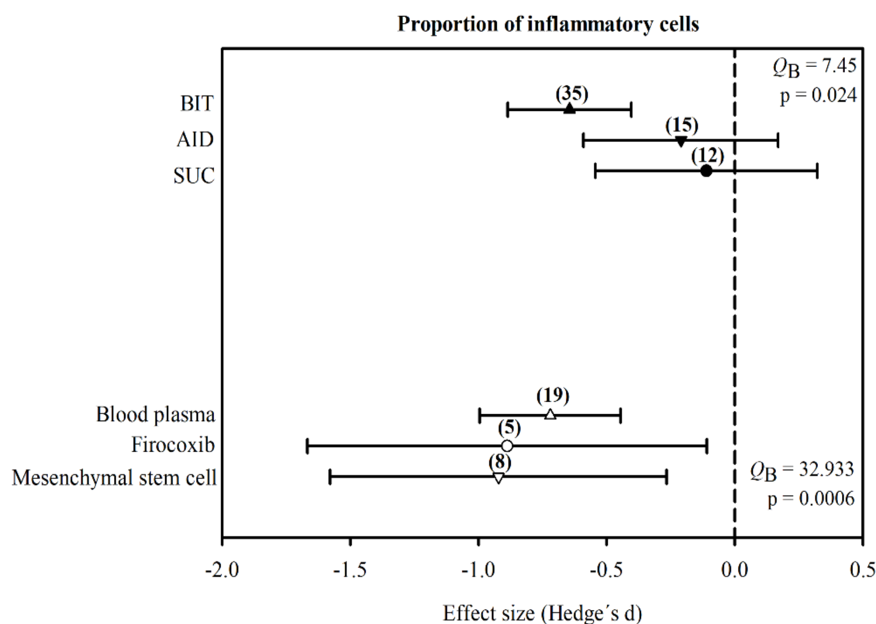


216

217 **Figure 2.** Effects of post-breeding endometritis treatments on uterine variables in mares.
218 PIC: Proportion of inflammatory cells; UCS: Ultrasound clinical signs; CIM:
219 Concentration of inflammatory markers; Histomorphometry: endometrial
220 histomorphometry; and Fertility. The cumulative effect size is reported with its 95 %
221 confidence interval and effects are significant if confidence intervals do not overlap with
222 zero. (n) = number of independent comparisons.

223

224 The impact of treatment categories on the proportion of inflammatory cells is
225 shown in the figure 3. According to the mixed-effects models, biological
226 immunomodulatory therapies exerted a significant reduction in the proportion of these
227 cells rather than anti-inflammatory drugs and support in uterine clearance (Fig. 3).
228 Mesenchymal stem cells were the mainly substances influencing this result, followed by
229 firocoxib and blood plasma (Fig. 3). The other treatments did not show any influence on
230 the proportion of inflammatory cells in uterine samples (Supplementary Table S5).
231

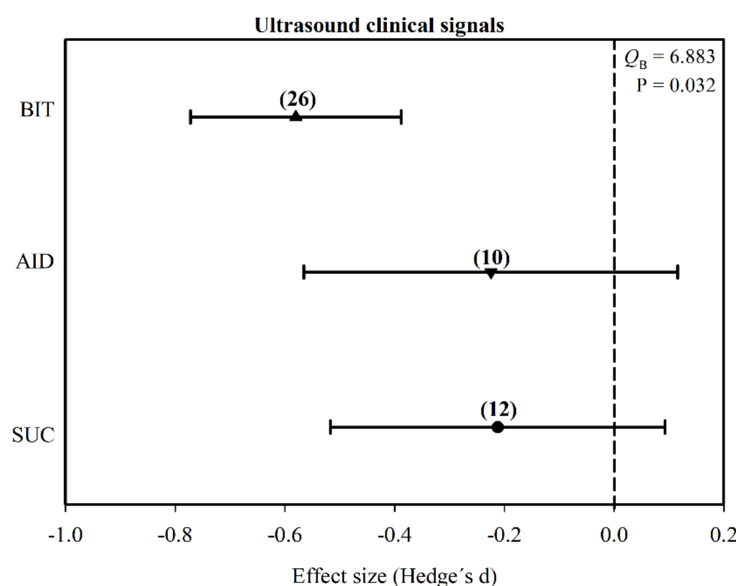


232

233 **Figure 3.** Effects of post-breeding endometritis treatments on the proportion of
234 inflammatory cells in mare's uterus. BIT: biological immunomodulators therapies; AID:
235 anti-inflammatory drugs; SUC: support in uterine clearance. The cumulative effect size
236 is reported with its 95 % confidence interval and effects are significant if confidence
237 intervals do not overlap with zero. (n) = number of independent comparisons.
238

239 Treatment with biological immunomodulators therapies also exerted a significant
240 moderate effect by ameliorating uterine parameters detected through ultrasound (Fig. 4),

241 differently to the observed for the other categories of treatments (Fig. 4). The variables
242 concentration of inflammatory markers (Q_B = 4.81; p = 0.44) and endometrial
243 histomorphometry (Q_B = 0.28; p = 0.87) were not influenced by the type of treatment
244 applied in the uterus of PBIE mares.



245

246 **Figure 4.** Effects of post-breeding endometritis treatments on uterine variable ultrasound
247 clinical signals in mares. BIT: biological immunomodulators therapies; AID: anti-
248 inflammatory drugs; and SUC: support in uterine clearance. The cumulative effect size is
249 reported with its 95 % confidence interval and effects are significant if confidence
250 intervals do not overlap with zero. (n) = number of independent comparisons.

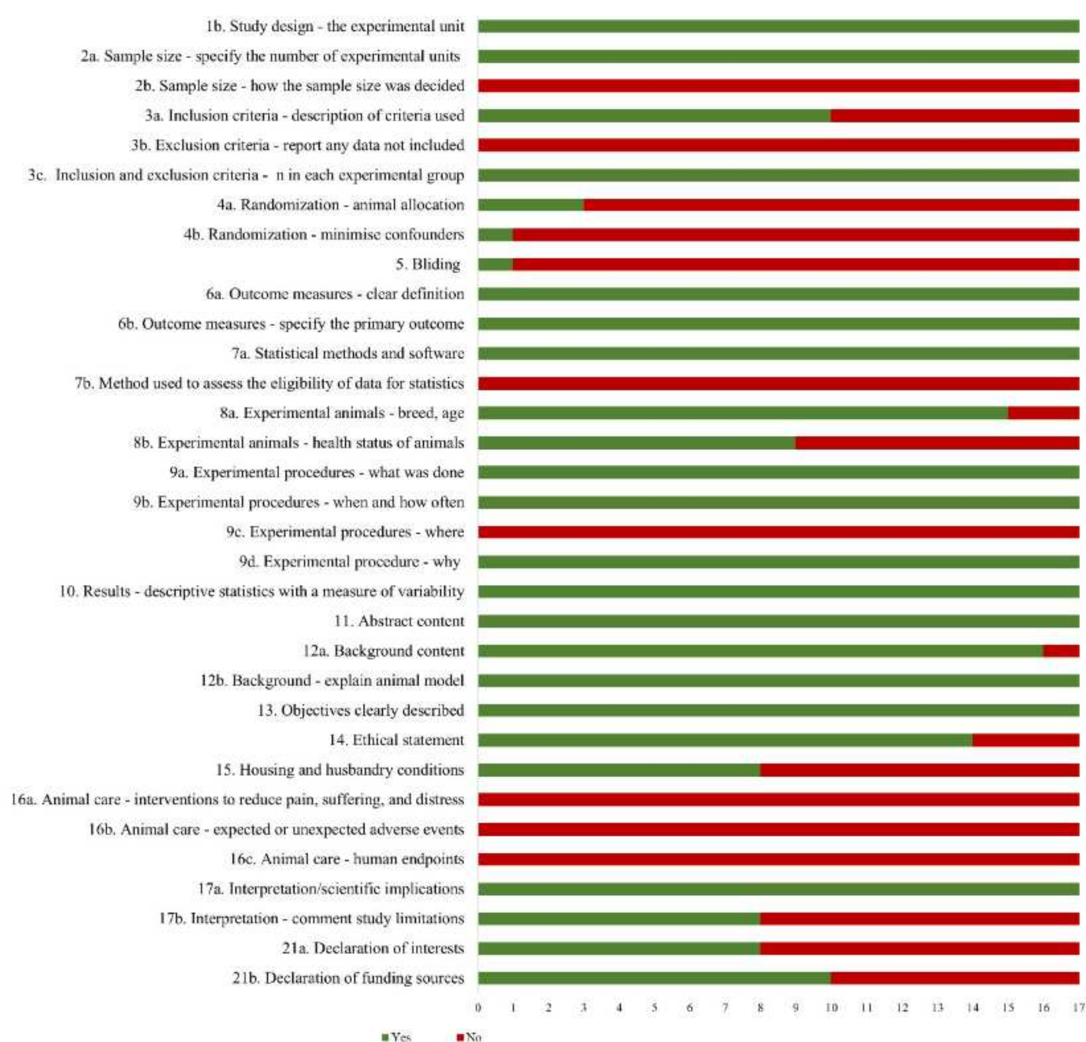
251

252 ***3.3. Risk of bias analysis***

253 According to our detailed analysis of the risk of bias, none of the 17 eligible
254 studies described neither how the sample size of animals was decided and nor justified
255 the lack of this information. Although in all studies the sample size of animals in each
256 experimental group was clearly described, only ten articles described the criteria used to
257 include the mares. None of the authors from the eligible studies reported if there was data

258 not included and the reason. Only three studies applied the randomization of the animals
259 prior to treatment management, and only one study described the blinding approach to
260 minimize the bias (Fig. 5). Although all studies mentioned the statistical method and
261 software used, none of them mentioned if there was applied any method to assess the
262 eligibility of data prior to statistical analysis. Regarding experimental procedures, in any
263 of the studies, the authors reported information about the local or acclimatization periods
264 before starting the treatments. Housing and husbandry conditions were reported in eight
265 of 17 studies. Although the interpretation of the results and scientific implications were
266 clearly reported by all authors, only eight studies commented on the limitations of the
267 studies (Fig. 5).

268



269

270 **Figure 5.** Bar chart displaying the frequencies of the options 'Yes' and 'No' among the
271 17 eligible studies included in the meta-analysis.
272

273 **4. Discussion**

274 This meta-analysis was undertaken to assess the influence of treatments for PBIE
275 on uterine variables, providing an analytical overview for a better understanding of this
276 condition and guides future research. Notably, when comparing individual treatments, the
277 mesenchymal stem cells demonstrated the most significant impact in reducing the
278 proportion of inflammatory cells, establishing themselves as the most effective treatment
279 in this particular aspect. The uterine infusion of mesenchymal stem cells was initially
280 used in degenerative chronic endometritis due to its ability to promote cell proliferation
281 and tissue remodeling (Mambelli et al., 2014). It is also described that these cells have
282 immunomodulatory functions in reducing neutrophil infiltration and increasing the
283 expression of anti-inflammatory cytokines in the uterine environment, which highlights
284 their potential use in PBIE (Ferris et al., 2014; Rink et al., 2018). However, there are still
285 controversies regarding the migration of these cells to the endometrium and their survival
286 in the inflamed uterine environment (Lange-Consiglio et al., 2023). Furthermore, while
287 holding promise for therapeutic applications, the stem cells encounter challenges in
288 practical implementation at the field level, since your acquisition and application is a
289 complex process. This practical limitation highlights the need to develop more accessible
290 strategies, such as new cultivation methods, lyophilization, and combination with
291 extracellular vesicles to increase the efficiency of this therapy (Perrini et al., 2016; Tongu
292 et al., 2021; Lange-Consiglio et al., 2023).

293 Another treatment that demonstrated a significant impact in reducing the
294 proportion of inflammatory cells was firocoxib. This selective non-steroidal anti-

295 inflammatory specifically targets the enzyme cyclooxygenase-2 while sparing
296 cyclooxygenase-1, resulting in a more targeted response and with fewer negative side
297 effects on fertility, ovulation, and uterine clearance (Friso et al., 2019). Further research
298 is needed to evaluate the potential effects of firocoxib and determine the optimal timing,
299 frequency, and dosage to ensure effectiveness without adverse effects.

300 Blood plasma also demonstrated a significant effect in reducing uterine
301 inflammatory cells, ranking as the third individually most effective in this variable. The
302 use of plasma-based therapies shows promise in the treatment of PBIE due to the release
303 of bioactive molecules by activated platelet granules, such as growth factors, cytokines,
304 and chemokines, which have regenerative properties and attenuate the uterine
305 inflammatory response (Metcalf et al., 2012; Segabinazzi et al., 2021; Colombo et al.,
306 2021). Furthermore, platelet rich plasma preparation using manual noncommercial
307 methods increases its popularity in veterinary practice, mainly due to the possibility of
308 production and use in the field conditions (Segabinazzi et al., 2021). Another method for
309 preparing plasma that has been investigated is the platelet lysate obtained by
310 cryogenically rupturing platelets of the rich plasma, offering storage advantages and
311 potentially enhancing platelet activation for more efficient immunomodulation
312 (Hauschild et al., 2017; Colombo et al., 2021). However, studies are needed to determine
313 the minimum platelet concentration necessary for immunomodulation, in addition to the
314 volume and timing of the most effective infusion for controlling PBIE (Schär et al., 2015;
315 Fantini et al., 2021; Pasch et al., 2021).

316 The meta-analysis also revealed that PBIE treatments had an impact on uterine
317 parameters assessed through ultrasound. Ultrasound clinical signs are commonly used in
318 routine reproductive practice due to their prompt results and practicality (Katila &
319 Ferreira-Dias, 2022). However, it is important to note that the presence of intrauterine

320 fluid evaluated using ultrasound may not always be indicative of PBIE, as some mares
321 may not accumulate fluid and demonstrate polymorphonuclears cells that are indicative
322 of inflammation (Cásseres de Borba et al., 2012; Katila & Ferreira-Dias, 2022).
323 Therefore, ultrasound clinical signs provide a preliminary and screening test rather than
324 a definitive diagnosis for PBIE (Morris et al., 2020). Additionally, combining multiple
325 diagnostic tests has been shown to enhance the accuracy and efficiency of diagnosing
326 endometritis (Diel de Amorim et al., 2016; Teixeira-Soares et al., 2022). Results from the
327 mixed-effects model indicated that only biological immunomodulatory therapies had a
328 significant effect on ultrasound clinical signs. While these therapies may not directly
329 target the elimination of intrauterine fluid, they indirectly contribute to its reduction by
330 alleviating inflammation and promoting uterine health (Buczkowska et al., 2015;
331 Scoggin, 2016).

332 The support in uterine clearance, removing the accumulation of intrauterine fluid,
333 is the main function of traditional treatments. However, these treatments did not elicit any
334 alteration in the parameter that evaluates fluid remotion, which was the ultrasound clinical
335 signs. This fact suggests that solely eliminating the fluid is not sufficient for endometritis
336 resolution. It is crucial to address the underlying cause, particularly uterine inflammation,
337 in order to prevent the formation and accumulation of fluid (Buczkowska et al., 2015;
338 Morrell & Rocha, 2022). Nevertheless, the importance of traditional treatments should
339 not be undermined, and therapy associations approaches are needed to enhance their
340 effectiveness (Canisso et al., 2020; Morrell & Rocha, 2022). Uterine lavage not only
341 facilitates uterine clearance but also reintroduces viable neutrophils into the stagnant
342 inflammatory uterine environment and serves as a carrier for other therapeutic agents
343 (Scoggin, 2016; Canisso et al., 2020). Despite being influenced by predisposing factors
344 for endometritis susceptibility, oxytocin remains widely used due to its cost-effectiveness

345 and practicality, while research is ongoing to develop more potent ecbolic agents, such as
346 carbetocin (Nikolakopoulos et al., 2000; Steckler et al., 2012; Scarlet et al., 2023).
347 Additionally, N-acetylcysteine, primarily recognized as a mucolytic agent aiding in fluid
348 removal, has also been investigated for its potential anti-inflammatory effects by
349 inhibiting neutrophil oxidative burst (Gores-Lindholm et al., 2013; Caissie et al., 2020).

350 The treatments for PBIE influenced the concentrations of inflammatory markers
351 in the uterus, but their impact on this parameter was not significant when analyzing the
352 treatment categories individually. In susceptible mares, the upregulation of pro-
353 inflammatory cytokines in the endometrium can result in tissue damage, increased nitric
354 oxide activity, and reduced myometrial function (Christoffersen et al., 2012; Troedsson
355 & Woodward, 2016). Treatment with anti-inflammatory drugs for PBIE aims to restore
356 the balance of inflammatory mechanisms by modulating the expression of inflammatory
357 cytokines and other markers involved in inflammation (Scoggin, 2016; Canisso et al.,
358 2020). In our study, we evaluated distinct classes of anti-inflammatory drugs, including
359 non-selective non-steroidal anti-inflammatory drugs (flunixin meglumine),
360 glucocorticoids (dexamethasone), and selective inhibitors of cyclooxygenase-2
361 (Firocoxib and Vedaprofen). Our results showed that none of them exerted a significant
362 effect on the concentration of inflammatory markers. Furthermore, there are still many
363 controversies regarding potential side effects, especially on myometrial contractions and
364 ovulation (Risco et al., 2009; Cuervo-Arango, 2011; Martínez-Boví et al., 2023).

365 The investigated treatments did not have a significant impact on the
366 histomorphometric parameter, which evaluates changes in the uterine structure and
367 provides information into the preservation of endometrial tissue (Herrera et al., 2020).
368 Histomorphometric analysis plays an important role in assessing treatment effectiveness,
369 offering assurance for their use in the absence of observable alterations, as certain

370 treatments for endometritis have historically been associated with uterine irritation and
371 excessive desquamation (Scoggin, 2016). An example of a treatment considered safe and
372 devoid of adverse effects on endometrial histomorphology is the intrauterine infusion of
373 ozone gas, known for its antimicrobial and immunostimulatory properties (Bocci, 2006;
374 Camargo et al., 2021; Köhne et al., 2023). The efficacy of ozone in treating endometritis
375 remains debatable, as it did not demonstrate significant effects in our study and failed to
376 eliminate completely pathogenic bacteria in infectious endometritis. (Loncar et al., 2017;
377 Köhne et al., 2023). Future research is needed to explore different combinations with
378 other therapies, such as uterine lavage, which has been shown to be efficient in the
379 treatment of infective endometritis (Ávila et al., 2022). Similarly, bacterial cell wall
380 extract, a biological immunomodulatory agent regulating inflammatory cytokine
381 production, has demonstrated safety without impacting endometrial histomorphology
382 (Rogan et al., 2007; Woodward et al., 2013; Herrera et al., 2020). However, similar to
383 ozone gas, it did not yield effects on other evaluative parameters employed in our study,
384 requiring additional experimental data to substantiate its efficacy in PBIE.

385 The meta-analysis revealed that the treatments did not significantly influence
386 fertility. Unfortunately, only two articles provided sufficient statistical data for
387 comparison. Fertility assessment is widely acknowledged as the gold standard for
388 monitoring reproductive success, bestowing information about the ability of the uterus to
389 sustain a pregnancy to term, albeit not yielding immediate results (Scarlet et al., 2023).
390 Hence, incorporating this indicator of reproductive success in studies is essential for a
391 comprehensive and reliable assessment of the effectiveness of treatments used for PBIE.
392 While our study did not find a direct impact on fertility, seminal plasma proteins have
393 been associated with potential to influence the duration and intensity of the uterine
394 inflammatory response (Woodward & Troedsson, 2013; Ferdoka et al., 2018; Morelli et

395 al., 2021). Proteins such as lactoferrin have properties including suppressing
396 inflammatory cytokines, promoting neutrophil binding to non-viable spermatozoa,
397 exerting bactericidal activity, and providing antioxidant effects (Ammons & Copie, 2013;
398 Ighodaro & Akinloye, 2018; Gabai et al., 2019). Exploring the impact of seminal proteins
399 in more depth could lead to promising advancements in enhancing PBIE treatments
400 outcomes, and consequently, fertility.

401

402 **5. Conclusion and perspectives**

403 Here, for the first time, we showed the effects of different PBIE treatments on key
404 uterine parameters in mares. Despite the continuous development of new treatments and
405 the evolution in the understanding of the inflammatory mechanisms of PBIE, the
406 methodological heterogeneity between the studies greatly limits the comparison. There is
407 a need to use as many parameters as possible to evaluate inflammation, in addition to
408 including fertility as the gold standard. Comparing the treatments used in our study,
409 mesenchymal stem cells, firocoxib and blood plasma showed the best results in the
410 control of post-coverage induced endometritis, by reducing the proportion of
411 inflammatory cells in the uterus. New approaches with combined therapies should be
412 studied, mainly associating traditional therapies with anti-inflammatories and biological
413 immunomodulators, and how these agents will act to restore the inflammatory balance so
414 important in endometritis.

415

416 **References**

417 Ávila, A. C. A., Diniz, N. C., Serpa, R. T., Chaves, M. M. B. de C., Viu, M. A. de O., &
418 de Oliveira, R. A. (2022). Effectiveness of Ozone Therapy in The Treatment of
419 Endometritis in Mares. Journal of Equine Veterinary Science, 112.
420 https://doi.org/10.1016/j.jevs.2022.103900

421 Bocci, V. A. (2006). Scientific and medical aspects of ozone therapy. State of the
422 art. Rivista Italiana Di Ossigeno-Ozonoterapia, 5(2), 93–104.
423 https://doi.org/10.1016/j.arcmed.2005.08.006

424 Brinsko, S. P., Rigby, S. L., Varner, D. D., & Blanchard, T. L. (2003). A practical method
425 for recognizing mares susceptible to post-breeding endometritis. Proc Am Assoc Equine
426 Pract, 49, 363–365.

427 Buczkowska, J., Kozdrowski, R., Sikora, M., Dzięcioł, M., & Matusz, A. (2015). Non-
428 traditional treatments for endometritis in mares. Bulgarian Journal of Veterinary
429 Medicine. Trakia University. https://doi.org/10.15547/bjvm.870

430 Caissie, M. D., Gartley, C. J., Scholtz, E. L., Hewson, J., Johnson, R., & Chenier, T.
431 (2020). The Effects of Treatment with N-Acetyl Cysteine on Clinical Signs in Persistent
432 Breeding-Induced Endometritis Susceptible Mares. Journal of Equine Veterinary
433 Science, 92. https://doi.org/10.1016/j.jevs.2020.103142

434 Camargo Ferreira, J., Silva Amaral, R., Mara Cardoso, L., Panelli Marchió, S., Aldrovani
435 Rodrigues, M., Fonseca Alves, C. E., & de Meira, C. (2021). Endometrial angiogenesis
436 induced by uterine insufflation with an oxygen–ozone gas mixture in
437 mares. Reproduction in Domestic Animals, 56(9), 1176–1183.
438 https://doi.org/10.1111/rda.13958

439 Canisso, I. F., Segabinazzi, L. G. T. M., & Fedorka, C. E. (2020, February 1). Persistent
440 breeding-induced endometritis in mares - a multifaceted challenge: From clinical aspects
441 to immunopathogenesis and pathobiology. International Journal of Molecular Sciences.
442 MDPI AG. https://doi.org/10.3390/ijms21041432

443 Carvalho, R. P. R., Lima, G. D. de A., & Machado-Neves, M. (2021, March 1). Effect of
444 eugenol treatment in hyperglycemic murine models: A meta-analysis. Pharmacological
445 Research. Academic Press. https://doi.org/10.1016/j.phrs.2020.105315

446 Cásseres de Borba, E.V.; Casanova Comozzato, G.; Malschitzky, E.; Bustamante-Filho,
447 I.; Afonso Martins, A.; Costa Mattos, R.; Pires Neves, A. Is the presence of uterine fluid
448 a reliable indicator of endometrial inflammation? Pferdeheilkunde 2012, 28, 27–29.

449 Christoffersen, M., Woodward, E., Bojesen, A. M., Jacobsen, S., Petersen, M. R.,
450 Troedsson, M. H. T., & Lehn-Jensen, H. (2012). Inflammatory responses to induced
451 infectious endometritis in mares resistant or susceptible to persistent endometritis. BMC
452 Veterinary Research, 8. https://doi.org/10.1186/1746-6148-8-41

453 Cohen, J. (1992). A power primer. Psychological Bulletin, 112(1), 155–159.
454 https://doi.org/10.1037/0033-2909.112.1.155

455 Colombo I., Mislei B., Mari G., Iacono E., Merlo B., Effect of platelet lysate on uterine
456 response of mares susceptible to persistent mating-induced endometritis,
457 Theriogenology, Volume 179, 2022, Pages 204-210,
458 https://doi.org/10.1016/j.theriogenology.2021.12.001

459 Cuervo-Arango J, Newcombe JR. Effect of changes in endometrial edema score
460 following mating on pregnancy rate in the mare. (2010). Animal Reproduction
461 Science, 121(1–2), 115–117. https://doi.org/10.1016/j.anireprosci.2010.04.034

462 Cuervo-Arango, J. (2011). The effect of treatment with flunixin meglumine at different
463 times relative to hCG administration on ovulation failure and luteal function in
464 mares. Animal Reproduction Science, 127(1–2), 84–90.
465 https://doi.org/10.1016/j.anireprosci.2011.07.008

466 Davies Morel, M. C. G. (2015). Equine reproductive physiology breeding and stud
467 management: 4th edition. Equine Reproductive Physiology Breeding and Stud
468 Management: 4th Edition (pp. 1–428). CABI International.

469 de Azevedo Viana, A. G., Ribeiro, I. M., Rodrigues Carvalho, R. P., Memili, E., Moura,
470 A. A., & Machado-Neves, M. (2021). Functional attributes of seminal proteins in bull
471 fertility: A systematic review. Reproduction, 161(4), 459–475.
472 https://doi.org/10.1530/REP-20-0392

473 Diel de Amorim, M., Gartley, C. J., Hill, A., Scholtz, E. L., Chenier, T. S., Foster, R. A.,
474 … Diel de Amorim, M. (2016). Comparison of Clinical Signs, Endometrial Culture,
475 Endometrial Cytology, Uterine Low-Volume Lavage, and Uterine Biopsy and
476 Combinations in the Diagnosis of Equine Endometritis. Journal of Equine Veterinary
477 Science, 44, 54–61. https://doi.org/10.1016/j.jevs.2015.10.012

478 Donato, G. G., Appino, S., Bertero, A., Poletto, M. L., Nebbia, P., Robino, P., … Nervo,
479 T. (2023). Safety and Effects of a Commercial Ozone Foam Preparation on Endometrial
480 Environment and Fertility of Mares. Journal of Equine Veterinary Science, 121.
481 https://doi.org/10.1016/j.jevs.2023.104222

482 du Sert, N. P., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., Browne, W. J., …
483 Würbel, H. (2020, July 1). Reporting animal research: Explanation and elaboration for
484 the arrive guidelines 2.0. PLoS Biology. Public Library of Science.
485 https://doi.org/10.1371/journal.pbio.3000411

486 Fantini, P., Jiménez, R., Vilés, K., Iborra, A., Palhares, M. S., Catalán, J., … Miró, J.
487 (2021). Simple tube centrifugation method for platelet-rich plasma (Prp) preparation in
488 catalonian donkeys as a treatment of endometritis-endometrosis. Animals, 11(10).
489 https://doi.org/10.3390/ani11102918

490 Fedorka, C. E., Scoggin, K. E., Boakari, Y. L., Hoppe, N. E., Squires, E. L., Ball, B. A.,
491 & Troedsson, M. H. T. (2018). The anti-inflammatory effect of exogenous lactoferrin on
492 breeding-induced endometritis in susceptible mares when administered post-breeding in
493 susceptible mares. Theriogenology, 114, 63–69.
494 https://doi.org/10.1016/j.theriogenology.2018.03.017

495 Fedorka, C. E., Scoggin, K. E., Woodward, E. M., Squires, E. L., Ball, B. A., &
496 Troedsson, M. H. T. (2017). The effect of select seminal plasma proteins on endometrial
497 mRNA cytokine expression in mares susceptible to persistent mating-induced
498 endometritis. Reproduction in Domestic Animals, 52(1), 89–96.
499 https://doi.org/10.1111/rda.12813

500 Felipe Navarrete, Fernando, S., Gabriela, C., Fernanda, R., Pablo, S. P., Lleretny, R. A.,
501 … Ovidio, C. F. (2020). Assessment of the anti-inflammatory and engraftment potential
502 of horse endometrial and adipose mesenchymal stem cells in an in vivo model of post
503 breeding induced endometritis. Theriogenology, 155, 33–42.
504 https://doi.org/10.1016/j.theriogenology.2020.06.010

505 Ferris, R. A., Frisbie, D. D., & McCue, P. M. (2014). Use of mesenchymal stem cells or
506 autologous conditioned serum to modulate the inflammatory response to spermatozoa in

507 mares. Theriogenology, 82(1), 36–42.
508 https://doi.org/10.1016/j.theriogenology.2014.02.015

509 Friso, A. M., Segabinazzi, L. G. T. M., Cyrino, M., Correal, S. B., Freitas-Dell'Aqua, C.
510 P., Teoro do Carmo, M., … Alvarenga, M. A. (2019). Periovulatory administration of
511 firocoxib did not alter ovulation rates and mitigated post-breeding inflammatory response
512 in mares. Theriogenology, 138, 24–30.
513 https://doi.org/10.1016/j.theriogenology.2019.06.045

514 Gores-Lindholm AR, LeBlanc MM, Causey R, Hitchborn A, Fayrer-Hosken RA, Kruger
515 M, et al. Relationships between intrauterine infusion of N-acetylcysteine, equine
516 endometrial pathology, neutrophil function, post-breeding therapy, and reproductive
517 performance. Theriogenology. 2013;80(3):218–227.
518 https://doi.org/10.1016/j.theriogenology.2013.03.026.

519 Hauschild, G., Geburek, F., Gosheger, G., Eveslage, M., Serrano, D., Streitbürger, A., …
520 Mischke, R. (2017). Short term storage stability at room temperature of two different
521 platelet-rich plasma preparations from equine donors and potential impact on growth
522 factor concentrations. BMC Veterinary Research, 13(1). https://doi.org/10.1186/s12917-
523 016-0920-4

524 Herrera, M. F., Otermin, M., Herrera, J. M., Simoy, M. V., Bianchi, C. P., Aguilar, J. J.,
525 & Fumuso, E. A. (2020). Effect of Mycobacterium cell wall fraction on endometrial
526 histomorphometry of mares resistant and susceptible to persistent breeding-induced
527 endometritis. Theriogenology, 156, 2–10.
528 https://doi.org/10.1016/j.theriogenology.2020.06.034

529 Katila, T., & Ferreira-Dias, G. (2022, March 1). Evolution of the Concepts of
530 Endometrosis, Post Breeding Endometritis, and Susceptibility of Mares. Animals. MDPI.
531 https://doi.org/10.3390/ani12060779

532 Lange-Consiglio, A., Gaspari, G., Funghi, F., Capra, E., Cretich, M., Frigerio, R., …
533 Cremonesi, F. (2023). Amniotic Mesenchymal-Derived Extracellular Vesicles and Their
534 Role in the Prevention of Persistent Post-Breeding Induced Endometritis. International
535 Journal of Molecular Sciences, 24(6), 5166. https://doi.org/10.3390/ijms24065166

536 Leblanc, S. J. (2012). Interactions of Metabolism, Inflammation, and Reproductive Tract
537 Health in the Postpartum Period in Dairy Cattle. Reproduction in Domestic
538 Animals, 47(SUPPL. 5), 18–30. https://doi.org/10.1111/j.1439-0531.2012.02109.x

539 Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P. A.,
540 … Moher, D. (2009, July). The PRISMA statement for reporting systematic reviews and
541 meta-analyses of studies that evaluate health care interventions: Explanation and
542 elaboration. PLoS Medicine. https://doi.org/10.1371/journal.pmed.1000100

543 Liu, I. K. M., & Troedsson, M. H. T. (2008). The diagnosis and treatment of endometritis
544 in the mare: Yesterday and today. Theriogenology, 70(3), 415–420.
545 https://doi.org/10.1016/j.theriogenology.2008.05.040.

546 Loncar, K. D., Ferris, R. A., McCue, P. M., Borlee, G. I., Hennet, M. L., & Borlee, B. R.
547 (2017). In Vitro Biofilm Disruption and Bacterial Killing Using Nonantibiotic
548 Compounds Against Gram-Negative Equine Uterine Pathogens. Journal of Equine
549 Veterinary Science, 53, 94–99. https://doi.org/10.1016/j.jevs.2017.02.003

550 Machado-Neves, M. (2022, March 1). Effect of heavy metals on epididymal morphology
551 and function: An integrative review. Chemosphere. Elsevier Ltd.
552 https://doi.org/10.1016/j.chemosphere.2021.133020

553 Mambelli, L. I., Mattos, R. C., Winter, G. H. Z., Madeiro, D. S., Morais, B. P.,
554 Malschitzky, E., … Kerkis, I. (2014). Changes in expression pattern of selected
555 endometrial proteins following mesenchymal stem cells infusion in mares with
556 endometrosis. PLoS ONE, 9(6). https://doi.org/10.1371/journal.pone.0097889

557 Martínez-Boví, R., Plaza-Dávila, M., & Cuervo-Arango, J. (2023). The effect of
558 dexamethasone and flunixin-meglumine on ovulation, endometrial oedema, and inter-
559 ovulatory interval length in the mare. Theriogenology, 197, 57–61.
560 https://doi.org/10.1016/j.theriogenology.2022.11.042

561 Metcalf, E. S., Scoggin, K., & Troedsson, M. H. T. (2012). The effect of platelet-rich
562 plasma on endometrial pro-inflammatory cytokines in susceptible mares following semen
563 deposition. Journal of Equine Veterinary Science, 32(8), 498.
564 https://doi.org/10.1016/j.jevs.2012.06.065

565 Morelli, M. A. C., Ostuni, A., Giangaspero, B., Cecchini, S., Carluccio, A., & Boni, R.
566 (2021). Relationships between seminal plasma metabolites, semen characteristics and
567 sperm kinetics in donkey (Equus asinus). Animals, 11(1), 1–19.
568 https://doi.org/10.3390/ani11010201

569 Morrell, J. M., & Rocha, A. (2022). A Novel Approach to Minimising Acute Equine
570 Endometritis That May Help to Prevent the Development of the Chronic State. Frontiers
571 in Veterinary Science, 8. https://doi.org/10.3389/fvets.2021.799619

572 Morris, L. H. A., McCue, P. M., & Aurich, C. (2020, November 1). Equine endometritis:
573 A review of challenges and new approaches. Reproduction. BioScientifica Ltd.
574 https://doi.org/10.1530/REP-19-0478

575 Nikolakopoulos, E., Kindahl, H., & Watson, E. D. (2000). Oxytocin and PGF2alpha
576 release in mares resistant and susceptible to persistent mating-induced
577 endometritis. Journal of Reproduction and Fertility. Supplement, (56), 363–372.

578 Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C.
579 D., … Moher, D. (2021, March 29). The PRISMA 2020 statement: An updated guideline
580 for reporting systematic reviews. The BMJ. BMJ Publishing Group.
581 https://doi.org/10.1136/bmj.n71

582 Pasch, L., Schmidt, A., & King, W. (2021). Clinical Observations After Prebreeding
583 Intrauterine Plasma Infusion in 18 Mares Inseminated With Thawed Frozen
584 Semen. Journal of Equine Veterinary Science, 99.
585 https://doi.org/10.1016/j.jevs.2021.103389 LeBlanc, M. M. (2010). Advances in the
586 diagnosis and treatment of chronic infectious and post-mating-induced endometritis in
587 the mare. Reproduction in Domestic Animals, 45(SUPPL. 2), 21–27.
588 https://doi.org/10.1111/j.1439-0531.2010.01634.x

589 Perrini, C., Strillacci, M. G., Bagnato, A., Esposti, P., Marini, M. G., Corradetti, B., …
590 Cremonesi, F. (2016). Microvesicles secreted from equine amniotic-derived cells and
591 their potential role in reducing inflammation in endometrial cells in an in-vitro
592 model. Stem Cell Research and Therapy, 7(1), 1–15. https://doi.org/10.1186/s13287-
593 016-0429-6

594 Rink, B. E., Aurich, C., French, H., & Donadeu, F. X. (2018). The Fate of Endometrial
595 Mesenchymal Stem Cells After Application in the Healthy Equine Uterus. Journal of
596 Equine Veterinary Science, 66, 108. https://doi.org/10.1016/j.jevs.2018.05.154

597 Risco, A. M., Reilas, T., Muilu, L., Kareskoski, M., & Katila, T. (2009). Effect of
598 oxytocin and flunixin meglumine on uterine response to insemination in
599 mares. Theriogenology, 72(9), 1195–1201.
600 https://doi.org/10.1016/j.theriogenology.2009.07.012

601 Rogan D, Fumuso E, Rodríguez E, Wade J, Sanchez Bruni SF. Use of a myco-
602 bacterial cell wall extract (MCWE) in susceptible mares to clear experimen-
603 tally induced endometritis with Streptococcus zooepidemicus. J Equine Vet
604 Sci 2007;27:112e7. https://doi.org/10.1016/j.jevs.2007.01.010.

605 Rojer, H., & Aurich, C. (2010). Treatment of persistent mating-induced endometritis in
606 mares with the non-steroid anti-inflammatory drug vedaprofen. Reproduction in
607 Domestic Animals, 45(6). https://doi.org/10.1111/j.1439-0531.2009.01572.x

608 Rosenberg, M.S., Adams, D.C., Gurevitch, J., 2000. MetaWin: Statistical Software for
609 Meta-Analysis: Version 2.0. Sinauer Associates

610 Rosenthal, R. (1979). The file drawer problem and tolerance for null
611 results. Psychological Bulletin, 86(3), 638–641. https://doi.org/10.1037/0033-
612 2909.86.3.638

613 Scarlet, D., Malama, E., Fischer, S., Knutti, B., & Bollwein, H. (2023). Relationship
614 between Clinical Uterine Findings, Therapy, and Fertility in the Mare. Veterinary
615 Sciences, 10(4), 259. MDPI AG. Retrieved from
616 http://dx.doi.org/10.3390/vetsci10040259

617 Schär, M. O., Diaz-Romero, J., Kohl, S., Zumstein, M. A., & Nesic, D. (2015). Platelet-
618 rich Concentrates Differentially Release Growth Factors and Induce Cell Migration In
619 Vitro. Clinical Orthopaedics and Related Research, 473(5), 1635–1643.
620 https://doi.org/10.1007/s11999-015-4192-2

621 Scoggin, C. F. (2016, December 1). Endometritis: Nontraditional Therapies. Veterinary
622 Clinics of North America - Equine Practice. W.B. Saunders.
623 https://doi.org/10.1016/j.cveq.2016.08.002

624 Segabinazzi, L. G. T. M., Canisso, I. F., Podico, G., Cunha, L. L., Novello, G., Rosser,
625 M. F., … Alvarenga, M. A. (2021). Intrauterine blood plasma platelet-therapy mitigates
626 persistent breeding-induced endometritis, reduces uterine infections, and improves
627 embryo recovery in Mares. Antibiotics, 10(5).
628 https://doi.org/10.3390/antibiotics10050490

629 Segabinazzi, L. G. T. M., Podico, G., Rosser, M. F., Nanjappa, S. G., Alvarenga, M. A.,
630 & Canisso, I. F. (2021). Three manual noncommercial methods to prepare equine platelet-
631 rich plasma. *Animals*, *11*(6). https://doi.org/10.3390/ani11061478

632 Segabinazzi, L. G., Friso, A. M., Correal, S. B., Crespilho, A. M., Dell'Aqua, J. A., Miró,
633 J., … Alvarenga, M. A. (2017). Uterine clinical findings, fertility rate, leucocyte
634 migration, and COX-2 protein levels in the endometrial tissue of susceptible mares treated
635 with platelet-rich plasma before and after AI. Theriogenology, 104, 120–126.
636 https://doi.org/10.1016/j.theriogenology.2017.08.007

637 Steckler, D., Naidoo, V., Gerber, D., & Kähn, W. (2012). Ex vivo influence of carbetocin
638 on equine myometrial muscles and comparison with oxytocin. Theriogenology, 78(3),
639 502–509. https://doi.org/10.1016/j.theriogenology.2012.02.030

640 Swegen, A. (2021, May 1). Maternal recognition of pregnancy in the mare: Does it exist
641 and why do we care? Reproduction. BioScientifica Ltd. https://doi.org/10.1530/REP-20-
642 0437

643 Teixeira-Soares, C. M., Viana, A. G. de A., Ribeiro, I. M., Silva, K. D. P., Sancler-Silva,
644 Y. F. R., & Machado-Neves, M. (2022). Comparison Between Gynecological
645 Examination Methods and Sample Collection Techniques for the Diagnosis of
646 Endometritis in Subfertile Mares. Journal of Equine Veterinary Science, 119.
647 https://doi.org/10.1016/j.jevs.2022.104147

648 Tongu, E. A. de O., Segabinazzi, L. G. T. M., Alvarenga, M. L., Monteiro, A., Papa, F.
649 O., & Alvarenga, M. A. (2021). Allogenic mesenchymal stem cell-conditioned medium
650 does not affect sperm parameters and mitigates early endometrial inflammatory responses

651 in mares. Theriogenology, 169, 1–8.
652 https://doi.org/10.1016/j.theriogenology.2021.03.019

653 Troedsson, M. H. T. (1999). Uterine clearance and resistance to persistent endometritis
654 in the mare. Theriogenology, 52(3), 461–471. https://doi.org/10.1016/S0093-
655 691X(99)00143-0

656 Troedsson, M. H. T., & Woodward, E. M. (2016). Our current understanding of the
657 pathophysiology of equine endometritis with an emphasis on breeding-induced
658 endometritis. Reproductive Biology, 16(1), 8–12.
659 https://doi.org/10.1016/j.repbio.2016.01.003

660 Vanderwall, D. K., & Woods, G. L. (2003). Effect on fertility of uterine lavage performed
661 immediately prior to insemination in mares. Journal of the American Veterinary Medical
662 Association, 222(8), 1108–1110. https://doi.org/10.2460/javma.2003.222.1108

663 Woodward, E. M., & Troedsson, M. H. T. (2015, July 1). Inflammatory mechanisms of
664 endometritis. Equine Veterinary Journal. Equine Veterinary Journal Ltd.
665 https://doi.org/10.1111/evj.12403.

666 Woodward, E. M., Christoffersen, M., Campos, J., Betancourt, A., Horohov, D., Scoggin,
667 K. E., … Troedsson, M. H. T. (2013). Endometrial inflammatory markers of the early
668 immune response in mares susceptible or resistant to persistent breeding-induced
669 endometritis. Reproduction, 145(3), 289–296. https://doi.org/10.1530/REP-12-0452

670 Woodward, E. M., Christoffersen, M., Horohov, D., Squires, E. L., & Troedsson, M. H.
671 T. (2015). The effect of treatment with immune modulators on endometrial cytokine
672 expression in mares susceptible to persistent breeding-induced endometritis. Equine
673 Veterinary Journal, 47(2), 235–239. https://doi.org/10.1111/evj.12266

674 Zivotkov H. I. (1938). Racianalizacja I perestrojka slucki iiskustvenogo osemenenija
675 kobil na novih proizvodstvenik inaucnih obosnovanijah. Moscow: Seljhozgiz.

Supplementary Table S1. Characterization of studies included in the meta-analysis, such as the country where the study was developed, horse breed, mare age, number (n) of susceptible and resistant mares to endometritis, semen storage condition (SSC), and sperm concentration used for artificial insemination.

Authors	Country	Horse breed	Age (year-old)	Number of mares (n)		SSC	Sperm concentration
				Susceptible	Resistent		
Vanderwall & Woods, 2003	USA	Crossbred	03-12	20	-	Cooled	1.0×10^9
Risco et al., 2009	Finland	Finnhorse and Standardbred	03-16	31	-	Fresh	0.5×10^9
Rojer & Aurich, 2010	Austria	Warmblood and Welsh Cob	12-14	17	-	Cooled	Unknown
Woodward et al., 2013	USA	Crossbred	15-24	6	-	Freeze	1.0×10^9
Woodward et al., 2015	USA	Crossbred	15-26	6	-	Freeze	1.0×10^9
Fedorka et al., 2017	USA	Crossbred	10-25	6	-	Fresh	1.0×10^9
Segabinazzi et al., 2017	Brazil	Crossbred	08-20	13	-	Fresh	0.8×10^9
Fedorka et al., 2018	USA	Crossbred	05-20	6	-	Fresh	0.5×10^9
Friso et al., 2019	Brazil	Crossbred	12-22	9	-	Fresh	1.0×10^9
Herrera et al., 2020	Argentina	Criollo	04-12	9	10	Freeze	0.5×10^9
Navarrete et al., 2020	Chile	Unknown	Unknown	-	9	Freeze	0.5×10^9
Caissie et al., 2020	Canada	Standardbred and Crossbred	07-19	9	-	Freeze	1.0×10^9
Tongu et al., 2021	Brazil	Crossbred	05-13	7	13	Fresh	1.0×10^9
Segabinazzi et al., 2021	USA	Light-breed	05-18	12	-	Freeze	2.0×10^9
Colombo et al., 2022	Italy	Standardbred	03-16	14	-	Freeze	0.8×10^9
Donato et al., 2023	Italy	Standardbred	08-19	24	-	Cooled	Unknown
Lange-Consiglio et al., 2023	Italy	Unknown	Unknown	16	-	Fresh	0.5×10^9

Supplementary Table S2. Characterization of the treatment protocols used by the eligible studies for the meta-analysis, including substances used, administration via, dose, and moment of application in relation to artificial insemination (AI).

Authors	Treatment agents	Administration via	Dose	Time of treatment
Vanderwall & Woods, 2003	Uterine lavage (Ringer Lactato)	Intra-uterine	4,000 mL	Immediately before AI
Risco et al., 2009	Oxytocin	Injectable	0.01 IU/kg	At AI; 4, 8, and 25 h after AI
	Flunixin meglumine	Injectable	1.1 mg/kg	At AI
Rojer & Aurich, 2010	Vedaprofen	Oral	1 and 2 mg/kg	ovulation induction day and one day after AI
Woodward et al., 2013	Dexamethasone	Injectable	50 mg	6 h post-breeding
	Mycobacterial cell wall extract	Injectable	1.5 mg	6 h post-breeding
Woodward et al., 2015	Dexamethasone	Injectable	50 mg	6 h after AI
	Mycobacterial cell wall extract	Injectable	1.5 mL	6 h after AI
Fedorka et al., 2017	Lactoferrin	Intra-uterine	1 mg/mL in 10 mL semen	At AI
	CRISP-3	Intra-uterine	150 µg/mL in 10 mL semen	At AI
Segabinazzi et al., 2017	Platelet-rich plasma	Intra-uterine	20 mL	24 h before AI or 4 h after AI
Fedorka et al., 2018	Lactoferrin	Intra-uterine	50, 250 or 500 µg/mL in 10 mL ringer lactate	6 h after AI
Friso et al., 2019	Firocoxib	Oral	0.2 mg/kg	ovulation induction, AI and one day after AI
Herrera et al., 2020	Mycobacterial cell wall extract	Injectable	1.5 mL	After AI
Navarrete et al., 2020	Mesenchymal stem cells	Intra-uterine	20 million in 20 mL 0,9% saline	3 h after AI
Caissie et al., 2020	N-acetil cystein	Intra-uterine	30 mL	12 h before AI
Tongu et al., 2021	Adipose mesenchymal stem cells - conditioned medium	Intra-uterine	30 mL or 3:4 (MSCs:semen)	24 h before; 6, 24, and 48 h after; or at AI

Segabinazzi et al., 2021	Platelet-rich plasma	Intra-uterine	40 mL	48 h before; 6, 24, 72, and 96 h after; or at AI
	Platelet-poor plasma	Intra-uterine	40 mL	48 h before; 6, 24, 72, and 96 h after; or at artificial insemination
Colombo et al., 2022	Platelet lysate	Intra-uterine	10 mL	24 h before AI
Donato et al., 2023	Ozone (Riger Spray®)	Intra-uterine	Keeping the applicator of the can pressed for five seconds	During estrus
Lange-Consiglio et al., 2023	Aminiotic mesenchymal stem cells - conditioned medium	Intra-uterine	20 x 10^9 MSCs in semen	At AI

Supplementary Table S3. Sample collection, parameters assessed, and the moment by which the analysis was performed in relation to ovulation and/or artificial insemination (AI). The parameters extracted from the studies were categorized into variables to be analyzed under the meta-analytical approach as follows: Ultrasound clinical signs (UCS), the proportion of inflammatory cells (PIC), concentration of inflammatory markers (CIM), endometrial histomorphometry, and fertility.

Authors	Sample collection	Parameters assessed	Variable Category	Time of analysis
Vanderwall & Woods, 2003	Ultrasonography	Diameter of embryonic vesicle	Fertility	12 d after ovulation
Risco et al., 2009	Ultrasonography	Uterine contractions and intra-uterine fluid	UCS	2, 4, 8, and 25 h after AI
	Endometrial biopsy	Edema, glandular contents, and dilation	Histomorphometry	2, 4, 8, and 25 h after AI
	Endometrial biopsy	Proportion of polymorphonuclear cells	PIC	2, 4, 8, and 25 h after AI
	Endometrial cytology	Proportion of polymorphonuclear cells	PIC	2, 4, 8, and 25 h after AI
Rojer & Aurich, 2010	Ultrasonography	Intra-uterine fluid	UCS	until 24 h after AI
	Endometrial cytology	Proportion of polymorphonuclear cells	PIC	until 24 h after AI
Woodward et al., 2013	Endometrial biopsy	Nitric oxide synthase mRNA and nitric oxide	CIM	6 h after AI
Woodward et al., 2015	Endometrial biopsy	Interleucin (IL)-1β, IL-6, IL-7, IL-10, IL-11, IL-1RA, and Interferon (INF)-γ	CIM	6 h after AI
Fedorka et al., 2017	Endometrial biopsy	IL-1β, IL-6, IL-8, IL-10, IL-1RN, INF-γ, and Tumor necrosis factor (TNF)-α	CIM	6 h after AI
Segabinazzi et al., 2017	Ultrasonography	Conception rate	Fertility	14 d after ovulation
	Endometrial biopsy	Proportion of polymorphonuclear and lymphocyte cells; and COX-2 score	PIC	24 h after AI
	Endometrial cytology	Proportion of polymorphonuclear cells	PIC	24 h before and after AI
Fedorka et al., 2018	Ultrasonography	Intra-uterine fluid	UCS	24 h after AI
	Low-volume lavage	Proportion of polymorphonuclear cells	PIC	24 h after AI
Friso et al., 2019	Endometrial biopsy	Proportion of polymorphonuclear and lymphocyte cells	PIC	24 h after AI
	Endometrial cytology	Proportion of polymorphonuclear cells	PIC	24 h after AI

Herrera et al., 2020	Endometrial biopsy	Glandular epithelium height, area, density, and diameter	Histomorphometry	07 d after ovulation
Navarrete et al., 2020	Endometrial cytology	Proportion of polymorphonuclear cells	PIC	48 h after AI
	Low-volume lavage	IL-6 and TNF-α	CIM	48 h after AI
Caissie et al., 2020	Ultrasonography	Endometrial edema score	UCS	12, 36, 60, and 84 h after AI
	Endometrial biopsy	Inflammation score	PIC	12 and 60 h after AI
	Endometrial cytology	Proportion of polymorphonuclear cells	PIC	12 and 60 h after AI
Tongu et al., 2021	Ultrasonography	Intra-uterine fluid	UCS	6 and 24 h after AI
	Endometrial cytology	Proportion of polymorphonuclear cells	PIC	6 and 24 h after AI
	Endometrial cytology	IL-6, IL-10, and TNF-α	CIM	6 and 24 h after AI
Segabinazzi et al., 2021	Ultrasonography	Intra-uterine fluid	UCS	48 h before and 24, 48, and 72 h after AI
	Endometrial cytology	Proportion of polymorphonuclear cells	PIC	48 h before and 24, 48, and 72 h after AI
	Endometrial cytology	IL-1β, IL-6, IL-10, TNF-α, and CXCL8	CIM	48 h before and 24, 48, and 72 h after AI
Colombo et al., 2022	Ultrasonography	Endometrial edema score and intra-uterine fluid	UCS	24 h after and before; and at AI
	Endometrial cytology	Endometrial cytology score	PIC	24 h after AI
Donato et al., 2023	Endometrial biopsy	Inflammation score	PIC	24 h and 18 to 27 days after treatment.
	Endometrial cytology	Proportion of polymorphonuclear cells	PIC	24 h, one week, two weeks and 18 to 27 days after treatment.
Lange-Consiglio et al., 2023	Endometrial cytology	IL-6, IL-10, TNF-α	CIM	0, 6, 12, 18 and 24 h after AI

Supplementary Table S4. Cumulative effects, heterogeneity analysis (Q statistic), number of independent comparisons, confidence intervals, and fail-safe numbers from the random-effects model of meta-analyses regarding the effect of treatments on uterine parameters from mares with post-breeding induced endometritis.

Variables	Proportion of inflammatory cells	Ultrasound clinical signals	Concentration of inflammatory markers	Endometrial histomorphometry	Fertility
Cumulative effect (d^{++})	-0.4322	-0.4160	-0.2808	0.0025	0.4343
Heterogeneity	0.20104	0.47010	0.00002	0.99986	0.0
Number of comparisons (k)	61	47	90	21	3
Confidence intervals	-0.6145 to -0.2499	-0.5615 to -0.2704	-0.4699 to -0.0918	-0.1641 to 0.1691	-4.6769 to 5.5456
Rosenthal's Method (N = 5k + 10)	477.4	549.1	297.8	0.0	25.8

Conventionally, d_{++} values around 0.2, 0.5, and 0.8 are considered to indicate weak, moderate, and strong effects, respectively (Cohen, 1992)

Supplementary Table S5. Effect sizes, heterogeneity analysis (*Q* statistic) and Rosenthal's fail-safe number for the analyzed variables according to methodological variations of the effect for the treatments of post-breeding endometritis in mares.

Variable	Heterogeneity	*p*-value	Cumulative effect (d++)	Number of comparisons (k)	Confidence intervals (CI) 95%	Rosenthal's Method (N = 5*k* + 10)
Proportion of inflammatory cells	32.9328	0.00006				667.2
Support in uterine clearance			-0.1115	11	-0.5443 to 0.3214	
Anti-inflammatory drugs			-0.2105	14	-0.5901 to 0.1692	
Biological immunomodulatory therapies			-0.6451	34	-0.8860 to -0.4041	
N-acetil cystein			0.2727	3	-0.6986 to 1.2440	
Firocoxib			-0.8882	4	-1.6673 to -0.1092	
Seminal plasma proteins			-1.1752	2	-2.9992 to 0.6487	
Blood plasma			-0.7206	18	-0.9947 to -0.4464	
Vedaprofen			0.2125	1	-5.3913 to 5.8163	
Oxytocin			-0.2941	7	-0.7897 to 0.2014	
Flunixin meglumine			0.0728	7	-0.4161 to 0.5617	
Mesenchymal stem cell			-0.9223	7	-1.5783 to -0.2662	
Ozone			0.1677	4	-0.5518 to 0.8871	
Ultrasound clinical signal	9.7149	0.13718				595.5
Support in uterine clearance			-0.2121	11	-0.5170 to 0.0927	
Anti-inflammatory drugs			-0.2245	09	-0.5649 to 0.1159	
Biological immunomodulatory therapies			-0.5800	25	-0.7718 to -0.3881	
Concentration of inflammatory markers	4.8136	0.43905				295.2
Anti-inflammatory drugs			-0.6003	06	-1.4629 to 0.2622	
Biological immunomodulatory therapies			-0.2557	83	-0.4524 to 0.0591	

Histomorphometry	0.2798	0.86945				0.0
Support in uterine clearance			0.0666	05	-0.4046 to 0.5378	
Anti-inflammatory drugs			0.0430	05	-0.4166 to 0.5025	
Biological immunomodulatory therapies			-0.0310	09	-0.2634 to 0.2014	
Fertility	Turned off	Turned off				Turned off

CAPÍTULO 5

CONSIDERAÇÕES FINAIS

A endometrite é reconhecidamente a principal causa de subfertilidade em éguas e nosso estudo teve como objetivo esclarecer seus mecanismos de desenvolvimento, métodos de diagnóstico e tratamentos. Ao realizar um exame ginecológico abrangendo diversos métodos avaliativos, constatamos que a endometrite foi a principal causa de exclusão de éguas receptoras em programas de transferência de embriões comerciais, com uma prevalência de 60% dos animais subférteis acometidos. Observamos a importância do fluido intrauterino como achado clínico, a presença frequente de infecções uterinas causadas principalmente pela bactéria *Escherichia coli* e a relevância do exame histopatológico para o diagnóstico final. Esta avaliação abrangente foi fundamental para o diagnóstico mais preciso da endometrite e deve ser utilizada para tomar decisões adequadas em relação às éguas receptoras.

A análise do proteoma do fluido uterino proporcionou informações valiosas. Identificamos diferenças significativas nos perfis proteômicos de éguas saudáveis, com endometrite infecciosa e com endometrite pós-cobertura. A detecção de proteínas específicas relacionadas à integridade uterina, resposta inflamatória e imunológica trouxe informações importantes sobre a endometrite e possíveis biomarcadores de diagnóstico.

Em relação aos tratamentos, terapias não tradicionais com imunomoduladores biológicos mostraram-se promissoras. Essas terapias reduziram significativamente a proporção de células inflamatórias no útero e os sinais clínicos ultrassonográficos da endometrite. Destacamos os tratamentos com células-tronco mesenquimais, firocoxib e plasma sanguíneo como os mais eficazes no controle da inflamação uterina. Esses achados abrem novas perspectivas para o diagnóstico, tratamento e compreensão dos mecanismos envolvidos na endometrite em éguas. Futuras pesquisas devem se concentrar na validação dos possíveis biomarcadores, na padronização dos métodos de diagnóstico e na avaliação de combinações terapêuticas para o controle da inflamação persistente.