



Original Research Paper

Biologics in Competition: Comparing Anti-Inflammatory (ACS) and Regenerative (PRP) Approaches for Intra-Articular Treatment of Canine Traumatic Arthritis

Laith Mohanad Jabbar¹, Ahmed Hassan Salh², Mustafa Sabbar Mhr³, Mohammed R. Abduljaleel^{4*}

¹Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Al-Qadisiyah, Al Diwaniyah, Iraq

²Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Al-Qasim Green, Babil, Iraq

³Department of Public Health, College of Veterinary Medicine, University of Al-Qadisiyah, Al Diwaniyah, Iraq

⁴Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Basrah, Basrah-Iraq.

*Corresponding author: Mohammed R. Abduljaleel. ⁴Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Basrah, Basrah-Iraq, 61004, Email: Mohammed.resen@uobasrah.edu.iq.

Key Words

Abstract

Platelet-Rich Plasma;
Autologous Conditioned Serum;
Interleukin-1 Receptor Antagonist; Growth Factors;
Traumatic Arthritis;
Regenerative Medicine.

Background: Traumatic arthritis in the dog results in osteoarthritis. Autologous Conditioned Serum (ACS) and Platelet-Rich Plasma (PRP) have promise but have not been directly compared in terms of pro-regenerative vs anti-inflammatory mechanisms.

Objectives: To compare intra-articular PRP and ACS in a canine model of traumatic arthritis based on clinical, biochemical, and radiographic outcomes.

Methods: 15 dogs (5/group) were surgically induced to have arthritis of the left stifle by crushing the CCL. Groups were injected 48h after with one intra-articular dose of autologous PRP, ACS or saline (control). The baseline and day 2 evaluations included lameness, swelling, range of motion, synovial fluid analysis and radiography, as well as at weeks 1–3.

Results: Both treatments were significantly better than the control treatment ($p < 0.05$). PRP enhanced the joint flexion angle and synovial fluid viscosity at week 3 ($p < 0.01$ vs. ACS/control). ACS was more effective in reducing synovial WBC count ($p < 0.01$ vs. PRP/control) and resolving edema/redness earlier. ACS lowered inflammatory markers more; PRP normalized glucose and protein levels.

Conclusion: Both PRP and ACS work but in different ways: PRP stimulates functional and structural repair via growth factors; ACS blocks acute inflammation (IL-1Ra) rapidly. Treatment should be based on clinical presentation: use PRP for regeneration and ACS for severely inflamed joints. This aids in individualized treatment strategies in veterinary regenerative medicine.

Introduction

Inflammatory and traumatic joint diseases, particularly those that progress to osteoarthritis, are an important health and economic burden in companion animal medicine, causing significant functional limitation and ongoing discomfort (Kuyinu et al., 2016; Roitner et al., 2024). While traditional therapies like corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) can reduce symptoms, they frequently have systemic side effects and do not address the disease's biological causes or promote healing of damaged

tissues (Wehling et al., 2007; Alrafas et al., 2023; Jeyaraman et al., 2024). Because of these limitations, much research has been conducted on alternative biological therapies that seek to enhance intrinsic healing mechanisms and alter the diseased joint milieu. In this regard, autologous treatments made from the patient's own blood have become a potential area of regenerative medicine.

The two main biological treatments under consideration are autologous conditioned serum (ACS) and platelet-rich plasma (PRP). Platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor-beta (TGF- β) are just a few of the growth

*Corresponding Author:

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factors that are released when platelets from whole blood are activated. When taken as a whole, these elements promote angiogenesis, extracellular matrix formation, and cell proliferation, which fosters the healing of soft and bony tissues (Towfik and Mutar, 2021; Civinini *et al.*, 2013; Toholova *et al.*, 2024; Jasim *et al.*, 2025). ACS therapy, on the other hand, is based on a different idea: autologous blood is incubated with stimulants such as glass beads, which causes white blood cells to create and release large concentrations of anti-inflammatory substances, most notably the Interleukin-1 Receptor Antagonist (IL-1Ra). By competitively preventing interleukin-1 α and β from binding to their receptors, IL-1Ra suppresses a key inflammatory pathway that causes cartilage deterioration and the advancement of arthritis (Wehling *et al.*, 2007; Gabay *et al.*, 2010; Alrafas *et al.*, 2023; Goodrich *et al.*, 2024).

The effectiveness of each treatment on its own has been shown in earlier research. According to a study by Towfik and Mutar (2021), intra-articular injection of autologous PRP significantly improved the biochemical characteristics of synovial fluid and clinical indicators (such as lameness and joint angle) in dogs with traumatic arthritis. Similar to this, earlier studies conducted in the same study setting showed that utilizing ACS also improved inflammatory state and that substituting autologous normal synovial fluid for inflammatory synovial fluid had a positive therapeutic impact.

But despite mounting evidence that both treatments are beneficial, there is still a crucial information gap: there is almost no direct, systematic comparison between them in a single, identical experimental paradigm. This kind of direct head-to-head comparison is the only way to distinguish the subtle variations in the effectiveness of each, show which may be better at enhancing particular clinical or biochemical indications, and ultimately offer significant, evidence-based recommendations for clinical decision-making.

Thus, by directly comparing the effectiveness of intra-articular injection of autologous PRP and autologous ACS in treating experimentally produced traumatic arthritis in dogs, this work seeks to close this gap. In order to compare the "enhanced repair" paradigm represented by PRP with the "inflammation suppression" model represented by ACS, the study will assess clinical outcomes (lameness, swelling, range of motion), biochemical

outcomes (synovial fluid analysis), and radiographic outcomes. It is anticipated that the results of this study will play a major role in creating a more accurate road map for customized biological therapy in veterinary regenerative medicine.

Materials and Methods

Ethical Approval

This experimental study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Veterinary Medicine, University of Al-Qadisiyah (Approval No.: 3018 in 15-5-2024). All procedures were conducted in strict accordance with international guidelines for the care and use of laboratory animals.

Study Design and Animals

A prospective, randomized, controlled, blinded experimental trial was conducted. Fifteen healthy, adult local breed dogs of both sexes, weighing 15-25 kg and aged 9 ± 3 months, were used. All the dogs came from a control breeding program and were judged clinically normal at the time of entry by a physical examination, complete blood count (CBC), and serum biochemistry. For two weeks, they were acclimatized in separate pens under standardized conditions (12-hour light/dark cycle, $22 \pm 2^\circ\text{C}$ temperature, balanced commercial diet and water *ad libitum*).

Randomization and Grouping

A random number table was used to randomly assign the dogs to three experimental groups ($n=5$) with the aid of a computer. The groups were:

1. Control Group (GC): Induced arthritis + intra-articular injection of 2 ml sterile 0.9% normal saline.
2. PRP Group (G-PRP): Induced arthritis + injection of 2 ml autologous Platelet-Rich Plasma into the joint.
3. ACS Group (G-ACS): Induced arthritis + intra-articular injection of 2 ml of the patient's own Conditioned Serum.

Induction of Traumatic Arthritis

Surgery was performed under general anesthesia, pre-medicated with xylazine 2% (2 mg/kg), then ketamine 10% (15 mg/kg) was administered as the anaesthetic (Abduljaleel *et al.*, 2025). Aseptic technique was carefully adhered to. The left stifle joint was exposed using the lateral parapatellar approach. As described, the cranial cruciate ligament (CCL) was seen and was completely crushed with a hemostatic forceps to induce instability and acute post-traumatic arthritis. The joint capsule, the overlying subcutis and the skin were stitched in layers.

Preparation of Autologous Therapies

Dogs in the G-PRP group: The venous blood was aseptically collected from the cephalic vein of the 5 dogs to which 3.2% sodium citrate was added for anticoagulation and then prepared into Platelet-Rich Plasma (PRP). PRP was prepared according to a standardised double centrifugation protocol (Dhurat & Sukesh, 2014; Hamroyev et al., 2024; Muthuprabakaran et al., 2021; Xue et al., 2025). The blood was first centrifuged at $200 \times g$ for 15 minutes. The supernatant plasma containing platelets was collected and transferred to a sterile tube, leaving the red blood cell pellet. This plasma was then centrifuged at $400 \times g$ for 10 minutes. The upper platelet-poor plasma (PPP) layer was removed, and the lower 2 ml, constituting the concentrated PRP, was aspirated. The PRP was activated *ex vivo* by adding 0.2 ml of 10% calcium chloride solution just prior to injection (Jasim et al., 2025).

Autologous Conditioned Serum (ACS): For the G-ACS dogs, 10 ml of whole blood was aseptically collected into special sterile syringes (Orthogen, Germany) pre-filled with sterile medical-grade glass beads ($\varnothing$ 4mm). The syringes were incubated for 8 hours at 37°C in a 5% CO₂ atmosphere. After incubation, the syringes were centrifuged at $3000 \times g$ for 10 minutes. The supernatant (the ACS) was filtered through a 0.2 μ m filter into a sterile vial and used within 2 hours of preparation (Curtis *et al.*, 2024; Della Tommasa *et al.*, 2025; Jeyaraman *et al.*, 2024; Shih *et al.*, 2024).

Treatment Protocol

Forty-eight hours after the CCL-crushing surgery, the dogs were re-anesthetized. Under aseptic conditions and ultrasound guidance, the left stifle joint was approached for arthrocentesis. Approximately 1.5 ml of synovial fluid (SF) was aspirated (for baseline analysis). Immediately after aspiration, 2 ml of the assigned treatment (saline, activated PRP, or ACS) was injected intra-articularly.

Assessment Parameters and Timeline

Assessments were performed at the following time points: Day 0 (pre-induction baseline), Day 2 (pre-treatment, post-induction), and weeks 1, 2, and 3 post-treatment. The primary investigator performing clinical assessments and the laboratory analyst were blinded to the group allocation.

Clinical Evaluation

Lameness Score: Assessed using a validated 5-point scale (0 = normal gait; 5 = non-weight bearing) (AlDirawi et al., 2018).

Stifle Joint Circumference (cm): Measured

at the level of the patellar tendon with a flexible tape measure.

Range of Motion (ROM): The stifle joint's passive flexion angle was measured using a universal goniometer.

Synovial Fluid (SF) Analysis

Physical Properties: Color, clarity, and viscosity (string test >5 cm).

Biochemical Analysis: Total protein (g/dL) by biuret method, Glucose (mg/dL) by glucose oxidase method, Alkaline Phosphatase (ALP, U/L) by kinetic method, and total White Blood Cell count (WBC/ μ L) using an automated hemocytometer.

Radiographic Evaluation

Mediolateral and craniocaudal radiographic views of the left stifle joint were taken. Radiographs were evaluated by a board-certified veterinary radiologist blinded to the groups for signs of joint effusion, periarticular soft tissue swelling, and osteophytosis using a standardized scoring system (0–3). (AlDirawi et al., 2018)

Statistical Analysis

All data were analyzed using SPSS software (Version 28.0, IBM Corp.). Normality of distribution was tested using the Shapiro-Wilk test ($W > 0.91$, $p > 0.05$ across variables). Data are presented as mean $\pm$ standard deviation (SD). For comparisons between the three groups across multiple time points, a two-way repeated measures Analysis of Variance (ANOVA) was used, followed by Tukey's post-hoc test for pairwise comparisons. A p-value of less than 0.05 was considered statistically significant (Alrafas et al., 2023).

Results

Clinical Outcomes

Both autologous treatment groups (G-PRP and G-ACS) demonstrated statistically significant improvement in all clinical parameters compared to the Control Group (GC) over the 3-week observation period ($p < 0.001$ for time $\times$ group interaction effect).

Lameness Score: Lameness was severe in all groups at Day 2 post-induction (mean scores: 4.2 ± 0.4). A significant divergence was observed from Week 1. G-ACS showed the most rapid reduction in lameness, reaching a near-normal score (0.6 ± 0.5) by Week 3. G-PRP showed a more gradual but consistent improvement, achieving a score of 1.4 ± 0.5 by Week 3, which was still significantly better than GC (3.8 ± 0.4) ($p < 0.01$ between G-ACS and G-PRP at Week 2; $p < 0.05$ at Week 3). (Figure 1A)

Joint Circumference: Swelling peaked at Day 2. While both treatment groups showed significant reduction, G-ACS again led to a faster resolution. Joint circumference in G-ACS normalized to

baseline levels by Week 2, whereas G-PRP reached similar levels by Week 3. The GC remained significantly more swollen throughout the study ($p < 0.001$ vs. both treatment groups at

all post-treatment points) (Figure 1B).

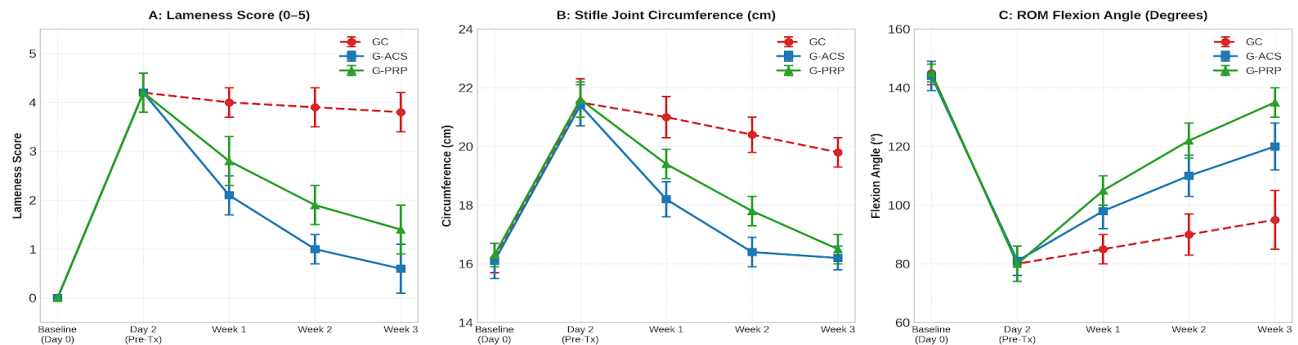


Figure 1: Longitudinal Path of Clinical Outcomes

(A) Lameness Score (0-5): Rapid reduction in G-ACS

(B) Stifle Joint is show resolution of periarticular swelling in G-ACS

(C) ROM showed restoration of fuction in G-PRP

Range of Motion (Flexion Angle): This parameter revealed a clear advantage for G-PRP. While both G-PRP and G-ACS improved from the severely restricted angle at Day 2, dogs in G-PRP achieved a significantly greater flexion angle ($135^\circ \pm 5^\circ$) compared to G-ACS ($120^\circ \pm 8^\circ$) and GC ($95^\circ \pm 10^\circ$) by Week 3 ($p < 0.001$ for

G-PRP vs. G-ACS and GC) (Figure 1C).

Synovial Fluid Analysis

Synovial fluid analysis revealed distinct patterns of response corresponding to the different therapeutic mechanisms (Table 1).

Table 1. Synovial Fluid Biochemical Parameters (Mean $\pm$ SD) at Day 2 and Week 3 Post-Treatment

Parameter	Group	Day 2 (Pre-Tx)	Week 3	p-value (Day 2 vs. W3 within group)	p-value (Between groups at W3)
TP (g/dL)	G-C	7.8+0.7	6.9+0.5	0.13	-
	G-PRP	7.5+0.6	3.5+0.3	0.001>	<0.01(vs.GC&G-ACS)
	G-ACS	7.6+ 0.5	4.8+0.4	0.001>	<0.01(vs.GC)
Glucose (mg/Dl)	G-C	42+9	48+7	0.45	-
	G-PRP	40+7	62+5	0.001>	<0.05(vs.GC & G-ACS)
	G-ACS	41+8	55+6	0.01>	<0.05(vs.GC)
ALP (U/L)	G-C	195+22	130+15	0.01>	-
	G-PRP	205+19	165+12	0.001>	<0.01(vs.GC)
	G-ACS	200+16	145+10	0.001>	NS (vs.GC)
WBC (cells/ μ L)	G-C	15000+2300	12000+1900	0.07	-
	G-PRP	16000+2600	4500+800	0.001>	<0.001(vs.GC)
	G-ACS	15800+2100	1100+450	0.001>	<0.001(vs.GC & G-PRP)

Abbreviations: TP=Total Protein; ALP=Alkaline Phosphatase; WBC=White Blood Cell; GC=Control; G-PRP=PRP Group; G-ACS=ACS Group.

Symbols: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to the group's own Day 2 value. NS = Not Significant.

Physical Properties: SF viscosity, assessed by the string test, was severely reduced in all groups at Day 2. By Week 3, G-PRP restored near-normal, strong viscosity (string length > 5 cm) in 4 out of 5 dogs. G-ACS showed moderate (viscid) restoration. GC SF remained thin and watery. SF color progressed from dark yellow (inflammatory) to pale yellow in G-ACS and yellow in G-PRP, while remaining dark yellow in GC.

Biochemical Markers

Total Protein (TP): TP was markedly elevated at Day 2. Levels decreased significantly in both treatment groups, with G-PRP showing the lowest final value (3.5 ± 0.3 g/dL), closely resembling healthy SF, compared to G-ACS (4.8 ± 0.4 g/dL) and GC (6.9 ± 0.5 g/dL) ($p < 0.01$ G-PRP vs. G-ACS at Week 3).

Glucose: Synovial fluid glucose was depleted at Day 2. G-PRP treatment led to a significant and sustained increase, normalizing values (62 ± 5 mg/dL) by Week 3. G-ACS showed a moderate increase (55 ± 6 mg/dL), while GC levels remained low (48 ± 7 mg/dL) ($p < 0.05$ G-PRP vs. G-ACS at Week 3).

Alkaline Phosphatase (ALP): ALP activity

was high initially. It decreased in all groups but remained highest in G-PRP at Week 3 (165 ± 12 U/L), consistent with active tissue remodeling, compared to G-ACS (145 ± 10 U/L) and GC (130 ± 15 U/L).

White Blood Cell (WBC) Count: The most striking difference was observed in WBC count. G-ACS caused a dramatic and rapid decrease in SF WBCs, down to near normal levels ($1,200 \pm 350$ cells/ μ L) by Week 2. G-PRP also decreased the number of WBCs, albeit at a slower rate, with a final count of $4,500 \pm 800$ cells/ μ L at Week 3. GC remained elevated at $> 12,000$ cells/ μ L throughout the study ($p < 0.001$ G-ACS vs. G-PRP at Weeks 1, 2 and 3).

Radiographic Evaluation

At Day 2, severe joint effusion and soft tissue swelling were confirmed on radiographs of all animals. Both G-ACS and G-PRP showed significantly decreased effusion at Week 3. Qualitative evaluation, however, indicated earlier improvement in soft tissue swelling in G-ACS, and G-PRP joints had better preservation of the radiographic joint space width. In all groups no osteophyte formation was seen in the 3 weeks studied (Figure 2, 3, 4 and 5).

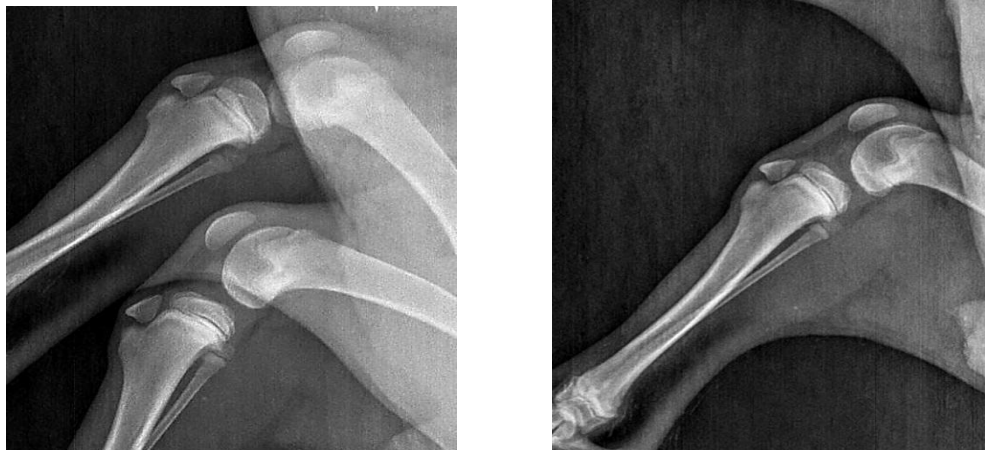


Figure 2. Radiographic views at Week 1 and Week 3 (right) of Group 1 (Control, G-C).



Figure 3. Radiographic views of Group 2 (G-PRP) at Week 1 and Week 3 (right).

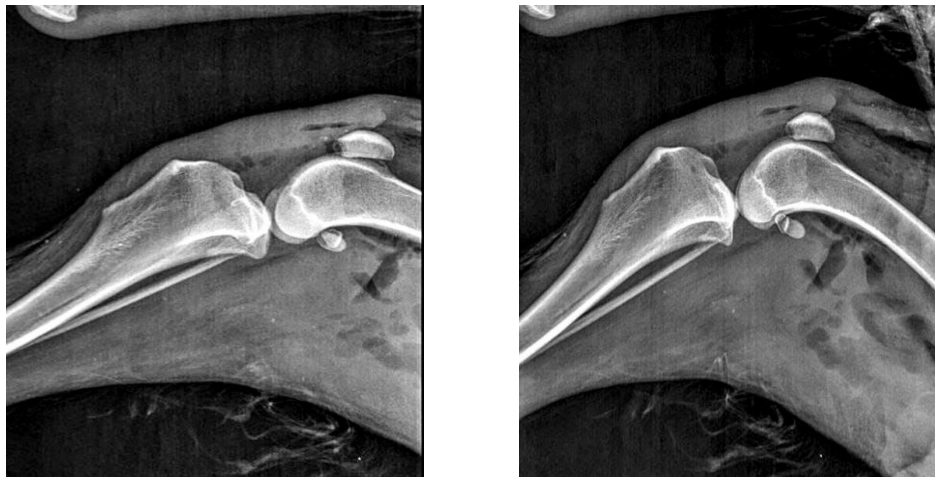


Figure 4. Radiographic views of Group 3 (G-ACS) at Week 1 and Week 3 (right).

Figure 5: Longitudinal Radiographic Scores Across Groups

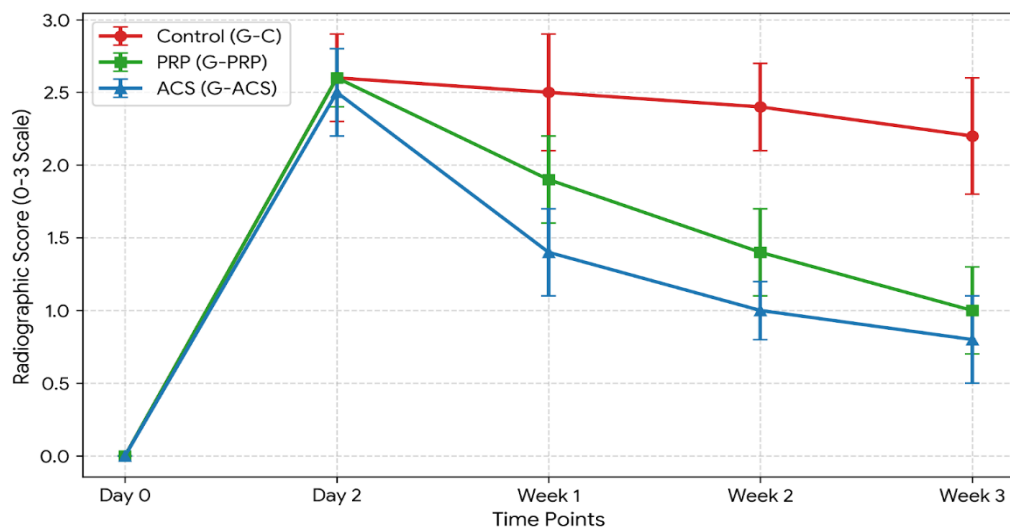


Figure 5. Radiographic Scores in all groups (0-3 Scale: 0= normal, 1= Mild swelling, 2= Moderate joint swelling, 3= Sever swelling with osteophytosis)

Discussion

The authors conducted this study to make a head-to-head comparison of the two main biological therapies that are used in an autologous manner, namely PRP and ACS, within a standardized model of acute traumatic arthritis. The data clearly show that both are far more effective than placebo, but have different, complementary, main mechanisms of action, which account for their different clinical and biochemical effects. This confirms our main hypothesis and the evidence-based selection of therapeutics.

The most remarkable result was in the difference between the effects on synovial inflammation (SF WBC count). The G-ACS group had a significant and swift decrease in WBCs and returned to normal levels by the 2nd week. This is a direct proof of the mechanism of

action ACS is designed to achieve – to provide high levels of IL-1Ra. IL-1 is a master pro-inflammatory cytokine which has a strong effect on synovial proliferation, leukocyte infiltration and production of cartilage-degrading enzymes (Gabay et al., 2010; Wehling et al., 2007; Wang et al., 2025). The rapid resolution of clinical swelling and lameness observed in this group is attributable to ACS's ability to block the IL-1 pathway and create a potent anti-inflammatory microenvironment. This is consistent with the clinical indications of the use of ACS in conditions with a predominant intense synovitis (Alvarez-Camino et al., 2013; Szarpak et al., 2025).

The G-PRP group had a more moderate and gradual decrease in the number of WBC. This is consistent with the known biology of PRP that consists of a complex mixture of pro- and anti-inflammatory modulators which are released from

platelet alpha-granules. This was clearly shown with the better functional restoration results. The considerable improvement in joint flexion angle and resurgence of near-normal SF viscosity in the G-PRP group are hallmark features of improved tissue integrity and joint biomechanics. The growth factors present in PRP, such as TGF- β and PDGF, have been shown to promote the migration of mesenchymal stem cells, proliferation of chondrocytes, and production of important components of the cartilage matrix, including hyaluronic acid and type II collagen (Civinini et al., 2013; Towfik and Mutar, 2021; Murugesan et al., 2025). Arthritis is associated with reduced glucose in the synovial environment — partly because cartilage is avascular — and this depletion is a marker of joint dysfunction. The increased metabolic and barrier function of the synovium as a result of the PRP therapy, which was less marked when using ACS, is suggested by the restoration of the glucose level. (Shirokova et al., 2020)

The pattern of ALP activity will also give some indication of the healing phases. ALP may reflect a marker of tissue mineralization (osteoblast activity) but it may also reflect cellular turnover and repair. The ALP in the G-PRP group was higher at Week 3 than in both the G-ACS and GC groups, which may be due to an ongoing anabolic/remodeling process rather than purely because of the resolution of inflammation, stimulated by growth factors. This indicates that PRP was stimulating a reparative response at endpoint of the study while the inflammatory phase was mostly completed in the G-ACS group.

Our clinical outcomes are completely in line with these biochemistry results. Having lameness and swelling resolved quickly in the G-ACS group highlights its potency as an "inflammatory firefighter". Therefore, ACS is a good choice in the acute phase of traumatic arthritis or any inflammatory arthropathy where pain and swelling predominate. On the other hand, the better recovery of the ROM in the G-PRP group suggests that it has a role as a "tissue engineer" and is most effective in the subacute to early chronic phase, when the aim is to restore structure and prevent long-term degeneration (Kuyinu et al., 2016; Piening et al., 2025).

Study Limitations

The 3-week observation period is a limitation, covering only the acute and early subacute healing phases but not long-term outcomes such as osteoarthritis progression. Further research with longer follow up is

required to see if the initial functional benefit of PRP is true when it comes to preventing joint degeneration in the long term. Furthermore, although functional and biochemical changes were measured, direct histopathological examination of cartilage and synovium would yield a definitive answer as to the effect of each therapy, in terms of morphology. Those study on the young age which preferred study on the old age, and single sex. Also small sample size (n=5) limited the statistical calculations.

Conclusions

Both PRP and ACS work but in different ways: PRP stimulates functional and structural repair via growth factors; ACS blocks acute inflammation (IL-1Ra) rapidly. Treatment should be based on clinical presentation: use PRP for regeneration and ACS for severely inflamed joints. This aids in individualized treatment strategies in veterinary regenerative medicine.

Competing Interest

The authors declare no conflict of interest

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