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EDITORIAL

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Clinical insights: Regenerative therapies in equine practice: Top 10 EVJ papers 2019–2024

1 | OVERVIEW

In the past two decades, the field of veterinary regenerative medicine has advanced considerably, with multiple therapies available to equine practitioners to treat joint and soft tissue disorders as well as a variety of other conditions such as wounds, ophthalmic or theriogenology indications. The term 'regenerative therapies' is a broad definition for innovative medical therapies that enable the body to repair, replace, restore and regenerate damaged or diseased cells, tissues and organs, while 'biological products' are those made from living material (human, animal, plant, microorganisms) used to treat or prevent disease. Options available to clinicians in equine practice include point-of-care blood or tissue-derived products (autologous) including platelet-rich plasma (PRP), autologous conditioned serum (ACS or interleukin receptor antagonist protein/IRAP), autologous protein solution (APS), bone marrow aspirate concentrate (BMAC or stromal vascular fraction) or cultured stromal cell-based products (autologous or allogeneic). Treatments in this area are rapidly evolving and clinicians are often asked for guidance as to the selection of one treatment over another for a specific clinical indication. However, the true efficacy of regenerative therapies remains controversial in some instances due to lack of rigour in clinical study design, lack of demonstrated consistency in product formulation and lack of regulatory oversight that would assure appropriate standards. The *Equine Veterinary Journal* (EVJ) papers included in the editorial review below have been assembled in a 'Special Collection', which aims to identify the 'Top 10' recent publications from EVJ (2019–2024) (Table 1) related to regenerative therapies and to highlight adjacent literature in other journals, which may aid practitioners in this field.

2 | CURRENT BIOLOGIC USE IN EQUINE PRACTICE

The first manuscript in our collection, by **Zanotto and Frisbie (2022)**, describes a survey of the American Association of Equine Practitioners updating on current practices in joint therapy, highlighting changes in the past 10 years.[1] A total of 407 completed surveys were returned, with the majority of respondents (90.9%) indicating that they spend most of their time (>75%) dedicated to equine practice, and most commonly working with racehorses (52.4%) and hunter/jumper (50.6%) disciplines. The findings overall indicated clear differences in the use of joint therapies over time, with an increase in prevalence of biological therapies since the previous survey 10 years prior.[2] The majority of respondents (87%) did report that they had treated patients who benefited from intra-articular biologic therapies. The most common reason cited for selecting a biological therapy over corticosteroids for intra-articular use was perceived long-term efficacy (45.7%), followed by safety (19.4%), client request (10.8%) and short-term efficacy (5.6%). The majority of respondents (83.3%) reported using ACS or interleukin receptor antagonist protein (IRAP), followed by PRP (72.5%), APS (53.8%), stromal cell therapy (53.7%) and BMAC (22.4%). The likelihood of respondents reporting having used ACS in particular was substantially higher in 2019 than in the previous survey performed in 2009.[1,2] The three most commonly reported factors influencing the selection of biological therapy were scientific data on efficacy, cost, and severity or chronicity of the condition to be treated. Biologic therapies were more commonly used in cases that were not previously responsive to corticosteroid treatment or when treating diseased soft tissues within a joint such as meniscus or ligament. Respondents reported the perspective that the joints that responded best to biological therapies were the stifle (33.2%), fetlock (23.6%) and coffin joints (22.6%). The authors went on to discuss and summarise that these findings highlight the importance of a cost-effectiveness analysis among biological therapies and other commonly used treatments such as corticosteroids and hyaluronic acid for long-term management of joint disease in performance horses.

These findings reflect both similarities and differences to another recent survey of orthobiologic use of the American College of Veterinary Surgeons (ACVS) and American College of Veterinary Sports Medicine and Rehabilitation (ACVSMR) diplomates.[3] In this study, 154 surveys were analysed, with respondents most commonly treating sport horses (44%) followed by Western performance (24%), racehorses (15%), pleasure horses (9%) and show horses (8%). The most common reasons for selecting one biologic treatment over another were based on scientific articles and data demonstrating efficacy (56%), personal experience (20%) and due to inefficacy of previous treatments evaluated (10%). Overall, PRP was the most commonly used biologic therapy by respondents (87.5%), followed by ACS (79.7%), bone marrow-derived mesenchymal stromal cell (MSC; 72.1%), APS (46.2%) and adipose-derived MSCs (19.5%). An additional 24% of respondents indicated that they used stromal cell therapy that was neither adipose- nor bone marrow-derived, but the cell source was not specified. Regarding indication or route of administration, respondents reported MSCs and PRP being more commonly administered intralesionally, while ACS and APS were more frequently

TABLE 1 Summary of highlighted studies evaluating equine biological therapies published in *Equine Veterinary Journal* in the past 5 years.

Reference	Investigator	Title	Methods	Main findings
1	Zanotto and Frisbie (2022)	Current joint therapy usage in equine practice: changes in the last 10 years	Electronic survey of American Association of Equine Practitioners	Biologic therapy use, particularly ACS, increased in frequency in the past decade
				Most common reasons for use over corticosteroids were long-term efficacy, safety and client request
				Most commonly used products were ACS (83%), PRP (72.5%), APS (53.8%) and MSC (53.7%)
5	Peng et al. (2024)	A systematic review and meta-analysis of the efficacy of platelet-rich plasma products for treatment of equine joint disease	Systematic review and meta-analysis	Most studies were associated with high risk of bias and there was high variability between PRP compositions
				Overall estimated effect indicated improvement with PRP for osteoarthritis, potentially septic arthritis
				Recognised classification systems and utilisation of randomised, blinded trials are required to better evaluate efficacy
6	Lofgren et al. (2023)	Conditioned serum in vitro treatment of chondrocyte pellets and osteoarthritic explants	In vitro experimental study	IL-1Ra concentrations in incubated serum were higher than conditioned serum using commercially available kits
			Chondrocytes, cartilage explants	In vitro treatment with conditioned serum did not alleviate IL-1ß induced responses in chondrocyte pellets
			Serum, incubated serum, conditioned serum	In vitro treatment with conditioned serum did not lead to morphological improvement in osteoarthritic cartilage explants
22	Hansen et al. (2024)	Racing performance of Thoroughbred racehorses with suspensory ligament branch desmitis treated with mesenchymal stem cells (2010–2019)	Retrospective case series	MSC treatment resulted in majority (71%) Thoroughbred racehorses with SLBD racing post-treatment
			Thoroughbred racehorses diagnosed with suspensory ligament branch desmitis	Racing pre-injury and being male were positively associated with racing post-injury
			Treated with allogeneic MSC and 3–4 treatments with autologous MSC	Number of races, earnings, and earnings per start did not differ pre- and post-injury in horses that raced post-injury
23	Salz et al. (2023)	Treatment of racehorse superficial digital flexor tendonitis: a comparison of stem cell treatments to controlled exercise rehabilitation in 213 cases	Retrospective cohort study of clinical treatment and race records	BM-MSC compared to CERP was associated with increased odds of returning to racing with at least five races post-injury
			Racehorses with superficial digital flexor tendonitis	Older age and increasing lesion length were associated with reduced likelihood to return to racing
			Controlled exercise rehabilitation protocol (CERP) +/− BM- or A-MSC	Male sex and increased number of pre-injury starts were associated with increased odds of returning to racing
24	Murphy et al. (2022)	Intralesional bone marrow and superior check desmotomy is superior to conservative treatment of equine superficial digital flexor tendonitis	Retrospective and case-controlled study	Horses that had surgery and intralesional bone marrow were more likely to return to racing versus conservative management
			Racehorses with superficial digital flexor tendonitis	No difference was seen between number placings, wins or post-injury earnings between surgical versus conservative management

(Continues)

TABLE 1 (Continued)

Reference	Investigator	Title	Methods	Main findings
			Managed conservatively or surgically with desmotomy and intralesional bone marrow	Average number of lifetime starts post-injury did not differ between surgery, conservative management or control groups
35	Broeckx et al. (2019)	The use of equine chondrogenic-induced mesenchymal stem cells as a treatment for osteoarthritis: a randomised, double-blinded, placebo-controlled proof-of-concept study	Randomised, double-blinded, placebo-controlled experimental osteoarthritis model	Improvement in visual and objective lameness was seen with chondrogenic-induced MSC + plasma versus control
			Intra-articular allogeneic chondrogenic-induced MSC + plasma versus saline at 5 weeks	Synovial fluid had higher viscosity and lower glycosaminoglycan concentration in MSC + plasma versus control
			Lameness, synovial fluid cytology, biomarker analysis, histology	Cartilage oligomeric matrix protein, collagen type II and glycosaminoglycans were higher in cartilage in MSC + plasma treated
36	Carlier et al. (2023)	Equine allogeneic tenogenic primed mesenchymal stem cells: a clinical field study in horses suffering from naturally occurring superficial digital flexor tendon and suspensory ligament injuries	Multicentre, blinded, randomised, placebo-controlled clinical trial	Horses receiving tenogenic primed MSC had improved fibre alignment score and echogenicity and lesion size decreased
			Horses with SDFT and SL injuries received either intralesional tenogenic primed MSC or saline	Re-injury rate was reduced in horses receiving tenogenic primed MSC compared to saline control
			Clinical and ultrasonographic scoring was performed at day 0, 56, and 112	Intralesional tenogenic primed MSC administration was safe and improved quality of healing and long-term outcomes
37	Colbath et al. (2020)	Mesenchymal stem cells for treatment of musculoskeletal disease in horses: relative merits of allogeneic versus autologous stem cells	Review article of published data on allogeneic versus autologous MSC therapies	Arguments against allogeneic MSC use include risk of immunological reaction and shorter cell survival following injection
				Arguments in favour of allogeneic MSC include reduced time to treatment, use of young healthy donors, and ability to manipulate cells
				Large, appropriately designed, randomised trials evaluating local and systemic immune responses are needed to resolve issue
43	Arevalo-Turrubiarte (2022)	Extracellular vesicles from equine mesenchymal stem cells decrease inflammation markers in chondrocytes in vitro	In vitro experimental study evaluating MSC-derived extracellular vesicles	Particles isolated from MSCs derived from different tissues did not differ in size and concentration
			Extracellular vesicles co-cultured with chondrocytes treated with pro-inflammatory cytokines	EVs from bone marrow-derived MSCs reduced metalloproteinase 13 gene expression
			Transmission electron microscopy of particles, real-time PCR of chondrocytes	EVs derived from MSCs can reduce inflammation and potentially be used as adjuvant treatments in articular pathologies

Abbreviations: A, adipose; ACS, autologous conditioned serum; APS, autologous protein solution; BM, bone marrow; EVs, extracellular vesicles; MSC, mesenchymal stromal cell; PRP, platelet-rich plasma; SDFT, superficial digital flexor tendon; SL, suspensory ligament.

administered intralesionally. Treatment of ACS was repeated commonly within 2 weeks of initial injection (48.1%), while MSCs (43.3%) and PRP (38%) were re-injected less frequently at 1–2 months after initial injection. APS was typically repeated more than 4 months after initial injection (39.6%). Local inflammation and expense were the most common adverse effects and limitation to use discussed. Protocols for repeated administration varied widely, and the lack of consensus regarding the timing of first injection following diagnosis or optimal treatment interval for repeated injections was discussed further. Overall, the authors discussed that, similarly to that reported by Zanotto and Frisbie[1] as well as another recent international survey of rehabilitation modalities by equine veterinarians,[4] the findings of this survey reflect a general increase in the use of biologic therapies over the past decade with high prevalence of the use of ACS and PRP. Their findings further suggested a similar level of use among large animal specialists, regardless of duration in practice or practice type, which was attributed to increased commercial availability and literature surrounding biological therapies for musculoskeletal disease, specifically in recent years.

The information collected by these surveys provide a baseline of current understanding of common practices surrounding the use of equine biological products for musculoskeletal disease at this time and help to support the continued use and investigation of these products. However, relatively little work has been done to evaluate and compare the biological activities of these compounds more fully or to support optimal processing and storage conditions, recommended dosages, or evidence-based protocols for the application of biologics clinically. Therefore, the decision on which biologic to use in a specific disease condition is often based on incomplete information on the specific pathological physiology. Larger case-controlled studies and clinical trials are warranted to determine treatment efficacy, optimal treatment intervals and to compare available therapies for specific indications. Below, we will further discuss the recent literature for each of the most commonly used biologic therapies (PRP, ACS, MSC) individually.

3 | PLATELET-RICH PLASMA

PRP has been used for the longest time and is the most extensively studied of the orthobiologics available. It exerts an effect based on platelet α-granule release of growth factors and chemokines, cytokines, and fibrinogen in plasma that stimulates macrophage chemotaxis, angiogenesis, proliferation, fibroblast migration and collagen synthesis to direct cellular processes integral to healing. In the second paper in our collection, **Peng et al. (2024)** aimed to systematically review the current evidence on PRP products used for osteoarthritis and septic arthritis in equine practice, as well as the efficacy of PRP products for the treatment of OA using a meta-analysis of the available literature (2013–2023).[5] Randomised controlled trials, non-randomised trials and controlled laboratory studies that used at least one type of PRP product were included. This study identified a total of 21 publications by systematic review and five for meta-analysis, which evaluated various different types of PRP products. The authors concluded that most of the studies were associated with a high risk of bias, but that the overall estimated effect indicated improvement in groups treated with PRP products for equine OA, and that PRP had potential in treating septic arthritis. These conclusions were noted to be tempered by the limited number of randomised controlled studies and high variability between the types of PRP products in terms of platelet and leukocyte count. To better evaluate PRP efficacy, a recognised classification system of products and utilisation of randomised, blinded, equivalency or non-inferiority trials would be necessary.

4 | AUTOLOGOUS CONDITIONED SERUM

ACS, also known as interleukin-1 receptor antagonist protein (IRAP), was developed to counteract the effects of the cytokine interleukin-1 (IL-1ß) produced in sites of inflammation, such as joints with osteoarthritis. In the third paper in our collection, **Lofgren et al. (2023)** evaluated the effects of ACS further in an in vitro experiment where chondrocytes stimulated with IL-1ß and cartilage explants with mild osteoarthritis were treated with either equine serum, serum incubated for 24 h or serum incubated for 24 h using a commercially available ACS container.[6] Chondrocytes were then evaluated using microarray, polymerase chain reaction, and for matrix metallopeptidase-13, and cartilage explants were assessed using the OARSI grading scale for histological evaluation of articular cartilage. In chondrocytes, inflammation- and cartilage matrix degradation-related genes, as well as growth factor signalling genes, were upregulated in all treatment groups versus untreated controls, while in explants, the histological OARSI scores did not differ between groups. This study is the first to assess the global gene expression associated with inflammation and cartilage matrix degradation in IL-1ß-stimulated chondrocytes and histological staging of OA cartilage explants to evaluate treatment effect. Additionally, the investigators found that serum incubated for 24 h (in the absence of using the commercial kit) contained significantly higher levels of IL-1Ra than ACS, which questions the reported necessity to use commercial systems to achieve elevated levels of IL-1Ra. However, this study did not indicate that either the incubated serum nor commercial ACS product alleviates IL-1ß induced responses in chondrocyte pellets or led to morphological improvement in osteoarthritic cartilage explants. The results do not support a beneficial disease-modifying effect of ACS on articular cartilage, although the information gleaned from these in vitro models cannot be directly translated to the in vivo scenario in which ACS is commonly used. Future studies to determine efficacy of ACS specifically to treat joint pain and its potential ability to reduce clinical lameness are still warranted.

5 | MESENCHYMAL STROMAL CELL THERAPY

Stromal cells are undifferentiated cells capable of self-renewal and differentiation into specific lineages. Multipotent cells (e.g., mesenchymal,

haematopoietic) give rise to more than one cell type but are often restricted to a single germ layer, while pluripotent cells (e.g., embryonic, fetal-derived embryonic-like, or induced pluripotent) give rise to all cell types of the body from all three germ layers. Sources of stromal cells in equine practice include bone marrow, adipose, blood, umbilical cord blood, amniotic tissue, dental pulp and synovial fluid or synovial lining. Typical reported dose ranges are from 10 to 30 million stem cells per joint or site. However, knowledge gaps remain regarding optimal cell number, timing of administration during inflammation, optimal pre-activation techniques to direct differentiation or cytokine secretion and use of autologous versus allogeneic preparations. Recent studies have focused on optimising cell preparations to increase efficacy or decrease antigenicity for allogeneic use[7–9] and to expand clinical use to include extracellular vesicle-based formulations.[10] In this section on stromal cell therapy, we will highlight recent evidence for efficacy in equine practice, investigation of immune licensing of MSC to enhance their immunomodulatory and antimicrobial effects and discussion of autologous versus allogeneic use, and future directions for cellular-based therapies including extracellular vesicle (EV)-based approaches or minimally manipulated biologic therapeutic options.

Evidence for cellular therapy efficacy: Preclinical studies support modest improvement in healing with MSC therapy for tendon lesions,[11,12] cartilage grafting[13] and intra-articular[14–16] and more recently for their antimicrobial properties to treat infection.[17–19] Clinical retrospective studies have provided evidence for reduced re-injury rate in National Hunt racehorses treated with MSCs for superficial digital flexor tendinopathy injury,[20] and that a higher percentage of horses with stifle injury, particularly meniscal injury, returned to work following intra-articular MSC injection.[16] A recent systematic review and meta-analysis of the use of stem cells and platelet-rich plasma for the treatment of naturally occurring equine tendon and ligament injuries indicated that MSCs and MSCs administered concurrently with PRP provided a reduced risk of re-injury.[21] This study did not reveal an overall increase in the likelihood of return to performance with any of the biologic treatments assessed; however, the authors concluded that these findings should be interpreted with consideration of the heterogeneity of findings, high risk of bias and poor study design in the majority of studies reported.

In the fourth paper in our series, **Hansen et al. (2024)** built upon this body of work to evaluate the racing performance of Thoroughbred racehorses with suspensory ligament branch desmitis (SLBD) treated with mesenchymal stem cells (2010–2019).[22] All horses were treated with allogeneic stem cells injected locally at the time of diagnosis and subsequently received three to four treatments with autologous bone marrow-derived MSCs. The authors concluded that treatment with MSCs resulted in the majority of Thoroughbred racehorses (71%) racing post treatment, with factors including having previously raced pre-injury and being male also positively associated with racing post-injury. In horses that raced post-injury, the number of races, earnings and earnings per start did not differ from pre-injury.

In the fifth paper in our series, **Salz et al. (2023)** further evaluated the association between treatment with either autologous bone marrow-derived (BM) MSC, allogeneic adipose-derived MSC or controlled exercise rehabilitation in 213 racehorses diagnosed with superficial digital flexor tendonitis (SDFT) with return to racing and completion of at least five races post-injury.[23] Follow-up was a minimum of 2 years after return to race training. Compared with controlled exercise rehabilitation programme alone, BM (but not adipose-derived) MSC treatment was associated with an increased odds of returning to racing, with at least five races post-injury. It was acknowledged that the treatment group receiving adipose-derived MSC included a limited number of horses compared with BM-MSC, and that due to the retrospective nature of the study, it was not possible to determine how strictly rehabilitation protocols were followed in every case.

Finally, in the sixth paper in our series, **Murphy et al. (2022)** compared the post-injury performance of Thoroughbred and Standardbred racehorses diagnosed with SDFT tendonitis treated with intralesional bone marrow and superior check desmotomy or managed conservatively and further to compare this performance with that of uninjured racehorses matched for age, sex and number of starts pre-injury.[24] BMAC is available as a minimally manipulated alternative to cultured cells, which contains concentrations of MSCs and interleukin receptor antagonist protein higher than that in serum, distinguishing it from other orthobiologic products.[25] Bone marrow aspirate may be appropriate to deliver autologous anabolic molecules, stromal cells and scaffold in the treatment of osteoarthritis, desmitis, and articular cartilage or bone defects.[25] In this study, horses that received the combination surgical intervention and bone marrow treatment were found to be more likely to return to racing than those managed conservatively, although of those that returned to racing in both groups, there was no difference in the average number of placings, wins or post-injury earnings between those surgically treated versus conservatively managed. While these findings support a potential beneficial effect for intralesional bone marrow injection, this treatment was only evaluated in combination with surgical intervention, and therefore the direct effect of the bone marrow aspirate therapy alone cannot be ascertained.

Immune licensing of MSC to enhance immunomodulatory and antimicrobial properties: The heterogeneity within and between stromal cell populations has been proposed to be partially responsible for the observed variability in therapeutic responses.[26] Pre-activation or 'inflammatory licensing' of MSC through priming with various cytokines or agonists including TGF-ß, Toll- and Nod-like receptor agonists or selection for expression of integrin $\alpha 10\beta 1$ has been described in a number of studies as a means to generate a homogeneous population of immunomodulatory MSCs, and thereby potentially improve the consistency of MSC therapy and response to treatment.[7,8,19,27–34]
In the seventh paper in our series, **Broeckx et al. (2019)** built on these concepts to evaluate the use of chondrogenic-induced MSC to treat osteoarthritis in a randomised, double-blinded, placebo-controlled proof-of-concept study.[35] Osteoarthritis was induced in the metacarpophalangeal joint using an osteochondral fragment-groove model and horses received intra-articular injection with allogeneic chondrogenic-induced MSC and equine allogeneic plasma or 0.9% saline at 5 weeks postoperatively. Improvement in visual and

objective lameness was seen with MSC intervention compared with control. Cartilage oligomeric matrix protein, collagen type II and glycosaminoglycans were elevated in the articular cartilage of horses receiving MSCs. Synovial fluid displayed higher viscosity and lower glycosaminoglycan levels in the MSC-treated group. Other synovial fluid biomarkers or cytology parameters did not differ between groups. Importantly, no adverse events or drug reactions were noted. These authors concluded that allogeneic equine chondrogenic-induced MSC combined with equine plasma may be a promising treatment for osteoarthritis in horses.

In the eighth paper in our series, this investigative group furthered the field in MSC licensing to evaluate allogeneic tenogenic primed MSC (tpMSC) in a clinical field study in horses with naturally occurring SDFT and suspensory ligament (SL) injuries in **Carlier et al. (2023)**.[36] In this multi-centre, blinded, randomised, placebo-controlled clinical trial, 100 client-owned horses with SDFT and SL injuries received either intralesional tpMSC or saline injections, and clinical and ultrasonographic evaluations were performed at the time of injection and subsequently at days 56 and 112. Horses receiving tpMSC treatment achieved improvement in fibre alignment scores and echogenicity and lesion size was significantly decreased compared with control. Re-injury rate was significantly lower in tpMSC-treated horses. These findings provide further support for intralesional administration of tpMSCs to improve healing quality and long-term outcomes in sport horses with naturally occurring SDFT and suspensory injuries.

As noted in the two previous papers, allogeneic cell sources have the potential to treat musculoskeletal injuries and may allow for improved consistency in product administration and accelerate time to first treatment. In the second to last paper in our series, **Colbath et al. (2020)** discussed the currently available published data regarding the therapeutic use of autologous versus allogeneic MSCs in horses and relative merits of each to treat musculoskeletal disease in horses.[37] Arguments advanced against the use of allogeneic MSC include the risk for immunological reaction following injection and potentially shorter cell survival, while arguments in favour of allogeneic MSC use include reduced time to treatment, collection from young healthy donors and ability to manipulate or licence cells prior to administration for a more homogeneous product. These authors concluded that while this area has not been exhaustively studied, accumulating evidence from studies in horses suggests that allogeneic MSCs may be a safe alternative and that large, appropriately designed, randomised trials including immunological evaluation of local and systemic immune responses are necessary to more fully resolve this issue.

Future directions for cellular-based therapies: Further investigation of MSC extracellular vesicle (EV)-based therapies or minimally manipulated biologic therapeutic options represents future directions in veterinary regenerative medicine to take advantage of the paracrine influence of MSC while reducing practical considerations with autologous use and regulatory concerns with cell-based therapies.

BMAC represents a minimally manipulated option available that is unique among biologic therapies in that it fulfils the triad of tissue engineering to provide MSCs, biophysical and chemical signals, and scaffolding for healing.[25] Bone marrow mononuclear cells specifically have been shown to modulate joint homeostasis in an equine model of synovitis.[38] Furthermore, erythrocyte removal from BMAC prior to intra-articular administration has been shown to improve treatment efficacy and reduce joint inflammation compared with unseparated BMAC.[39]

Additionally, EVs produced by MSCs play a role in intercellular communication through transfer of proteins, lipids and RNAs, and there is now a consensus that the EVs produced by MSCs are responsible for their reported anti-inflammatory and regenerative effects.[10,40–42] Due to their small size, exosome and microvesicle-based therapies offer several therapeutic advantages over the use of whole MSC as potential cell-free therapy to minimise side effects with systemic use such as pulmonary embolism or regulatory concerns with cell-based approaches.[40] While the use of EVs to treat equine musculoskeletal conditions are in their infancy, initial results from in vitro, in vivo and preclinical studies demonstrate EV administration has potent anti-inflammatory and pro-regenerative effects and enhances biomechanical and qualitative properties in repair tissue, which has been attributed to their capacity for immunomodulation.[10] Furthermore, the acellular nature of EVs would allow for allogeneic application and long-term storage for immediate clinical application.[42]

In the 10th and final paper in our series, **Arevelo-Turrubiarte et al. (2022)** present an in vitro investigation of EVs from equine mesenchymal stem cells to decrease inflammatory markers in chondrocytes.[43] In this study, MSC from bone marrow, adipose and synovial fluid were cultured in vitro, culture medium was centrifuged and filtered, and isolated particles were analysed for size and concentration. Healthy equine chondrocytes were treated with inflammatory cytokines IL-1ß and TNF-α and MSC-derived EVs from bone marrow and synovial fluid cells were added as co-treatments in culture. Gene expression analysis by real-time PCR was performed to evaluate the effects of EVs, which revealed that EVs from bone marrow MSCs reduced metalloproteinase 13 gene expression, which encodes an enzyme related to cartilage degradation in inflamed chondrocytes in vitro. These findings suggest that EVs derived from MSCs can reduce inflammation and potentially be used as an adjuvant treatment to improve tissue and cartilage repair in articular pathologies. Further investigation of EVs and other acellular and/or minimally manipulated MSC-based therapeutic options in large animal models of disease is warranted.

6 | SUMMARY

The articles summarised here highlight advancements in our understanding of biologic therapies in equine practice over the past 5 years, which can be used to assist clients in decision-making and cost-effectiveness when choosing between biologics and other available therapies such as corticosteroids and hyaluronic acid. However, relatively little work has been done to evaluate and compare the functional activity of available treatments more fully or to support optimal

processing and storage conditions, recommended dosages, and timing of initial and re-dosing to provide evidence-based protocols for clinical application. Further studies in these areas may allow for treatment of equine disorders that have previously limited performance.

KEYWORDS

biologic, horse, regenerative, therapy

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Lynn Pezzanite: Conceptualization; investigation; writing – original draft; writing – review and editing; methodology; data curation.

ETHICAL ANIMAL RESEARCH

Not applicable.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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