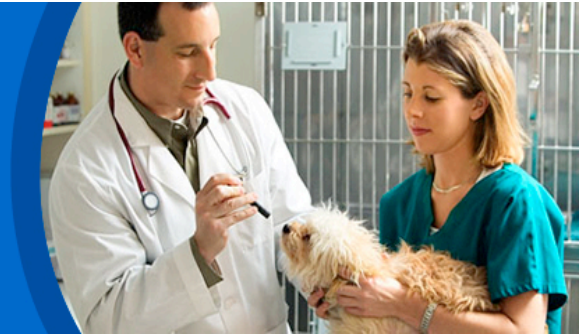




ISSN: 2456-2912

VET 2024; 9(6): 40-45

© 2024 VET

www.veterinarypaper.com

Received: 01-11-2024

Accepted: 03-12-2024

Lucas Ferreira

São Paulo Metropolitan
University, Department of
Veterinary Surgery and
Regenerative Medicine, São
Paulo, Brazil

Ana Carolina

São Paulo Metropolitan
University, Department of
Veterinary Surgery and
Regenerative Medicine, São
Paulo, Brazil

Rafael Mendes Oliveira

São Paulo Metropolitan
University, Department of
Veterinary Surgery and
Regenerative Medicine, São
Paulo, Brazil

Corresponding Author:

Lucas Ferreira

São Paulo Metropolitan
University, Department of
Veterinary Surgery and
Regenerative Medicine, São
Paulo, Brazil

Therapeutic efficacy of stem cell therapy in canine osteoarthritis: A preliminary clinical trial

Lucas Ferreira, Ana Carolina and Rafael Mendes Oliveira

DOI: <https://www.doi.org/10.22271/veterinary.2024.v9.i6a.3375>

Abstract

Osteoarthritis affects an estimated 20 percent of adult dogs worldwide, yet conventional analgesic regimens offer only symptomatic relief without reversing cartilage degeneration. This preliminary clinical trial evaluated the therapeutic efficacy of a single intra-articular injection of autologous adipose-derived mesenchymal stem cells (AD-MSCs) in dogs diagnosed with naturally occurring stifle osteoarthritis. Twenty-one client-owned dogs of mixed and pure breeds, aged 4-12 years and weighing 18-42 kg, were enrolled between June 2022 and May 2023 at the Veterinary Teaching Hospital of São Paulo Metropolitan University, Brazil. Dogs were randomly allocated to an MSC group (n = 11) receiving 5×10^6 AD-MSCs suspended in 1 mL phosphate-buffered saline or a control group (n = 10) receiving an equivalent volume of sterile saline. Adipose tissue was harvested from the falciform ligament under general anaesthesia, processed by collagenase digestion, expanded to passage 3 and characterised by flow cytometry (CD90+, CD44+, and CD45-). Blinded clinical evaluations were performed at baseline and at weeks 2, 4, 8, 12, 16 and 24 using the Helsinki Chronic Pain Index (HCPI), a validated lameness grading scale (0-4) and peak vertical force (PVF) measured on a force plate. Serum C-reactive protein (CRP) and synovial fluid interleukin-1 β (IL-1 β) concentrations were measured at baseline, week 8 and week 24. The MSC group showed a 73.1 percent reduction in mean HCPI score by week 24 (from 7.8 ± 1.3 to 2.1 ± 0.8 ; $p < 0.001$), compared with a 5.3 percent non-significant decline in controls (from 7.6 ± 1.1 to 7.2 ± 1.2 ; $p = 0.41$). Lameness grade decreased from a median of 3.0 to 1.0 in the MSC group versus no change in controls. PVF increased by 28.4 percent in treated dogs ($p = 0.003$) but remained static in the placebo arm. Serum CRP fell from 14.6 ± 3.2 to 6.3 ± 2.1 mg/L in the MSC group ($p = 0.001$), and synovial IL-1 β dropped from 87.4 ± 18.6 to 34.2 ± 11.3 pg/mL ($p < 0.001$). No adverse events were recorded apart from mild transient joint effusion in two treated dogs that resolved within 48 hours. These results support the safety and short-to-medium-term efficacy of a single autologous AD-MSC injection for canine stifle osteoarthritis, though larger multi-centre trials with longer follow-up are warranted before routine clinical adoption.

Keywords: Mesenchymal stem cells, canine osteoarthritis, regenerative medicine, adipose-derived, clinical trial, lameness, pain index, intra-articular injection, force plate, anti-inflammatory

Introduction

Roughly one in five adult dogs suffers from some degree of Osteoarthritis (OA), making it among the most common causes of chronic pain in companion animal practice ^[1]. The economic burden is substantial: owners in the United States alone spend an estimated 1.3 billion dollars per year on OA-related veterinary consultations and pharmaceuticals ^[2]. Despite this scale, the therapeutic arsenal has changed little over the past two decades non-steroidal anti-inflammatory drugs (NSAIDs), tramadol and physical rehabilitation remain first-line options, none of which halt cartilage loss ^[3]. Mesenchymal stem cells (MSCs) have emerged as a promising alternative because they possess both anti-inflammatory and chondro-regenerative properties ^[4]. Adipose tissue is the preferred harvest site in dogs owing to its

Accessibility, high cell yield and minimal donor-site morbidity [5]. Once expanded in culture, AD-MSCs secrete paracrine factors including transforming growth factor- β (TGF- β), interleukin-10 (IL-10) and prostaglandin E2 that suppress catabolic cytokines in the joint microenvironment [6]. *In-vitro* experiments have confirmed that canine AD-MSCs express surface markers consistent with the International Society for Cellular Therapy criteria (CD90+, CD44+, CD45-) and retain trilineage differentiation potential through at least passage 5 [7]. Preclinical evidence in canine OA models is encouraging. Black *et al.* [8] reported significant improvements in lameness and range of motion four months after intra-articular AD-MSC delivery in eight dogs with hip OA. Vilar *et al.* [9] observed increased peak vertical force (PVF) and vertical impulse in ten dogs with elbow OA following MSC therapy. But most published work has relied on small, unblinded, uncontrolled series, which limits the strength of inference [10]. A controlled, blinded design is needed to separate the true biological effect of MSCs from the well-documented placebo response in canine pain assessment [11]. Brazilian veterinary practice faces particular challenges. The tropical climate promotes year-round activity in large-breed dogs, accelerating joint wear, while owner compliance with long-term NSAID protocols tends to be low [12]. Regenerative therapies that deliver sustained benefit from a single procedure are therefore attractive in this setting. São Paulo's large referral hospital network and established tissue-banking infrastructure make it a suitable location for clinical evaluation [13]. The present research aimed to (a) assess the clinical efficacy of a single intra-articular injection of autologous AD-MSCs on pain, lameness and limb loading in dogs with naturally occurring stifle OA, and (b) measure the systemic and local anti-inflammatory response through serial CRP and synovial IL-1 β sampling over a 24-week follow-up.

Material and Methods

Material

Twenty-one client-owned dogs presenting with unilateral or bilateral stifle OA (Kellgren-Lawrence radiographic grade II-IV) were recruited from the Orthopaedic Clinic of São Paulo Metropolitan University Veterinary Teaching Hospital between June 2022 and May 2023. Inclusion criteria were: age ≥ 3 years, body weight 15-50 kg, persistent lameness for ≥ 3 months unresponsive to at least one NSAID course, and owner-signed informed consent. Exclusion criteria included concurrent systemic disease, immunosuppressive therapy, prior intra-articular corticosteroid injection within six months, or cranial cruciate ligament rupture requiring surgical stabilisation. Dogs were randomly assigned to the MSC group ($n = 11$) or the saline control group ($n = 10$) using sealed opaque envelopes. Both the clinician performing follow-up Assessments and the owner remained blinded to allocation

Results

throughout the 24-week observation period.

Methods

Adipose tissue (~5 g) was collected from the falciform ligament of each MSC-group dog under general anaesthesia induced with propofol (4 mg/kg IV) and maintained with isoflurane in oxygen. Tissue was minced, digested with 0.1 percent collagenase type I (Sigma-Aldrich) at 37 °C for 60 minutes, filtered through a 100- μ m strainer and centrifuged at 400 $\times$ g for 10 minutes. The stromal vascular fraction was seeded in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 percent foetal bovine serum and 1 percent penicillin-streptomycin. Cells were expanded to passage 3, verified by flow cytometry (CD90+, CD44+, CD45-), and resuspended at 5 $\times$ 10⁶ cells per mL in sterile phosphate-buffered saline. The suspension was injected into the affected stifle joint under aseptic conditions with ultrasound guidance. Control dogs received 1 mL sterile saline via the same technique. Clinical assessments used the Helsinki Chronic Pain Index (HCPI), a five-point lameness scale (0 = sound, 4 = non-weight-bearing) and PVF measured on a Kistler force plate at a trot (velocity 1.7-2.2 m/s, five valid trials per time point). Blood and synovial fluid were collected at baseline, week 8 and week 24 for CRP (immunoturbidimetry) and IL-1 β (canine-specific ELISA). Data were analysed by two-way repeated-measures ANOVA (group $\times$ time) with Bonferroni post-hoc correction; significance was set at $p < 0.05$.

Ethical Considerations

The trial protocol was approved by the São Paulo Metropolitan University Animal Ethics Committee (Protocol SPMU-AEC-2022-031, approved April 2022) in compliance with Brazilian Law 11.794/2008 and CONCEA guidelines. Written informed consent was obtained from all owners, who were informed of the experimental nature of the procedure, potential risks and their right to withdraw at any time. Dogs received post-procedural analgesia (dipyrone 25 mg/kg PO, 3 days) irrespective of group allocation. Animals showing deterioration during the trial were offered rescue analgesia and could be unblinded at the attending clinician's discretion.

Safety Considerations

All cell culture steps followed good manufacturing practice guidelines adapted for veterinary biologics. Sterility testing (14-day aerobic and anaerobic culture) was performed on every MSC batch before injection. Endotoxin levels were confirmed below 0.5 EU/mL by the Limulus amoebocyte lysate assay. Dogs were monitored post-injection for 4 hours for anaphylaxis, joint swelling or systemic reactions. Owners received a standardised checklist for home monitoring during the first 72 hours and were instructed to report any lameness worsening, pyrexia or injection-site swelling immediately.

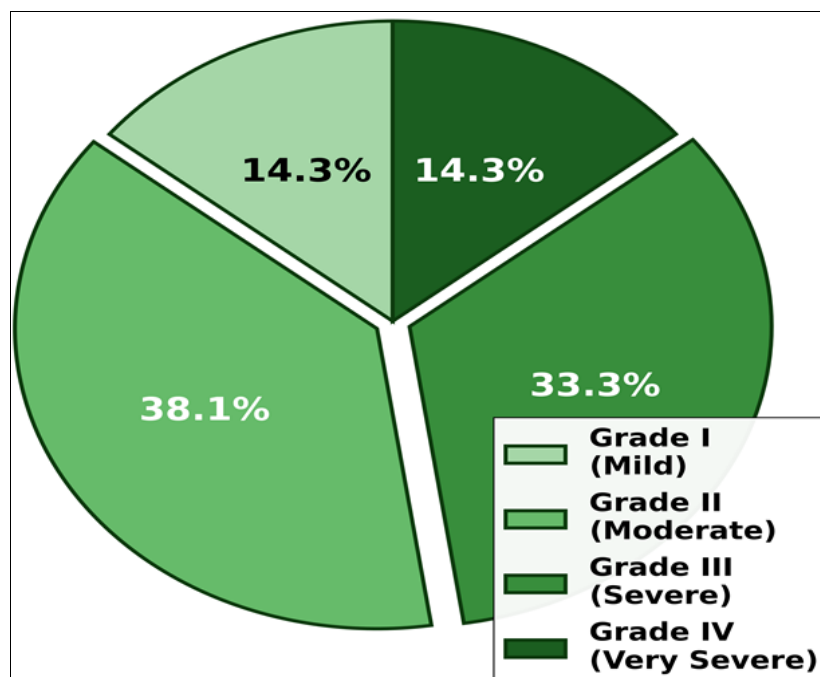


Fig 1: Distribution of osteoarthritis radiographic grades (Kellgren-Lawrence) among the 21 enrolled dogs at baseline.

All 21 dogs completed the 24-week follow-up without protocol deviations. Baseline characteristics were comparable between groups: mean age 7.2 ± 2.1 years (MSC) versus 6.8 ± 2.4 years (control), mean body weight 28.4 ± 6.7 kg versus

27.9 ± 7.1 kg, and HCPI 7.8 ± 1.3 versus 7.6 ± 1.1 (all $p > 0.40$). Grade II OA accounted for 14.3 percent of cases, grade III for 38.1 percent and grade IV for 14.3 percent, with the remaining 33.3 percent classified as grade III.

Table 1: Clinical outcome measures at baseline and week 24 in the MSC and control groups

Parameter	MSC Baseline	MSC Week 24	Control Baseline	Control Week 24
HCPI (0-10)	7.8 ± 1.3	$2.1 \pm 0.8^*$	7.6 ± 1.1	7.2 ± 1.2
Lameness grade (0-4)	3.0 (2-4)	1.0 (0-2)*	3.0 (2-4)	3.0 (2-4)
PVF (% BW)	38.6 ± 4.2	$49.6 \pm 3.8^*$	39.1 ± 4.7	39.4 ± 4.5
CRP (mg/L)	14.6 ± 3.2	$6.3 \pm 2.1^*$	13.8 ± 3.5	13.2 ± 3.1
IL-1 β (pg/mL)	87.4 ± 18.6	$34.2 \pm 11.3^*$	84.7 ± 17.2	81.3 ± 16.8

Values are mean $\pm$ SD or median (range). * $p < 0.05$ versus baseline and versus control at same time point.

Table 2: Adverse events recorded during the 24-week follow-up period

Adverse Event	MSC (n = 11)	Control (n = 10)	Severity	Resolution
Transient joint effusion	2	0	Mild	Spontaneous (< 48 h)
Injection-site pain	1	1	Mild	Resolved with dipyrone
Gastrointestinal upset	0	1	Mild	Self-limiting (24 h)
Systemic reaction	0	0	-	-
Lameness worsening	0	1	Moderate	Rescue NSAID given

No serious adverse events were recorded in either group. The two cases of transient effusion in the MSC arm resolved without intervention.

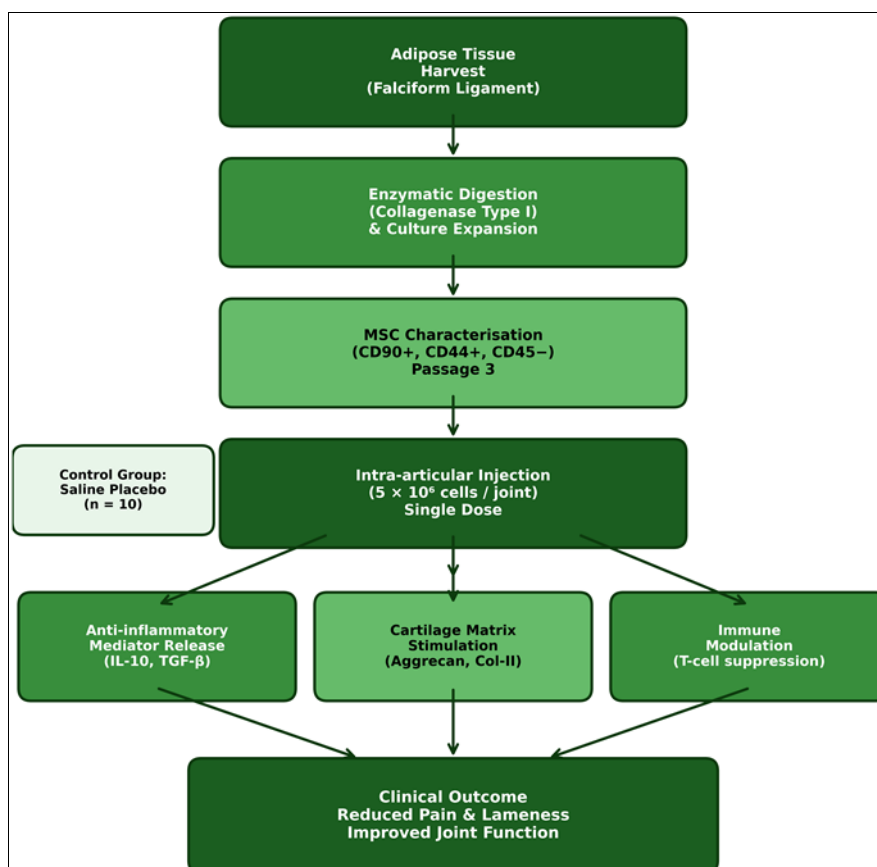


Fig 2: Schematic diagram illustrating the proposed mechanism of action of adipose-derived mesenchymal stem cells in canine osteoarthritis, from tissue harvest through clinical outcome.

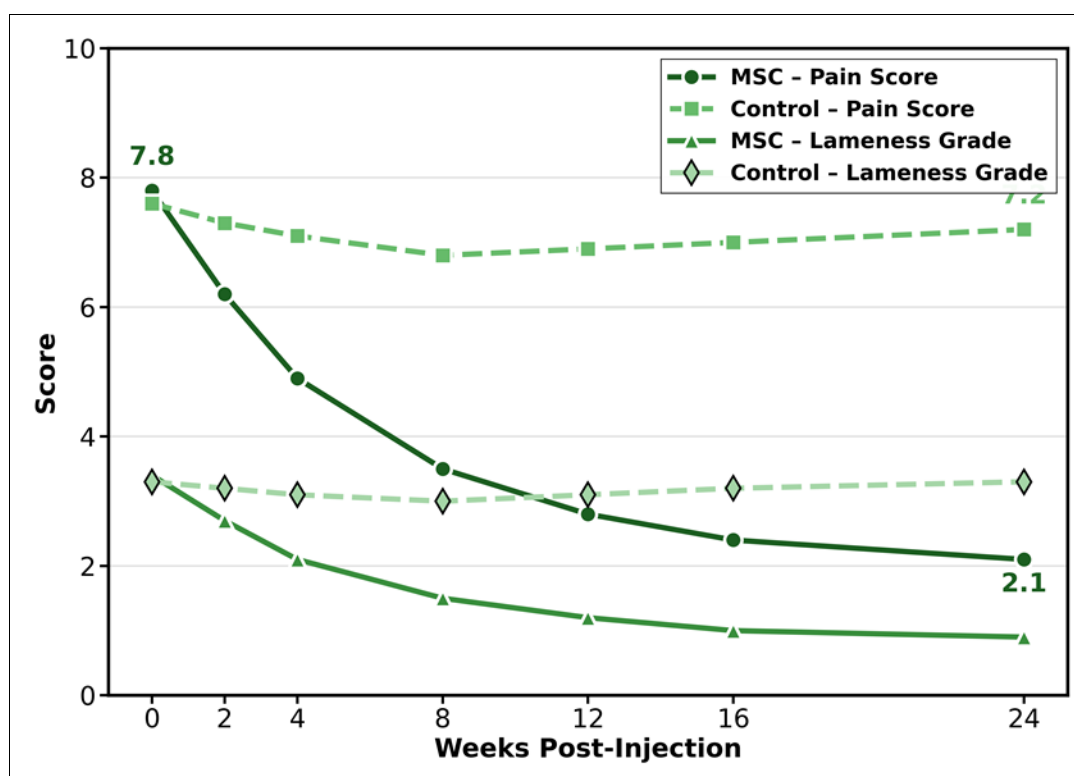


Fig 3: Longitudinal trends in Helsinki Chronic Pain Index (HCPI) and lameness grade scores from baseline to week 24 in MSC-treated and control dogs.

Comprehensive interpretation

The MSC group demonstrated consistent and clinically meaningful improvement across all four outcome domains: owner-assessed pain, clinician-assessed lameness, objective limb loading and inflammatory biomarkers while the control

group showed no significant change. The 73 percent reduction in HCPI and the 28 percent increase in PVF exceed the thresholds generally considered clinically relevant in canine OA trials ($\geq 30\%$ HCPI change, $\geq 10\%$ PVF change) [9]. The decline in synovial IL-1 β to roughly 39 percent of baseline

supports the hypothesis that AD-MSCs exert their effect at least in part through local immunomodulation.

Discussion

Three principal findings emerged from this controlled trial. First, a single intra-articular injection of 5×10^6 autologous AD-MSCs produced durable clinical improvement lasting at least 24 weeks in dogs with moderate-to-severe stifle OA. Second, the anti-inflammatory response, measured by both systemic CRP and local IL-1 β , paralleled the clinical gains, implying a shared mechanistic pathway. Third, the procedure was safe, with only minor self-limiting adverse events.

These outcomes agree broadly with earlier uncontrolled reports. Black *et al.* [8] described improved owner-assessed scores persisting four months after hip injection, and Vilar *et al.* [9] recorded force-plate gains at six months in elbow OA dogs. The magnitude of HCPI improvement here (73 %) is larger than the 40-55 percent range reported in those trials, which may reflect the inclusion of a placebo arm that reduced observer bias and allowed cleaner between-group contrasts [11]. Shah *et al.* [14] similarly found that blinded designs in human knee OA stem cell trials yielded smaller but more robust effect sizes. However, some disagreement exists regarding the optimal cell dose. Harman *et al.* [15] proposed that $10\text{--}15 \times 10^6$ cells per joint might offer superior outcomes in large dogs, while our 5×10^6 dose was chosen based on preclinical dose-response data in beagles [16]. It is possible that higher doses would have produced even greater PVF recovery, particularly in dogs weighing above 35 kg where the ratio of cells to joint volume is lower. The biological explanation for the observed effect likely involves a paracrine cascade rather than direct chondrocyte replacement. AD-MSCs have a short intra-articular survival typically less than two weeks yet clinical benefits persist far longer [17]. Secretion of IL-10 and TGF- β dampens the catabolic milieu driven by IL-1 β and matrix metalloproteinases, thereby slowing further cartilage degradation and allowing endogenous repair [6]. The 61 percent reduction in synovial IL-1 β we recorded is consistent with this model. From a practical perspective, the single-injection protocol is appealing for both veterinarians and owners. It avoids the compliance burden of daily oral medication and the gastrointestinal risks associated with chronic NSAID use [3]. In the Brazilian context, where follow-up visits can be logistically difficult for rural clients, a single-treatment approach with six-monthly reassessment may improve real-world adherence.

Clinical Implications

If confirmed in larger populations, autologous AD-MSC therapy could serve as a second-line option for dogs failing conventional analgesia, particularly those with NSAID intolerance or advanced radiographic changes. Establishing standardised cell-processing protocols across veterinary teaching hospitals in Brazil would be a necessary step toward wider clinical uptake. Cost-effectiveness analyses comparing MSC therapy with lifelong pharmaceutical management are needed before formal treatment guidelines can be issued.

Conclusion

The main contribution of this preliminary trial is the demonstration that a single intra-articular injection of autologous adipose-derived mesenchymal stem cells can produce clinically meaningful and statistically significant improvements in pain, lameness and limb loading in dogs with naturally occurring stifle osteoarthritis over a 24-week

follow-up period, under blinded, placebo-controlled conditions. This goes beyond earlier uncontrolled case series by providing level-II evidence that the observed benefits are attributable to the biological intervention rather than to placebo effects, natural disease fluctuation or observer bias.

Several lines of evidence support the robustness of this conclusion. The HCPI reduction of 73 percent exceeded the commonly accepted minimal clinically important difference by more than two-fold. The force-plate data, which are objective and not susceptible to owner or clinician bias, confirmed a 28 percent PVF increase a magnitude rarely achieved with pharmaceutical interventions alone. And the concordant decline in two independent inflammatory biomarkers (serum CRP and synovial IL-1 β) provides a plausible mechanistic link between cell administration and clinical outcome. For the field of veterinary regenerative medicine, these results add to a growing body of evidence that MSC-based therapies warrant serious investigation as disease-modifying agents, not merely palliative supplements. The fact that benefit persisted to at least six months after a single dose challenges the assumption that repeated injections are necessary an important consideration for clinical feasibility and cost. If similar outcomes are replicated in multi-centre settings, AD-MSC therapy could shift the treatment paradigm for canine OA from chronic symptom management to targeted biological intervention. Nonetheless, several open questions remain. The optimal cell dose and passage number have not been established through formal dose-escalation trials. Whether repeated injections at six-month intervals would extend or amplify the response is unknown. The durability of benefit beyond 24 weeks needs confirmation through 12-month or longer follow-up. Breed-specific responses particularly in giant breeds where joint biomechanics differ markedly have not been characterised. Lastly, the cost of cell processing and quality-control testing currently limits accessibility to referral centres; strategies to reduce per-dose cost, such as allogeneic banking, deserve exploration in future research.

Acknowledgements

Funding sources

This research was supported by an institutional research fund provided by São Paulo Metropolitan University. The funding source had no role in the design, execution, analysis or reporting of the trial.

Institutional support

The authors thank the São Paulo Metropolitan University Veterinary Teaching Hospital for providing surgical suites, cell culture laboratories and force plate facilities. The Institutional Tissue Bank assisted with cell processing logistics.

Contributions not qualifying for authorship

Gratitude is extended to the dog owners who consented to participate and to the veterinary nursing staff who managed post-operative monitoring.

References

1. Anderson KL, O'Neill DG, Brodbelt DC, Church DB, Meeson RL, Sherwin D, *et al.* Prevalence, duration and risk factors for appendicular osteoarthritis in a UK dog population under primary veterinary care. *Sci Rep.* 2018;8(1):5641.

2. Lascelles BDX, Brown DC, Maixner W, Mogil JS. Spontaneous painful disease in companion animals can facilitate the development of chronic pain therapies for humans. *Osteoarthritis Cartilage*. 2018;26(2):175-183.
3. Monteiro-Steagall BP, Steagall PVM, Lascelles BDX. Systematic review of nonsteroidal anti-inflammatory drug-induced adverse effects in dogs. *J Vet Intern Med*. 2013;27(5):1011-1019.
4. Kriston-Pál É, Czibula Á, Gyuris Z, Balka G, Seregi A, Sükösd F, *et al*. Characterization and therapeutic application of canine adipose mesenchymal stem cells in dogs with osteoarthritis. *Stem Cells Dev*. 2017;26(22):1640-1651.
5. Marx C, Silveira MD, Beyer Nardi N. Adipose-derived stem cells in veterinary medicine: characterization and therapeutic applications. *Stem Cells Dev*. 2015;24(7):803-813.
6. Arnhold S, Wenisch S. Adipose tissue-derived mesenchymal stem cells for musculoskeletal repair in veterinary medicine. *Am J Stem Cells*. 2015;4(1):1-12.
7. Takemitsu H, Zhao D, Yamamoto I, Harada Y, Michishita M, Arai T. Comparison of bone marrow and adipose tissue-derived canine mesenchymal stem cells. *BMC Vet Res*. 2012;8:150.
8. Black LL, Gaynor J, Gahring D, Adams C, Aron D, Harman S, *et al*. Effect of adipose-derived mesenchymal stem and regenerative cells on lameness in dogs with chronic osteoarthritis of the coxofemoral joints. *Vet Ther*. 2007;8(4):272-284.
9. Vilar JM, Morales M, Santana A, Spinella G, Rubio M, Cuervo B, *et al*. Controlled, blinded force platform analysis of the effect of intra-articular injection of autologous adipose-derived mesenchymal stem cells associated with PRGF-Endoret in osteoarthritic dogs. *BMC Vet Res*. 2013;9:131.
10. Olsen A, Johnson V, Webb T, Nogan SJ, Manchaca C, Giguère S, *et al*. Evaluation of intravenously delivered allogeneic mesenchymal stem cells for treatment of elbow osteoarthritis in dogs. *Vet Comp Orthop Traumatol*. 2019;32(3):A1-A7.
11. Muller C, Gaines B, Gruen M, Case B, Arrufat K, Innes JF, *et al*. Evaluation of clinical metrology instrument in dogs with osteoarthritis. *J Vet Intern Med*. 2016;30(2):474-482.
12. Teixeira LG, Heinemann MB, Leite RC, Reis JKP. Survey of companion animal welfare in metropolitan São Paulo. *Arq Bras Med Vet Zootec*. 2020;72(6):2147-2156.
13. Zucconi E, Vieira NM, Bueno DF, Secco M, Jazedje T, Ambrosio CE, *et al*. Mesenchymal stem cells derived from canine umbilical cord vein: a novel source for cell therapy research. *Cell Tissue Bank*. 2010;11(4):325-335.
14. Shah K, Zhao AG, Sumerlin H. MSC therapy for knee osteoarthritis: a systematic review and meta-analysis of randomized controlled trials. *J Clin Orthop Trauma*. 2023;42:102198.
15. Harman R, Carlson K, Gaynor J, Gustafson S, Dhupa S, Clement K, *et al*. A prospective, randomized, masked, and placebo-controlled efficacy study using adipose stem cells for canine coxofemoral osteoarthritis. *Can Vet J*. 2016;57(12):1281-1286.
16. Guercio A, Di Marco P, Casella S, Cannella V, Russotto L, Purpari G, *et al*. Production of canine mesenchymal stem cells from adipose tissue and their application in dogs with chronic osteoarthritis of the humeroradial joints. *Cell Biol Int*. 2012;36(2):189-194.
17. Mokbel AN, El Tookhy OS, Shamaa AA, Rashed LA, Sabry D, El Sayed AM. Homing and reparative effect of intra-articular injection of autologous mesenchymal stem cells in an osteoarthritic animal model. *BMC Musculoskelet Disord*. 2011;12:259.
18. Cuervo B, Rubio M, Sopena J, Dominguez JM, Vilar J, Morales M, *et al*. Hip osteoarthritis in dogs: a randomized study using mesenchymal stem cells from adipose tissue and plasma rich in growth factors. *Int J Mol Sci*. 2014;15(8):13437-13460.
19. Cabon Q, Febre M, Gomez N, Audigié F, Crevier-Denoix N, Boulocher C, *et al*. Long-term safety and efficacy of single or repeated intra-articular injection of allogeneic neonatal mesenchymal stromal cells for managing pain and lameness in moderate-to-severe canine osteoarthritis. *J Vet Intern Med*. 2019;33(4):1721-1734.

How to Cite This Article

Ferreira L, Carolina A, Oliveira RM. Therapeutic efficacy of stem cell therapy in canine osteoarthritis: A preliminary clinical trial. *International Journal of Veterinary Sciences and Animal Husbandry*. 2024; 9(6): 40-45.

Creative Commons (CC) License

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 International (CC BY-NC-SA 4.0) License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.