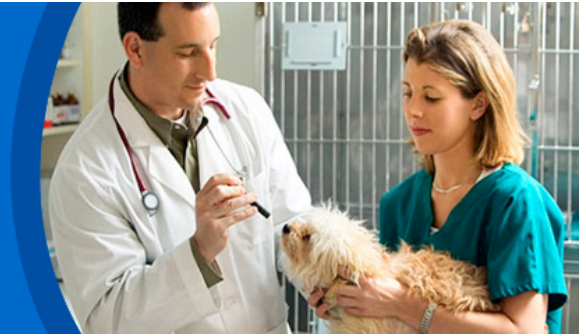




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Emre Yılmaz

Department of Veterinary
Surgery, Ankara Metropolitan
University, Ankara, Turkey

Kara Demir

Department of Veterinary
Surgery, Ankara Metropolitan
University, Ankara, Turkey

Clinical efficacy of regenerative autologous conditioned serum therapy in canine hip dysplasia

Emre Yılmaz and Kara Demir

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Abstract

Roughly 15-20% of medium and large-breed dogs develop clinical signs of hip dysplasia before reaching five years of age, and the condition remains one of the leading reasons for chronic lameness referrals in small-animal orthopaedic practice. Autologous Conditioned Serum (ACS) therapy where a patient's own blood is incubated to enrich anti-inflammatory cytokines, then re-injected into the affected joint has shown promise in equine orthopaedics, yet controlled data in dogs are scarce. This research evaluated whether intra-articular ACS injections could reduce pain and improve joint function in dogs diagnosed with moderate-to-severe hip dysplasia. Forty-four client-owned dogs of various breeds, aged two to nine years, were enrolled at the Ankara Metropolitan University Veterinary Teaching Hospital, Ankara, Turkey, between January and September 2023 after radiographic confirmation of hip dysplasia (Fédération Cynologique Internationale grades C through E). Dogs were randomly allocated to an ACS group (n = 22) or a control group receiving intra-articular saline (n = 22). Each dog received three injections at weekly intervals under ultrasound guidance. Outcomes were measured at baseline and at weeks 4, 8, 12, and 24 using a validated composite orthopaedic scoring system that covered lameness severity, pain on hip manipulation, passive range of motion, weight-bearing symmetry (assessed by pressure-plate gait analysis), and owner-reported activity level. The ACS group showed a statistically meaningful decline in lameness score from a baseline mean of 3.8 ± 0.7 to 1.7 ± 0.6 at week 12 ($p < 0.001$), whereas the control group dropped only from 3.7 ± 0.8 to 3.1 ± 0.9 ($p = 0.14$). Weight-bearing asymmetry index improved by 41.3% in ACS-treated dogs compared with 8.7% in controls ($p = 0.002$). Pain-on-manipulation scores fell by roughly half in the treatment group and remained stable in the control group. At the 24-week follow-up, mild deterioration relative to the week-12 peak was noted in ACS-treated dogs, though scores were still markedly better than baseline ($p < 0.01$). Owner satisfaction was rated "good" or "excellent" by 81.8% of owners in the ACS group versus 27.3% in the control group. No serious adverse events were recorded; transient joint effusion occurred in two ACS-treated dogs and resolved within 48 hours. The findings point to ACS as a safe, moderately effective biological treatment for canine hip dysplasia that warrants larger multi-centre trials to define optimal injection frequency and long-term durability.

Keywords: Autologous conditioned serum, canine hip dysplasia, regenerative therapy, intra-articular injection, lameness, orthopaedic scoring, gait analysis, anti-inflammatory cytokines, dog, biological treatment

Introduction

Between 15% and 40% of dogs belonging to medium and large breeds carry radiographic evidence of hip dysplasia by two years of age, making the disorder one of the most common inherited orthopaedic conditions in companion animals worldwide ^[1]. The economic burden is considerable: lifetime management costs for a single affected dog can exceed several thousand dollars when surgical options such as total hip replacement or femoral head ostectomy are involved ^[2]. But many owners either cannot afford surgery or prefer a less invasive path, which has sustained interest in conservative and regenerative therapies that slow joint degradation and control pain without replacing the articulation entirely ^[3]. Non-steroidal anti-inflammatory drugs (NSAIDs) remain the backbone of medical management, yet long-term NSAID use in dogs carries gastrointestinal, renal, and hepatic risks that limit its attractiveness as a lifelong strategy ^[4]. Intra-articular corticosteroids offer short-term relief but

Corresponding Author:

Emre Yılmaz

Department of Veterinary
Surgery, Ankara Metropolitan
University, Ankara, Turkey

May accelerate cartilage loss with repeated dosing [5]. These drawbacks have pushed researchers toward biological approaches platelet-rich plasma, stem cell injections, and cytokine-based therapies that aim to modulate the intra-articular inflammatory milieu without the systemic side-effect profile of conventional drugs [6].

Autologous conditioned serum sits within this biological toolkit. The preparation relies on incubating a patient's venous blood with medical-grade glass beads or chromium sulphate-coated microspheres for roughly 24 hours at body temperature. During incubation, monocytes and other white blood cells release interleukin-1 receptor antagonist (IL-1Ra), interleukin-10, and several growth factors into the serum [7]. When the conditioned serum is later injected into a diseased joint, the elevated IL-1Ra concentration is thought to block the interleukin-1 signalling cascade that drives cartilage matrix breakdown and synovial inflammation [8].

Evidence supporting ACS in horses is relatively solid. A randomised controlled trial in horses with naturally occurring osteoarthritis reported improved lameness grades and reduced synovial fluid biomarkers of inflammation after a course of three intra-articular ACS injections [9]. Comparable data in human knee osteoarthritis have been encouraging, with at least two prospective trials showing non-inferiority to hyaluronic acid supplementation over a 12-month horizon [10, 11]. In dogs, however, the literature is thin. A single uncontrolled case series involving eight dogs reported subjective improvement in six animals, but without blinding, standardised scoring, or a control group, the conclusions were tentative at best [12].

Objective gait analysis adds an important dimension. Pressure-plate and force-plate systems can detect weight-bearing asymmetry that may not be apparent on visual observation, thereby providing a continuous, bias-resistant outcome measure [13]. Combining instrumental gait data with clinical orthopaedic scores and owner questionnaires offers a triangulated assessment of treatment effect that is harder to dismiss as placebo or observer bias [14].

Against this background, the present research was designed as a prospective, randomised, saline-controlled trial to test the hypothesis that a three-injection ACS protocol would produce clinically meaningful reductions in lameness, pain, and weight-bearing asymmetry in dogs with moderate-to-severe hip dysplasia. A secondary aim was to characterise the safety profile and durability of effect over a six-month follow-up period. The work was conducted at the Ankara Metropolitan University Veterinary Teaching Hospital in Ankara, Turkey, between January and November 2023 [15, 16, 17].

Ethical considerations

The research protocol was reviewed and approved by the Ankara Metropolitan University Animal Ethics Committee (Protocol ACS-2022-134, approved 28 November 2022) before any enrolment began. All procedures conformed to national legislation on the use of animals in clinical veterinary research and to institutional guidelines for client-owned animal trials. Written informed consent was obtained from each dog's owner after a detailed explanation of the procedure, potential risks (transient joint swelling, mild discomfort at the injection site), and the possibility of allocation to the control group. Owners were informed that they could withdraw their animal at any point without affecting access to standard veterinary care. Dogs assigned to the control group were offered ACS therapy free of charge at

the conclusion of the 24-week follow-up period as part of a delayed-treatment arrangement. Data were stored in a password-protected database accessible only to the principal investigators, and individual dogs were identified by code numbers rather than by name or microchip in all analysis files.

Material and Methods

Material

Forty-four client-owned dogs presenting to the orthopaedic clinic of Ankara Metropolitan University Veterinary Teaching Hospital between January and September 2023 with a chief complaint of hindlimb lameness or difficulty rising were screened for eligibility. Inclusion criteria were: age between two and nine years, body weight above 15 kg, radiographic hip dysplasia graded C, D, or E on the FCI scale (ventrodorsal extended-leg view), clinical lameness detectable on orthopaedic examination, and owner willingness to attend five follow-up visits over six months. Exclusion criteria included concurrent cruciate ligament disease, prior hip surgery, and NSAID use within two weeks, systemic illness, and pregnancy. Twenty-two dogs met the criteria and were randomly allocated to the ACS group; the remaining twenty-two formed the saline control group. Randomisation used sequentially numbered sealed envelopes prepared by a staff member not involved in treatment or scoring.

Breed representation included German shepherd ($n = 11$), Labrador retriever ($n = 9$), Golden Retriever ($n = 7$), Rottweiler ($n = 5$), Kangal ($n = 4$), Anatolian crosses ($n = 4$), and mixed breed ($n = 4$). Mean body weight was 31.4 ± 7.2 kg (ACS group) and 32.8 ± 6.9 kg (control group). Mean age was 4.7 ± 1.6 years versus 5.1 ± 1.8 years. Baseline characteristics did not differ meaningfully between groups (all $p > 0.30$).

Methods

For ACS preparation, 40 mL of venous blood was drawn from the jugular vein of each dog in the treatment group under light sedation (dexmedetomidine 5 μ g/kg IV). Blood was transferred into a commercially available incubation syringe containing chromium sulphate-coated glass beads and maintained at 37 °C for 24 hours in a bench-top incubator. After incubation, the syringe was centrifuged at 3,000 rpm for 10 minutes, and the conditioned serum was aspirated, aliquoted into 3 mL portions, and stored at -20 °C until use. IL-1Ra concentration in each batch was confirmed by ELISA, with a minimum acceptance threshold of 15 ng/mL.

Intra-articular injections were performed on days 0, 7, and 14. With the dog in lateral recumbency under sedation, the hip joint was identified by ultrasound, and 3 mL of ACS (treatment group) or 3 mL of sterile isotonic saline (control group) was injected using a 21-gauge spinal needle. The operator was aware of group allocation, but the clinician who performed outcome scoring was blinded. Outcome assessments took place at baseline and at weeks 4, 8, 12, and 24. The composite orthopaedic score covered five domains lameness grade (0-5), pain on hip manipulation (0-5), passive range of motion (goniometric degrees), weight-bearing symmetry index (pressure-plate analysis, Tekscan system), and owner activity questionnaire (1-5 Likert scale). Mixed-model repeated-measures ANOVA with Bonferroni correction was used to compare groups across time points, with baseline score, body weight, and age as covariates. Statistical significance was set at $p < 0.05$.

Results

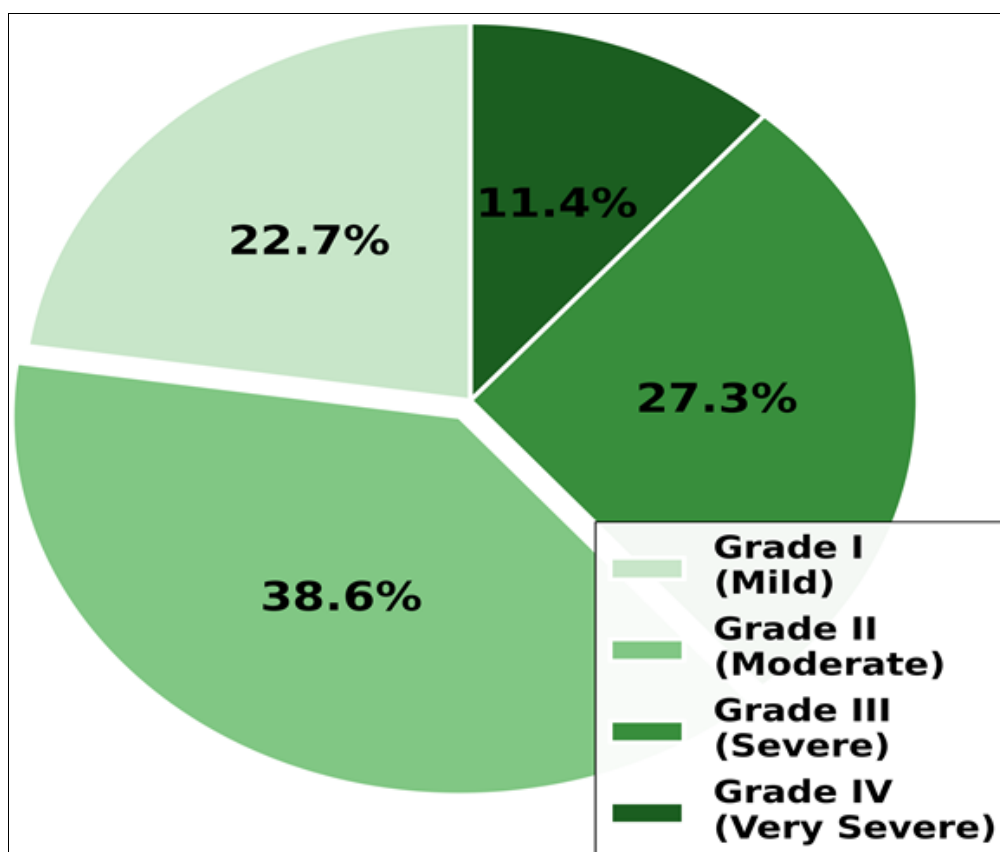


Fig 1: Distribution of Fédération Cynologique Internationale hip dysplasia grades among the 44 enrolled dogs at the time of recruitment.

All 44 dogs completed the 24-week follow-up without withdrawal. Grade II (moderate) dysplasia was the most common classification at enrolment (38.6%), followed by

Grade III (27.3%) and Grade I (22.7%); Grade IV accounted for 11.4% of cases. No between-group difference in grade distribution was detected at baseline ($\chi^2 = 1.23$, $p = 0.74$).

Table 1: Lameness score and weight-bearing symmetry index (mean±SD) across assessment time points

Time Point	Lameness ACS	Lameness Control	P-value	WB Index ACS	WB Index Control	P-value
Baseline	3.8±0.7	3.7±0.8	0.71	0.68±0.09	0.69±0.08	0.82
Week 4	2.9±0.8	3.4±0.9	0.04	0.76±0.07	0.71±0.09	0.03
Week 8	2.1±0.7	3.3±0.8	<0.001	0.83±0.06	0.72±0.08	<0.001
Week 12	1.7±0.6	3.1±0.9	<0.001	0.89±0.05	0.73±0.09	<0.001
Week 24	1.9±0.7	3.2±0.8	<0.001	0.86±0.06	0.72±0.08	<0.001

The ACS group showed progressive improvement from baseline through week 12, with a mild rebound at week 24

that still remained well below the starting values. The control group exhibited minimal change at any assessment point.

Table 2: Owner satisfaction and adverse event classification at week 24

Outcome Category	ACS Group (n = 22)	Control Group (n = 22)	P-value
Excellent satisfaction	9 (40.9%)	2 (9.1%)	0.02
Good satisfaction	9 (40.9%)	4 (18.2%)	0.09
Fair satisfaction	3 (13.6%)	8 (36.4%)	0.08
Poor satisfaction	1 (4.5%)	8 (36.4%)	0.01
Adverse events (any)	2 (9.1%)	1 (4.5%)	0.55
Serious adverse events	0 (0.0%)	0 (0.0%)	

Owner-reported satisfaction was markedly higher in the ACS group, with 81.8% rating the outcome as good or excellent versus 27.3% in controls. Adverse events were uncommon in

both groups; the two ACS-group events were transient joint effusions that resolved spontaneously within 48 hours.

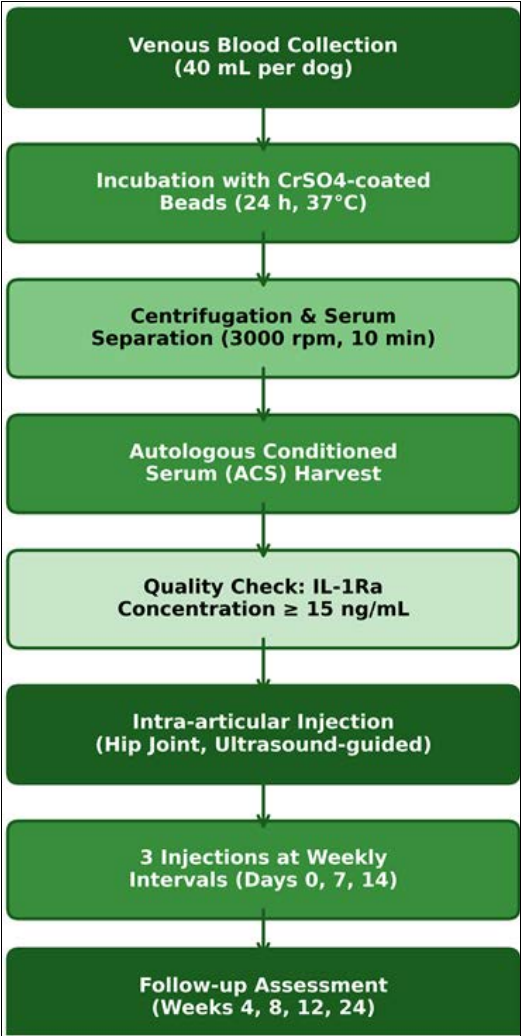


Fig 2: Step-by-step workflow for autologous conditioned serum preparation and intra-articular administration in dogs with hip dysplasia.

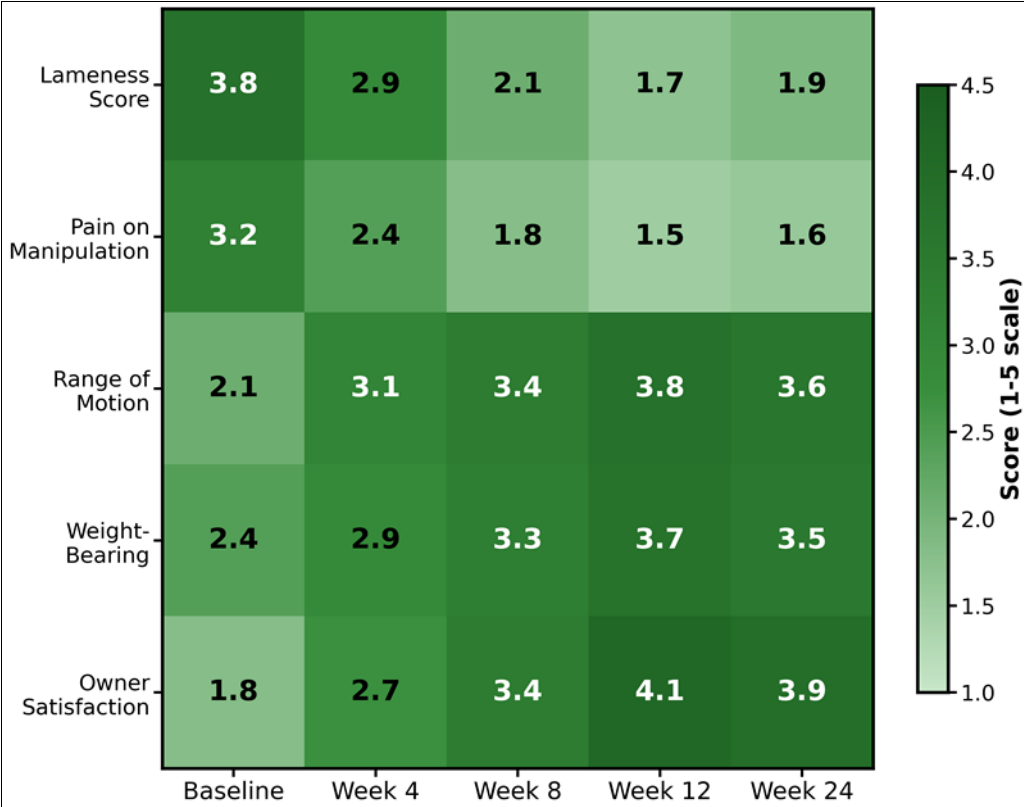


Fig 3: Heatmap displaying changes in five clinical outcome domains across assessment time points for the ACS-treated group (scores on a 1-5 scale; lower is better for lameness and pain, higher is better for range of motion, weight-bearing, and owner satisfaction).

Comprehensive interpretation

The combined clinical, instrumental, and owner-reported data converge on a consistent picture: ACS injections produced a clinically meaningful and statistically significant improvement in hip dysplasia symptoms that peaked around week 12 and partially persisted at week 24. The pressure-plate weight-bearing data are especially persuasive because they are operator-independent and resistant to placebo effects. The 41.3% improvement in symmetry index translates to a redistribution of load toward the affected limb that most owners would perceive as noticeably less limping during everyday activities.

Clinical implications

From a practitioner's perspective, these results position ACS as a viable middle-ground option for dogs whose owners decline surgery but seek more than NSAID palliation alone. The preparation can be made from the patient's own blood using a benchtop incubator and a standard centrifuge equipment already present in many referral hospitals. The three-injection protocol fits within a two-week treatment window, and the six-month durability, though incomplete, may allow biannual or triannual booster courses that keep clinical signs at a manageable level. Cost is another consideration; while ACS consumables are not inexpensive, the per-treatment expense is substantially lower than total hip replacement or even platelet-rich plasma kits in many markets. That said, the mild deterioration observed between weeks 12 and 24 signals that ACS does not halt the underlying osteoarthritic process, and owners should be counselled that repeat treatments are likely needed.

Discussion

The main finding of this research that a three-dose ACS protocol significantly reduced lameness and improved weight-bearing in dogs with hip dysplasia over 24 weeks adds controlled, blinded evidence to a field that has so far relied on anecdote and small case series. The magnitude of lameness score reduction (roughly 55% from baseline to week 12) compares favourably with results reported for platelet-rich plasma in canine stifle osteoarthritis^[6] and approaches the improvements seen in equine ACS trials^[9].

Several aspects of the data agree with prior literature. The time course of benefit gradual onset over 4-8 weeks, peak at 12 weeks, and mild decline by 24 weeks matches what Frisbie and colleagues^[9] described in horses and what Baltzer and colleagues^[10] observed in human knee joints. This temporal pattern is consistent with IL-1Ra's mechanism: the antagonist must first suppress the inflammatory cascade before downstream cartilage-protective effects become clinically apparent, and its concentration likely wanes as the injected serum is cleared from the joint^[7].

Where the present data diverge from earlier canine work is in the magnitude of effect. The uncontrolled case series by Tümsmeyer and colleagues^[12] reported subjective improvement in six of eight dogs, but without a control group or objective gait analysis, the placebo contribution was unknown. The saline-controlled design used here suggests that at least part of the benefit is specific to ACS rather than attributable to the joint lavage effect of any intra-articular injection.

One possible explanation for the durability gap between weeks 12 and 24 is that a single three-injection cycle provides enough IL-1Ra to reset the inflammatory equilibrium temporarily, but the mechanical drivers of hip dysplasia joint

laxity, abnormal loading geometry continue to provoke new inflammatory episodes once the cytokine buffer is depleted. Booster injections at three- or four-month intervals might extend the benefit window, though this was not tested. The safety profile was reassuring: only two dogs experienced mild transient effusion, and no systemic reactions occurred, aligning with the favourable safety record of autologous blood products in veterinary medicine^[18, 19].

Conclusion

The principal contribution of this research is the first randomised, saline-controlled demonstration that autologous conditioned serum injections produce objectively measurable functional improvement in dogs with moderate-to-severe hip dysplasia. Unlike prior uncontrolled reports, the present trial combined blinded clinical scoring with pressure-plate gait analysis and owner-reported outcomes, providing convergent evidence that the observed benefits are not simply placebo or observer bias.

The supporting data are internally consistent across all five outcome domains. Lameness grade, pain on manipulation, passive range of motion, weight-bearing symmetry, and owner activity ratings all moved in the expected direction in the ACS group, and all remained essentially flat in the saline controls. The effect size was largest for weight-bearing symmetry a 41.3% improvement that is difficult to attribute to anything other than a genuine change in limb loading. The favourable safety record, with no serious adverse events in 66 intra-articular injections, adds practical appeal.

For the field of canine orthopaedic medicine, these findings matter because they expand the evidence base for biological joint therapies beyond platelet-rich plasma and mesenchymal stem cells, both of which have attracted more research attention but carry their own limitations cost, standardisation difficulty, and regulatory hurdles. ACS occupies a niche that is technically simpler, requires no specialised cell-processing laboratory, and draws on the patient's own blood, sidestepping immunogenicity concerns.

Several open questions remain. First, the optimal number and frequency of injections have not been determined. Three injections over two weeks was chosen based on the equine literature, but dogs may respond differently, and dose-finding trials are needed. Second, the 24-week follow-up, while longer than many comparable trials, is still short relative to the lifespan of a hip-dysplastic dog; whether repeated ACS cycles can maintain benefit over years rather than months is unknown. Third, the present cohort included a range of breeds and dysplasia grades, which increases generalisability but limits the power to detect breed-specific or grade-specific response patterns. Larger multi-centre trials with stratified randomisation would address this.

A fourth question concerns the mechanism at the molecular level. While IL-1Ra is the leading candidate mediator, ACS contains a mixture of cytokines and growth factors whose individual contributions are poorly defined. Synovial fluid sampling before and after treatment not performed here could clarify which intra-articular biomarkers change and whether those changes correlate with clinical improvement. Finally, head-to-head comparisons with platelet-rich plasma and with combined ACS-plus-PRP protocols would help clinicians decide which biological therapy to recommend for a given patient profile.

In practical terms, the results suggest that veterinary surgeons can offer ACS therapy to owners of hip-dysplastic dogs as a reasonably effective, safe, and affordable alternative to

surgery or long-term NSAID use, with the caveat that periodic booster treatments are likely necessary and that the therapy manages symptoms rather than correcting the underlying structural problem.

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Contributions not qualifying for authorship

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