Original Article

Efficiency and clinical outcomes of platelet-rich plasma therapy on canine coxofemoral osteoarthritis

Patama Arunrattanakul[1] Kumpanart Soontornvipart[1*]

Abstract

The purpose of this study was to find out the optimal technique to prepare plasma with the highest platelet concentration in platelet rich plasma (PRP), as well as determine the result of autologous PRP from the dogs with osteoarthritis (OA) condition. It was shown that the PRP could reduce inflammation, cell regeneration and enhance angiogenesis to support the injured tissue. This study was composed of two phases; the first phase aimed to find the best centrifugation speed and time that could be able to produce the plasma with the highest platelet concentration by conducting the experiment from a total of 6 healthy dogs without OA condition, which was verified by physical examinations and blood tests. The blood from these dogs was collected with Arthrex® Double syringe. In the second phase, a total of 9 dogs with OA condition in both sides of coxofemoral were used to perform the experiment by intraarticular PRP injection. In addition, a video double blinded clinical evaluation on a pre- and a post-PRP injection state were performed, which was evaluated by radiographic findings, lameness score, kinematic data analysis (Kinovea®), and owner questionnaire (CBPI/LOAD) at the day before PRP and post-PRP injection at 8 weeks. The PRP would be processed by using the centrifugation speed and time results in the first phase. The result of the first phase indicated that using 1,500 rpm centrifugation speed for 3 min given the highest platelet concentration and significant in terms of statistics (P = 0.028), and the result of the second phase revealed that lameness score (P = 0.031), kinematic data analysis (P = 0.035), and the owner questionnaire (P = 0.016) post-PRP injection at 8 weeks were resulted in significantly improved in terms of statistics. However, there was no significant improvement in the results of the radiographic findings (P = 1.000). In conclusion, this study evaluated the optimal PRP preparation technique and its short-term clinical outcomes in dogs with coxofemoral osteoarthritis. Phase I identified 1,500 rpm for 3 min as the most effective centrifugation setting for achieving high platelet concentration using a sterile double syringe system. In Phase II, intra-articular PRP injection led to significant improvements in lameness scores, hip joint mobility, and owner-reported pain levels over an 8-week period, with no adverse effects observed. However, radiographic changes were not detected, and the long-term efficacy and safety remain unknown. Further research is needed to assess PRP's sustained benefits, optimal dosing frequency, and potential side effects.

Keywords: canine, coxofemoral joint, dog, kinematic analysis, osteoarthritis, platelet rich plasma

[1]*Department of Surgery, Faculty of Veterinary Science, Chulalongkorn University, Pathumwan, Bangkok 10330 Thailand*
***Correspondence:** *kumpanart.s@chula.ac.th* (K. Soontornvipart)

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Introduction

Platelet-rich plasma (PRP) has been recognized as one of the most widely used biological cell-based therapies in human medicine over the past several years. Numerous studies have supported the benefits of PRP treatment in various human medical fields, such as maxillofacial surgery (Merigo *et al*., 2018), recovery of tendon, ligament, and muscle injuries (Sammartino *et al*., 2005; Roubelakis *et al*., 2014), and the healing of chronic Achilles tendinopathy (de Vos *et al*., 2010). The key mechanisms of PRP include reducing inflammation, promoting tissue regeneration, enhancing cell differentiation, and stimulating angiogenesis.

In recent years, veterinary orthopedic surgeons at the Small Animal Hospital, Chulalongkorn University, have encountered a wide range of orthopedic conditions. Among them, one of the most frequently diagnosed conditions is coxofemoral osteoarthritis (OA), a degenerative disease of the hip joint commonly seen in aging, large-breed dogs (Sánchez-Molano *et al*., 2014). The development of this condition is often related to joint instability, which contributes to the progression of OA. As a result, affected dogs experience pain and reduced quality of life.

Previous studies have reported that approximately 20% of dogs are affected by OA (Anderson *et al*., 2018). Although the pathogenesis of OA involves inflammatory and metabolic pathways that impact the articular cartilage and subchondral bone (Vaughan-Scott and Taylor, 1997; Mobasheri and Batt, 2016), the complete pathophysiological process is still not fully understood.

Successful management of OA primarily focuses on pain control and improving the patient's quality of life. Early diagnosis and timely treatment have been shown to lead to better clinical outcomes. However, detecting OA in its early stages is challenging, as the condition progresses slowly and symptoms such as joint stiffness and lameness often develop gradually. Diagnostic methods for OA in dogs include physical examination, radiography, synovial fluid analysis, arthroscopy, magnetic resonance imaging, and computed tomography (Filet *et al*, 2005; Pettitt and German, 2015).

Treatment options for OA consist of medical therapy, surgical intervention, and physical rehabilitation. Common medical therapies include long-term use of corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs), and nutraceuticals, while physical rehabilitation focuses on weight control, exercise, and physiotherapy (Curtis *et al*., 2000). However, chronic NSAIDs use is associated with adverse effects on the gastrointestinal tract, kidneys, liver, and hematological system (Lascelles *et al*., 2005). As a result, newer treatment approaches have been explored, including hyaluronic acid, stem cell therapy, and PRP, which aim to promote cartilage regeneration and provide long-term pain and inflammation relief.

PRP contains a high concentration of platelets in plasma. Upon activation, platelet α-granules release growth factors and cytokines that can reduce inflammation and support cartilage regeneration (Alves and Grimalt, 2018; Gato-Calvo *et al*., 2019; Lee *et al*., 2019). If PRP is capable of reducing inflammation and enhancing tissue repair, as previously described, it may also improve gait and increase the range of motion (ROM) in affected joints. This is especially beneficial for elderly dogs with hip joint instability or luxation due to OA progression, in which surgery may not be ideal, particularly in patients with multiple comorbidities or high anesthetic risk.

Therefore, the objective of this study was twofold: to identify the optimal centrifugation speed and duration to prepare autologous PRP with the highest platelet concentration (Phase I), and to evaluate the short-term clinical outcomes of intra-articular PRP therapy in dogs diagnosed with coxofemoral OA (Phase II) using radiographic analysis, lameness scoring, gait kinematic data, and owner-based questionnaires.

Materials and Methods

Animals

Phase 1: This study incorporated 6 healthy dogs. Dogs with orthopedic or systemic concurrent diseases, during pregnancy and lactation periods were excluded from the study. The study was performed at the Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University. First and foremost, all the dogs were examined for orthopedics through physical examinations, blood collection before a deep sedative and blood collection. The 15 ml of blood of each syringe was centrifuged to find out the appropriate technique that make the highest concentration platelets and good characterized of plasma with different criteria and time to further use for dog coxofemoral osteoarthritis. Consent form was filled in and permitted by the owners to use their dogs prior to participating in this study.

***Phase 2*:** This study incorporated 9 dogs presented with coxofemoral OA. The research was performed at the same location as the first phase. First and foremost, all dogs were examined for orthopedics through physical examinations, blood collection, hips radiography in hip extended view and lateral view, and lameness assessment score. The severity of coxofemoral osteoarthritis was classified based on radiographic grading into mild (n = 2), moderate (n = 3), and severe (n = 4) cases.

Dogs with orthopedic or systemic concurrent diseases were excluded from the study. In particular, dogs diagnosed with cranial cruciate ligament rupture or patellar luxation were excluded to avoid confounding effects on gait and clinical outcomes. Dogs experiencing complications or adverse effects from arthrocentesis and PRP injection, as well as those in pregnancy or lactation periods, were also excluded.

The dogs' owners had to complete the dog brief pain inventory (CBPI) and Liverpool Osteoarthritis in Dogs (LOAD) before and after the study. A consent form was filled in and permitted by the owner to use their dogs prior to participating in this study. Dogs with orthopedics or systemic concurrent disease, complications and adverse effects from arthrocentesis and injection of PRP treatment, during pregnancy and lactation periods were excluded from the study.

This study was separated into 2 phases with samples of 15 dogs. In the first phases, the study mainly focused on 6 healthy dogs that have the owner and with a good health condition, which was verified by having a physical examination, as well as the blood examination to ensure that they had no orthopedic disease conditions and additional complications from the experienced veterinarian. The process was collecting the blood sample from each dog into 150 ml blood bag per single dog, then the blood from each dog would be centrifuged with 10 different centrifugation speeds and time. After that, the platelet concentration, and the additional components from the plasma with platelet concentration were collected. In the second phase, the study was performed by diagnosing coxofemoral OA, with the aim to compare before and after outcomes of PRP treatment for dog coxofemoral OA. The PRP preparation and treatment were done under deep sedative condition with dexmedetomidine (3–5 mcg/kg) and morphine (0.3 mg/kg) with appropriate aseptic technique. All dogs were evaluated by using lameness score, radiographic examination, active range of motion (AROM) from kinematic analysis, and questionnaires following the evaluation schedule below. Kinematic analysis including maximum extension angle (MEA), maximum flexion angle (MFA) and ROM, was assessed with 2D-kinematic analysis by Kinovea® software (Softonic company, United Kingdom) for 1 week prior to PRP injection and 8 weeks after the injection (Table 1). The participated patients were provided with antibiotics on the day of the injection. Area restrictions would be imposed for 3 days after the PRP injection. After that, the owners were able to walk the dogs as usual.

Platelet-rich Plasma Preparation

Phase 1: One-hundred-and-fifty-ml of whole blood sample were collected from jugular vein from healthy dog, under deep sedation and asepsis by 18 gauge 1½ -inch needle, which connected with a CPDA-1 single blood bag 150 ml, and it was separated into 15 ml double syringe system (Arthrex®, Device innovation Co., Ltd., Naples, Florida, USA) Afterwards, the sample were centrifuged by a centrifuge machine (Hettich Zentrifugen, Rotofix32A) with centrifugation speed (rpm) and time (min) (Table 2). After the centrifuge process was completed, the centrifuged blood would be separated into 3 layers as follows: Firstly, the upper contained plasma, which was concentrated platelets called PRP. Secondly, the middle layer is buffy coats containing white blood cells. Lastly, the bottom layer was red blood cell (Fig. 1). In the end of the process, a 1 ml sample of plasma (upper layer) was sent to Vet Clinical Center (VCC) Laboratory for platelet count with Wright-Giemsa stained technique with 100× magnification under light microscope (Fig. 2) and using the formula number of platelets × 10 field × 2 × 10^3/µL to collect platelet count and fluid analysis for further PRP treatment in dog coxofemoral osteoarthritis in phase 2.

Phase 2: According to the centrifuge technique in phase 1, For the plasma, which was concentrated platelets 2–11 times from baseline, a 1.5 ml sample of plasma (upper layer) were separated into three fractions: first, a 0.25 ml. EDTA tube which were sent to the VCC for platelets count and cytology, the second part 0.25 ml was collected in a sterile tube and sent to VCC for conventional bacterial identification. Lastly, the residuum fraction 1 ml of plasma in the double syringe was injected into the affected coxofemoral joint.

Table 1 Evaluation schedule

Clinical evaluation	First visit	1 week before injection	Post-PRP injection (Week)	
			0	8
Physical examination and orthopedics	✓			✓
Blood examination	✓			
Radiography	✓			✓
Lameness score	✓		✓	✓
Synovial fluid analysis			✓	✓
Owner questionnaire			✓	✓
Training the dog to walk and trot on a treadmill	✓			
Collect kinematic data		✓		✓

Table 2 The different methods of centrifugation speed (rpm) and time (minutes) for PRP

Centrifugation speed (rpm)	Time (min)
1,500	3
1,500	5
2,000	3
2,000	5
2,500	3
2,500	5
3,000	3
3,000	5
3,500	3
3,500	5

Figure 1 Separation of blood into 3 layers in the Arthrex® double syringe after centrifuging.

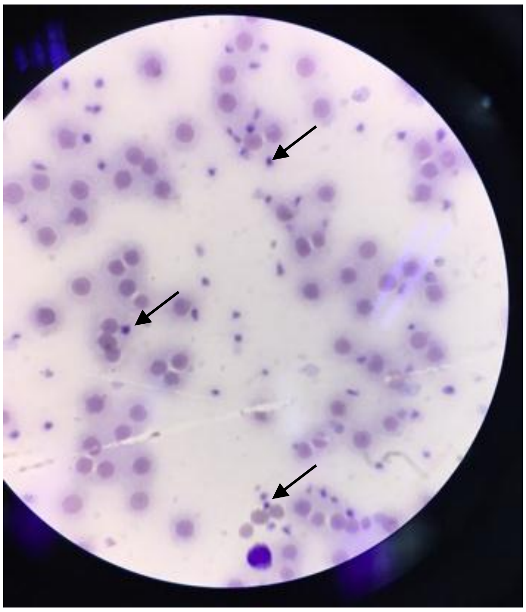

Figure 2 Cytology of blood smear and platelet in the Wright-Giemsa-stained technique slide (black arrow).

Clinical Evaluation

Radiographic osteoarthritis scoring: All the dog's hips were diagnosed by radiography in hip extended view. Grading of OA of coxofemoral joint was evaluated by a veterinary radiologist at the first visit and at 8 weeks after PRP injection. According to osteoarthritis grading (modified from Wessely *et al*., 2017), dogs were chosen in criteria of mild and severe grade of OA in this study.

Lameness score: Lameness score in this study was confirmed to the Lameness score criteria (modified from Impellizeri *et al*., 2000 and Hazewinkel *et al*., 2008). It was evaluated by 3 veterinarians on the first visit and 8 weeks after PRP injection via 2 planes video record (left and right view).

Kinematics data analysis: To precede the initial kinematic analysis by Kinovea® software at one week before PRP injection and 8 weeks after PRP injection, the dogs were trained to walk and trot on a treadmill. They were trained at a frequency of three to nine sessions per day, for approximately two or three repetitions of 3–5 mins per session (Miqueleto *et al*., 2013) throughout the week before the study.

For the preparation of video-based kinematic data collection, each dog was shaved at the tagged point with 12 retroflected 18-mm diameter spherical markers with adhesive tape (Miqueleto *et al*., 2013; Carr *et al*. 2016; Carr and Dycus, 2016). The marker was placed on the anatomical landmarks: dorsal border of wing of ilium, greater trochanter, lateral prominence of the ischial tuberosity, lateral epicondyle of femur, lateral malleolus of fibula, head of the fifth metatarsal bone. (Miqueleto *et al*., 2013; Souza *et al*., 2019) (Fig. 3).

To set up the video-based kinematic data collection, the iPhone 6 with a camera setting of 60 frame-per-second (fps) and a resolution of 1920×1080 wase used. The kinematic data was collected in the sagittal plan by

2 cameras positioned on the left and right sides, 1-meter away from the treadmill horizontally. Moreover, prior to the record, Kinovea® 0.8.15 software analysis was calibrated and marked the distance from treadmill and camera. Subsequently, the treadmill commenced from a lower speed and gradually increase until the dog achieved normal trotting gait maintained between 2.1 and 2.2 m/s of video record (Miqueleto *et al.*, 2013). Apart from that, the kinematic data derived from ROM, MEA and MFA of hip joint, stifle and tarsal joint would be measured with Kinovea® software at one week before the PRP injection and 8 weeks after PRP injection.

Statistical analysis: The parameters of gender, age, body weight, body condition score (BCS), hydration status, radiographic findings, lameness score, platelet concentration, and owner questionnaire were reported as descriptive statistics. Age and body weight were reported as mean ± standard deviation (SD), while the part of body condition score, radiographic findings, lameness score and owner questionnaire were reported as median and interval (IQR). Only the hydration status was reported as a percentage. Kolmogorov-Smirnov test and Shapiro-Wilk test were used for determining normal distribution of kinematic data analysis (Kinovea®). The kinematic data analysis with normal distribution were compared between before and after PRP injection by the paired T-test as well. Radiographic findings, comparisons between platelet baseline and platelet rich plasma, lameness score, and owner questionnaire were used Wilcoxon Signed Ranks test to differentiate the significant difference between before and after PRP injection. All statistical analysis would be performed using SPSS version 22 (IBM Corp., Armonk, NY, USA), and any *P*-value < 0.05 was considered as statistically significant.

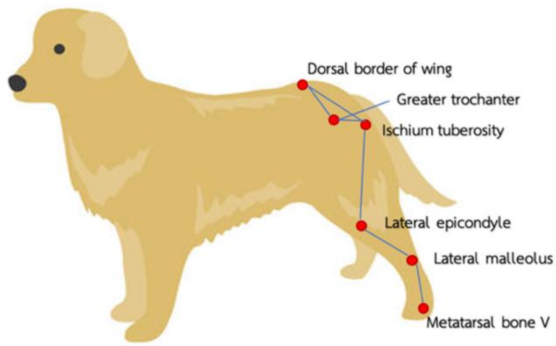

Figure 3 Anatomical landmarks for marker replacement.

Result

Animals Demographic Data

Phase 1: The total of 6 dogs were enrolled in this study to find the appropriate centrifugation speed and time for the first phase of the experiment. All enrolled dogs were verified that they were not having OA conditions. A mean ± SD of age was 29.53 ± 22.16 months, and a body weight was 32.67 ± 9.85. Among a total of 6 dogs, four of them were a male dog (66.7%) and another two were female dog (33.3%). The BCS was calculated as median and interval (IQR) as it was a range of data. The median and interval of all dogs were 3.0 (3.0–3.5). A hydration status was calculated as a percentage, and all dogs have hydration status less than 5%. The hematology and the blood chemistry of all dogs were normal.

Phase 2: After the first phase of discovering the proper value of centrifugation speed and time, the second phase was using the result of the first phase to produce the PRP and inject into a total of 9 dogs, whose were diagnosed to have OA conditions. The Golden Retriever was the most common breed (6/9 dogs), then the Chow Chow, Shetland sheepdog, Alaskan Malamute, and a mixed breed, respectively. The mean ± SD of age was 46.2 ± 23.33 months, and the body weight was 34.93 ± 12.38. A total of male dog was 7 (77.78%), and a female dog was 2 (22.22%). The BCS was calculated as median and interval (IQR) due to it was a range of data as the same as phase 1, which all 9 dogs were 3.5 (3.0–3.5). The ages, weight, BCS, and hydration status at the time before injecting PRP and at the time after injecting PRP were not many differences, so they were not taken to compare. Thus, only the data of at the time before injecting PRP was selected. The hydration status was calculated as a percentage, and all dogs have hydration status less than 5%. The hematology and the blood chemistry of all dogs were normal.

Platelet rich plasma preparation: 150 ml of blood from each 6 dogs would be separated into 15 ml of Arthrex® double syringe with different centrifugation speed (rpm) and time (min) in 10 techniques. Although the box plot below illustrated a total of 7 comparison between syringe and platelet baseline were significant difference (P < 0.05), the syringe with the highest median was the syringe number 3, its centrifugation was 1,500 rpm 3 mins. The median of platelet concentration would be inversely decreased follow the centrifugation speed. The other box plot revealed the overall comparison between platelet baseline and all of the 10 syringes that contained platelet PRP (Fig. 4).

The median and interval (IQR) of platelet concentration of 9 dogs compared between baseline and after centrifugation showed that the platelet concentration in PRP significant difference (P < 0.05) from platelet baseline. The total of platelet concentration after centrifugation was 2–11 times of the platelet baseline and all PRP before injection sent to VCC laboratory for bacterial identification and sensitivity test, the result of all PRP were no growth at 72 h after incubation.

Clinical Evaluation

Radiographic osteoarthritis scoring: 18 coxofemoral joints were diagnosed by radiography in hip extended view. Score would be evaluated at the first visit and at 8 weeks after PRP injection. The participating dogs were categorized into mild, moderate, and severe OA. The median (IQR) radiographic OA score at baseline was 2.0 (1.0–3.0), and 2.0 (1.0–3.0) at 8 weeks after PRP injection. The bar graph showed no statistically significant change (P = 1.000) between Days 0 and 8 weeks after PRP injection.

Lameness score: The evaluation of lameness score would be performed by 3 blinded evaluation veterinarians at first visit and 8 weeks after PRP injection via 2 planes video record (left and right view). Results revealed that the lameness score significantly improved in 8 weeks (P = 0.031). The median (IQR) lameness score at baseline was 2.0 (2.0–2.0) and 1.0 (1.0–2.0) post-injection, indicating reduced severity in clinical gait abnormality among the majority of dogs.

Kinematics data analysis: The kinematic data of 9 dogs would be collected in the sagittal plane by 2 cameras positioned on the left and right sides, Kinovea® 0.8.15 software was used for AROM data analysis at one week before the PRP injection and 8 weeks after PRP injection. The analysis of data was using statistics to calculate mean ± SD, which was grouped by comparing Maximum extension angle MEA, MFA, pre-PRP injection, and post-PRP injection was shown in Figure 5. The results of coxofemoral joints indicated that there was a significant difference (P = 0.035) between before the PRP injection and at 8 weeks after injection on all coxofemoral joints (Table 3). On the other hand, there was no significant difference in the results of stifle and tarsal joints (Tables 4 and 5).

Additionally, the corresponding median and interquartile range (IQR) values for the hip joint ROM are summarized as follows:

- Left Hip Extension (MEA):
  Pre-PRP: 119.0° (117.0–121.0°) → Post-PRP: 128.0° (126.0–131.0°)
- Left Hip Flexion (MFA):
  Pre-PRP: 97.0° (94.0–99.0°) → Post-PRP: 105.0° (101.0–108.0°)
- Right Hip Extension (MEA):
  Pre-PRP: 115.0° (113.0–117.0°) → Post-PRP: 129.0° (127.0–132.0°)
- Right Hip Flexion (MFA):
  Pre-PRP: 94.0° (91.0–97.0°) → Post-PRP: 110.0° (107.0–113.0°)

These values further demonstrate objective improvement in hip joint mobility following intra-articular PRP therapy.

Owner questionnaires: The owner of 9 dogs were assessed the modified questionnaire to Thai version of CBPI at Day 0 and at 8 weeks after PRP injection in this study. The median (IQR) pain severity score (PSS) improved from 5.0 (4.0–6.0) at baseline to 3.0 (2.0–4.0) at 8 weeks. The pain interference score (PIS) improved from 6.0 (5.0–7.0) to 3.0 (2.0–4.0). The changes were statistically significant (P = 0.016), indicating both reduced pain and improved quality of life from the owner's perspective.

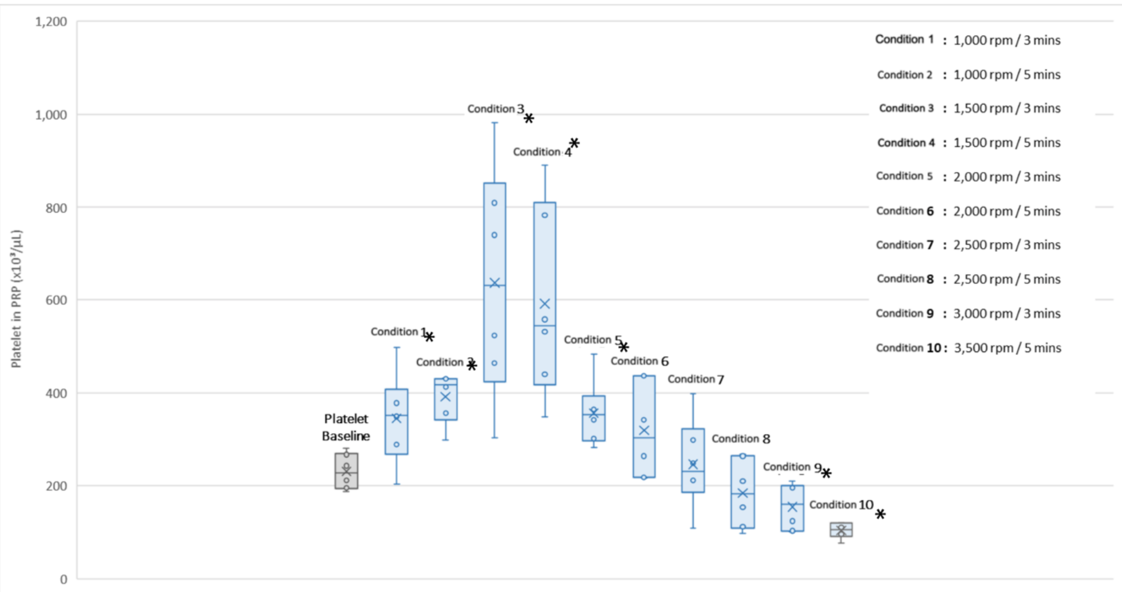

Figure 4 The box plot showed median and interval (IQR) compared between the concentration platelet in PRP and platelet baseline with different centrifugation speed (rpm) and time (min) in 10 techniques in 6 dogs. *P-value < 0.05 compared platelet concentration between baseline and after centrifugation, using the Wilcoxon Signed Ranks test.

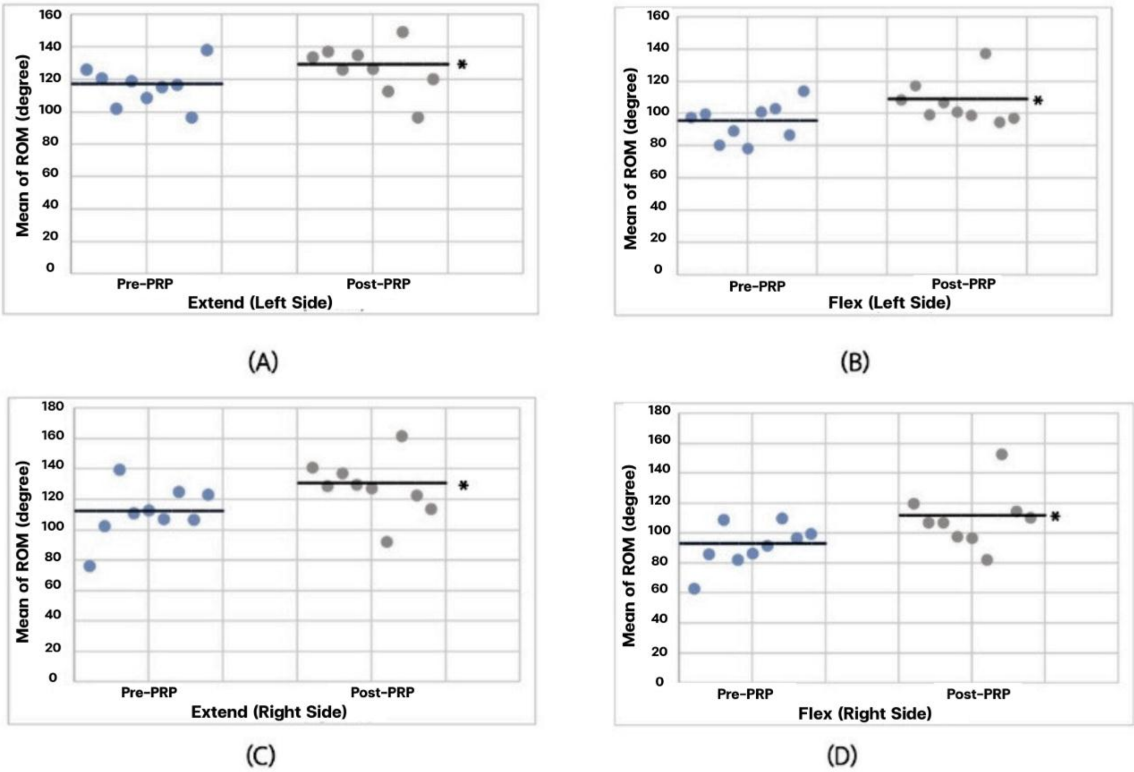

Figure 5 Scatter dot plot showed the mean of MEA and MFA angles of 9 coxofemoral OA dogs. (A) described the mean of MEA angle of left coxofemoral joint in pre-PRP and post-PRP treatment, (B) described the mean of MFA angle of left coxofemoral joint in pre-PRP and post-PRP treatment, (C) described the mean of MEA angle of right coxofemoral joint in pre-PRP and post-PRP treatment, and (D) described the mean of MFA angle of right coxofemoral joint in pre-PRP and post-PRP treatment. **P*-value < 0.05 compared between pre-PRP treatment and post-PRP treatment, using paired t-test.

Table 3 Comparison of the active range of motion (AROM) of the coxofemoral joint before and after PRP treatment with mean±SD.

Weeks	Right hip joint		Left hip joint	
	Extension	Flexion	Extension	Flexion
0	111.75 ± 18	91.69 ± 14.37	116 ± 13.33	94.42 ± 12.47
8	128.49 ± 18.59	110.04 ± 18.67	126.55 ± 15.59	106.87 ± 13.17

**p*-value < 0.05 compared between pre-PRP treatment and post-PRP treatment, using paired t-test

Table 4 Comparison of the active range of motion (AROM) of the stifle joint before and after PRP treatment with mean±SD.

Weeks	Right stifle joint		Left stifle joint	
	Extension	Flexion	Extension	Flexion
0	139.47 ± 11.99	99.04 ± 14.81	143.4 ± 12.53	104.6 ± 13.86
8	134.89 ± 12.64	99.73 ± 11.84	138.88 ± 12.81	103.62 ± 13.59

**p*-value < 0.05 compared between pre-PRP treatment and post-PRP treatment, using paired t-test.

Table 5 Comparison of the active range of motion (AROM) of the tarsal joint before and after PRP treatment with mean±SD.

Weeks	Right tarsal joint		Left tarsal joint	
	Extension	Flexion	Extension	Flexion
0	159.49 ± 12.66	119.14 ± 16.34	164.68 ± 11.76	121.09 ± 18.41
8	165.7 ± 11.16	121.14 ± 12.49	164.69 ± 12.66	120.33 ± 15.95

**p*-value < 0.05 compared between pre-PRP treatment and post-PRP treatment, using paired t-test.

Discussion

Significance of platelet-rich plasma in osteoarthritis therapy: PRP has become an increasingly popular biological therapy in both human and veterinary medicine, particularly for degenerative joint diseases such as OA (Aminkov *et al.*, 2021; Bansal *et al.*, 2021). In this study, intra-articular PRP treatment showed clinical efficacy in improving joint function, pain reduction, and limb mobility in dogs diagnosed with bilateral coxofemoral OA. The findings support previous studies suggesting PRP's ability to reduce inflammation, stimulate cellular regeneration, and promote angiogenesis (Dhillon *et al.*, 2017; Alves and Grimalt, 2018).

Optimization of platelet-rich plasma preparation: The study established that centrifugation at 1,500 rpm for 3 min using a closed double syringe system (Arthrex®) yielded the highest concentration of platelets (2–11 ×

baseline) with statistical significance ($P = 0.028$). This technique minimized contamination and exposure to anticoagulants, which may reduce the risk of adverse reactions, as suggested by Cook and Smith (2018). The platelet-rich layer was cytologically confirmed (Fig. 2), and all processed PRP samples were sterile after 72 h of incubation.

Clinical outcomes after platelet-rich plasma Injection: PRP treatment led to statistically significant improvements in lameness scores ($P = 0.031$), kinematic parameters ($P = 0.035$), and owner-reported pain and interference scores ($P = 0.016$). These outcomes reflect enhanced weight-bearing, joint range of motion, and daily activity levels post-treatment.

Importantly, no adverse effects or post-injection complications were observed. Although the radiographic OA score remained unchanged ($P = 1.000$), this was expected within the limited 8-week window, and aligns with previous studies where radiographic changes lag behind clinical improvement (Fahie *et al*., 2013; Arican *et al*., 2018).

Relevance of kinematic data: The 2D kinematic analysis using Kinovea® software revealed significant improvements in hip joint movement. MEA and MFA values increased post-PRP injection in both hind limbs. However, the values remained lower than those of healthy dogs (Souza *et al*., 2019), indicating partial restoration of function. These findings echo Hilty *et al*. (2017), who also reported increased ROM following PRP injection in canine OA cases.

The lack of significant improvement in stifle and tarsal joints (Tables 4 and 5) confirms the specificity of treatment effects to the coxofemoral joint, the direct target of PRP injection.

Interpretation of owner questionnaires: Owner-reported outcomes (CBPI and LOAD) showed reduced pain and improved function at 8 weeks. However, it is important to interpret these subjective metrics cautiously, as they may be influenced by bias. Brown *et al*. (2013) demonstrated weak correlation between owner questionnaires and objective gait analysis. Thus, combining subjective and objective assessments, as done in this study, provides a more holistic evaluation.

Pathophysiology and targeting osteoarthritis in large breeds: Most of the enrolled dogs were Golden Retrievers, a breed highly susceptible to OA and hip dysplasia (Bockstahler *et al*., 2007; Anderson *et al*., 2020). OA pathogenesis in these dogs involves chronic inflammatory cascades with cytokine release (IL-1, IL-6, TNF-α), enzymatic cartilage degradation, and mechanical instability (Freitag *et al*., 2016). PRP's growth factor content—including PDGF, VEGF, IGF-1, EGF, and bFGF—plays a therapeutic role by modulating inflammation and supporting cartilage repair (Dhillon *et al*., 2017; Kon *et al*., 2020).

Hydration status was controlled, and no dogs exhibited dehydration, which is known to affect platelet counts (Rinnovati *et al*., 2016; Borgman *et al*., 2019). This consistency minimized variability in platelet yield across samples.

Study limitations and future directions: The study is limited by a small sample size and short observation period. Some dogs were uncooperative during treadmill sessions, leading to possible variation in kinematic data. Additionally, more objective biomechanical metrics such as ground reaction force via force plate analysis (e.g., PVF and VI) could strengthen future evaluations.

Long-term studies are needed to determine the duration of PRP efficacy, radiographic changes, and the potential benefits of repeated PRP injections. Furthermore, exploring PRP's in vitro effects on canine chondrocytes or cartilage explants could elucidate mechanisms of action.

In conclusion, this study demonstrated that a single intra-articular PRP injection, prepared using an optimized centrifugation protocol, improves clinical signs, joint function, and quality of life in dogs with coxofemoral osteoarthritis. While radiographic changes were not observed over 8 weeks, functional and behavioral improvements were evident. PRP is a promising adjunct therapy for canine OA and warrants further investigation in larger, longer-term studies.

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