EFFICIENCY AND CLINICAL OUTCOMES OF PLATLET-RICH PLASMA THERAPY ON CANINE COXOFEMORAL OSTEOARTHRITIS

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ประสิทธิภาพและผลทางคลินิกของการใช้พลาสมาที่มีเกล็ดเลือดเข้มข้นต่อภาวะข้อสะโพกเสื่อมในสุนัข

น.ส.ปฐมา อรุณรัตนากุล

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Thesis Title	EFFICIENCY AND CLINICAL OUTCOMES OF PLATLET-RICH PLASMA THERAPY ON CANINECOXOFEMORAL OSTEOARTHRITIS
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ปฐมา อรุณรัตนากุล : ประสิทธิภาพและผลทางคลินิกของการใช้พลาสมาที่มีเกล็ดเลือดเข้มข้นต่อภาวะข้อสะโพกเสื่อมในสุนัข. (EFFICIENCY AND CLINICAL OUTCOMES OF PLATLET-RICH PLASMA THERAPY ON CANINECOXOFEMORAL OSTEOARTHRITIS) อ.ที่ปรึกษาหลัก : ผศ. ดร.กัมปนาท สุนทรวิภาต

การศึกษานี้มีวัตถุประสงค์เพื่อ หาความเหมาะสมของวิธีการเตรียมพลาสมาที่มีเกล็ดเลือดเข้มข้นและเปรียบเทียบผลการใช้พลาสมาที่มีเกล็ดเลือดเข้มข้นของสัตว์ที่มีภาวะข้อสะโพกเสื่อม เนื่องจากมีศึกษาพบว่าเกล็ดเลือดเข้มข้นมีผลในการลดการอักเสบ สร้างเซลล์ใหม่ เพิ่มหลอดเลือดมาเลี้ยงบริเวณเนื้อเยื่อที่บาดเจ็บ การศึกษานี้ประกอบด้วย 2 ขั้นตอน คือ ขั้นตอนที่ 1 เป็นขั้นตอนการศึกษาวิธีการเก็บเลือดและการทำ พลาสมาที่มีเกล็ดเลือดเข้มข้น ประกอบด้วยสุนัขที่มีสุขภาพแข็งแรงจากการตรวจร่างกาย และตรวจเลือดจำนวน 6 ตัว สุนัขทั้ง 6 ตัวจะถูกเก็บเลือดเพื่อนำมาปั่นที่แรงและเวลาที่เหมาะสมด้วยหลอดเก็บเลือดเฉพาะ(Double syringe) แบบระบบปิดเพื่อให้ได้พลาสมาที่มีความเข้มข้นของเกล็ดเลือดมากที่สุดเพื่อนำแรงและเวลาที่เหมาะสมไปใช้ในขั้นตอนที่ 2 ส่วนในขั้นตอนที่ 2 ประกอบด้วยสุนัขพันธุ์ใหญ่ที่มีภาวะข้อสะโพกเสื่อมทั้ง 2 ข้าง จำนวน 9 ตัว จากการวินิจฉัยของสัตวแพทย์ที่มีประสบการณ์ สุนัขจะถูกประเมินผลทางคลินิกก่อนและหลังฉีดพลาสมาที่มีเกล็ดเลือดเข้มข้นด้วยวิธีการประเมินผลทางภาพถ่ายรังสีเทคนิค ประเมินลักษณะการเดินกะเผลก ประเมินการเคลื่อนไหวของข้อสะโพกจากภาพเคลื่อนไหวบนลู่วิ่งโดยใช้โปรแกรมคอมพิวเตอร์ และประเมินความเจ็บปวดและการใช้ชีวิตจากเจ้าของสุนัขด้วยแบบสอบถาม ในวันแรกก่อนและหลัง 8 สัปดาห์ของการได้รับพลาสมาที่มีเกล็ดเลือดเข้มข้นเข้าข้อสะโพกทั้ง 2 ข้าง ผลการศึกษาพบว่าในขั้นตอนที่ 1 แรงและเวลาในการปั่นเหวี่ยงเลือดที่ 1,500 รอบต่อ 3 นาที ให้ปริมาณของเกล็ดเลือดเข้มข้นสูงที่สุดอย่างมีนัยสำคัญทางสถิติ และจากการศึกษาในขั้นตอนที่ 2 พบว่าข้อมูลของการประเมินลักษณะการเดินกะเผลก ประเมินการเคลื่อนไหวของข้อสะโพกจากภาพเคลื่อนไหวบนลู่วิ่งโดยใช้โปรแกรมคอมพิวเตอร์ และประเมินความเจ็บปวดและการใช้ชีวิตจากเจ้าของสุนัขด้วยแบบสอบถาม มีการพัฒนาที่ดีขึ้นหลังจากได้รับการฉีดพลาสมา 8 สัปดาห์อย่างมีนัยสำคัญทางสถิติ แต่อย่างไรก็ตามการประเมินผล

สาขาวิชา ศัลยศาสตร์ทางสัตวแพทย์ ลายมือชื่อนิสิต .....

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Patama Arunrattanakul : EFFICIENCY AND CLINICAL OUTCOMES OF PLATLET-RICH PLASMA THERAPY ON CANINECOXOFEMORAL OSTEOARTHRITIS. Advisor: Asst. Prof. KUMPANART SOONTORNVIPART, D.V.M., Ph.D., D.T.B.V.S.

The purpose of this study was to find out for the optimal technique to prepare plasma with 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 was 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 10 dogs with OA condition in both side of coxofemoral were used to perform the experiment by intraarticular PRP injection. In addition, a video double blinded clinical evaluation on a pre-PRP and a post-PRP injection state were performed, which was evaluated by radiographic finding, 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 minutes given the highest platelet concentration

Field of Study: Veterinary Surgery Student's Signature .....

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I am over helmed in all humbleness and gratefulness to acknowledge my depth to all clinicians at surgical unit of Small Animal Hospital, the Faculty of Veterinary Science, Chulalongkorn University and my classmates for their sincerity, friendship, and the great sense of humor. It was a great privilege and honor to work with them. I am over helmed in all humbleness and gratefulness to acknowledge my depth to all clinicians at surgical unit of Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University and my classmates for their sincerity, friendship, and the great sense of humor. It was a great privilege and honor to work with them.

Finally, I am extremely grateful to my friends and family to help me get through all the difficult times of this project, as well as standing beside me, especially in the moment of global Covid-19 pandemic situation.

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CHAPTER I

INTRODUCTION

1.1 Importance and rationale

Platelet-rich plasma (PRP) has been recognized as one of the most popular alternative biological cell-based therapies in human for several years. Furthermore, there were multiple supporting evidences in reference to the PRP treatment in human such as the Maxillofacial surgery (Merigo, et al., 2018), the recovery of tendon, ligament and muscle (Roubelakis, et al., 2014; Sammartino, et al., 2005), and the healing of human Achilles tendinopathy (de Vos RJ, et al., 2011). The aim of this PRP treatment was not only to reduce inflammation but also to enhance regeneration, to promote cell differentiation, and to induce angiogenesis.

In the past few years, veterinary orthopedic surgeons in the Small Animal Hospital, Chulalongkorn University have witnessed various orthopedics diseases. Among these, one of the most prevalent disease was the coxofemoral osteoarthritis (OA), which was a degenerative type of coxofemoral joint and was regularly found among aging large-breed dogs (Sanchez-Molano, 2014). The etiology of this disease relates to the instability of the coxofemoral joint which further provokes the progression of osteoarthritis. Consequently, patients tend to suffer from pain and from the deteriorated living conditions.

A large number of literatures review has discussed the prevalence of OA and has reported that, approximately, as much as 20% of dogs were affected by this illness (Anderson et al.,2018). However, even though the pathogenesis of the OA was influenced by inflammatory and metabolic pathways that impact the cartilage and subchondral bone (Mobasheri and Ball, 2016; Scott and Taylor, 1997), the complete and profound understanding of the disease was yet unknown.

One of the most successful treatments was associated with the suppression of pain occurring from OA to improve the patient's life quality. Despite the fact that early diagnosis and the immediate treatments of OA were reported to greatly ameliorate the clinical outcomes, the initial stage of OA in dog was difficult to identify because of a slowly progressive change and degeneration of cartilage. The dog was usually brought to a clinic with lameness and the stiffness of joint and pain. Hence, the common investigative methods of OA were physical examination,

radiographic, synovial fluid analysis, arthroscopy, magnetic resonance imaging(MRI) and computerized tomography scan (CT) (Pettitt and German, 2015).

Medical therapies, surgical, and physical managements were practical treatments required for OA. The common medical therapies were long term steroid, NSAIDs and nutraceuticals, while physical managements were weight control, exercise, and rehabilitation (Curtis, et al., 2000). Unfortunately, the adverse effects of using a long term NSAIDs therapies were evident in gastrointestinal tract, renal, hematology, and hepatic (Lascelles, 2005). Nonetheless, numerous recent studies regarding the effects of hyaluronic acid (HA), stem cell, and PRP aimed to regenerate the cartilage and to succeed the long-term reduction of pain and inflammation. For the further explanation, PRP was the highly concentrated platelet in plasma, a-granules in platelets degranulated and stimulate growth factor and cytokines to decrease inflammation and regeneration of cartilage (Alves and Grimalt, 2018; Calvo, et al., 2019; Lee, et al., 2019). If the PRP treatment could reduce inflammation as stated in previous studies, then, this may result in the significant improvement from locomotion gait and the range of motion (ROM). As aging dogs were especially exposed to hip joint instability or luxation because of the osteoarthritis progression of coxofemoral joint, therefore the best treatment of surgery was not always suitable, particularly when dog do was aged and has more than one concurrent disease which increase the risk during anesthesia.

So, this study aimed to evaluate the effects of PRP therapy to clarify clinical outcomes after coxofemoral intraarticular injection in dogs.

1.2 Objectives of study

1. To find out the optimal technique to obtain the highest concentration platelets and good characterized of plasma with different criteria and time for further use.

2. The purpose of this study was to evaluate the efficiency and clinical outcomes of PRP on dog coxofemoral osteoarthritis.

1.3 Research plan

This experimental study had a total of 15 samples dog, and the experiments were separated into 2 phases. The first phase was performed to find an appropriate technique to centrifuge the blood with a special Arthrex® Double syringe autologous conditioned plasma (ACP) that specifically selected for this experiment. In addition, this phase would like to determine the centrifugation speed and time to produce the suitable platelet concentration that sufficient to treat the coxofemoral osteoarthritis conditions by studying 6 dogs (N = 6) that has 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 veterinary. The process was collecting the blood sample from each dog into 150 ml blood bag per a single dog, then the blood from each dog would be centrifuged with a 10 different centrifugation speeds and time. After that, the platelet concentration, and the additional components from the plasma with platelet concentration would be collected. The second phase was conducting the experiment based on the total of 9 dogs (N = 9) with coxofemoral osteoarthritis conditions without additional complications from the experienced veterinarians. The dog would be retrieved the preliminary physical examination and the blood examination, then anesthetize them respectively. At the date of conducting the experiment, 15 ml of each dog's blood would be collected under the generalized anesthesia conditions and contained in the ACP double syringe. Then, the bloods would be centrifuged with the speed and time as a result from the first phase. Afterward, some of the plasma with platelet concentration would be injected into both side of the hip with 1 ml in each side of the hip, and the remains of it would be used to determine the platelet concentration including its components and cultured it by using the plasma contained in ACP double syringe as mentioned earlier. After the treatment, each dog would be retrieved an antibiotic, monitoring the allergy conditions, and grounded for a week. The clinical effectiveness after the injection of plasma with platelet concentration could be evaluated by a modified questionnaire Canine Brief Pain

Inventory (CBPI) and modified questionnaire Liverpool Osteoarthritis in dogs (LOAD) for the owner. The AROM calculation from the Kinovea application, the radiography of the hip in the ventro-dorsal view, and the lameness score evaluation would be performed from 3 experienced veterinarians by using blind technique at the week before and after 8 weeks of PRP injection.

1.4 Advantages of the study

This study can optimize technique of PRP preparation, composition of PRP and the clinical outcome of the intra articular in dog OA treatment.

CHAPTER II

REVIEW OF LITERATURES

PRP application were studied in veterinary field such as the effect of PRP in spinal cord injuries in rats (Chen, et al., 2018) and suppressing inflammatory effect of PRP in rheumatoid arthritis (RA) mice (Tong, et al., 2017). Moreover, there was sounded result with PRP treatment in OA dogs caused by cranial cruciate ligament rupture (Vilar, et al., 2018) as well.

2.1 Platelets rich plasma (PRP)

Platelets - rich plasma (PRP) is highly concentrated platelets in plasma. The platelets contain a-granules, releasing platelet-derived growth factor (PDGF), and can change transforming growth factor β1 (TGF- β1) to transform Growth Factor β2 (TGF-β2), and the anti-inflammatory cytokines (Carr et al., 2016; M.S. Dhilon et al., 2017). The cytokines stimulated by PRPs are vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), insulin-like growth factor-2 (IGF-2), epidermal growth factor (EGF), hepatocyte growth factor (HGF), and basic fibroblastic growth factor (bFGF) (Alves and Grimalt, 2018; Calvo, et al., 2019). All of these cytokines are responsible for decreasing catabolism, improving anabolism, as well as promote chondral remodeling (Dhillon, et al., 2017). Moreover, certain studies have revealed that the PRPs can suppress interleukin-1 (IL-1), the mediated nuclear factor (NF) light-chain, enhancing nuclear factor-kB (NF-kB) act as proinflammatory cytokine to stimulate catabolic function and cause of degradation of cartilage (Cook and Smith, 2018). It is noteworthy that PRP can be classified into 4 types according to the number of platelets, leukocyte, and the composition of plasma as follows: pure platelet rich plasma (pure PRP), leukocyte-rich platelet rich plasma (L-PRP), pure platelet rich fibrin(P-PRF), and leukocyte and platelet rich plasma combination (L-PRF) (Landsdown, et al., 2017).

2.2 Coxofemoral osteoarthritis

Osteoarthritis (OA) is a common joint condition that results of slowly progression cartilage degeneration was the caused of pain and physical disability. The common lesion were articular cartilage degradation, subchondral bone remodeling, osteophyte formation and synovial inflammation (He et al., 2020).. OA is influenced by the inflammatory and metabolic processes which generate catabolic more than anabolic (Mobasheri and Batt, 2016) and can be categorized into two types: the primary OA which is an idiopathic cause, and the secondary OA which occurred from abnormal stress loading via articular cartilage and multifactorial disease such as systemic diseases, drug-induced, obesity, aging and other orthopedic disease. In addition, the study has found that receiving steroid for a long period of time caused the cartilage cells decrease a synthesis of proteoglycan, which was a crucial of cartilage matrix in articular cartilage. Meanwhile, it synthesizes a matrix metalloproteinases (MMPs) enzyme, which its role was to decompose a part of collagen type II that was another component of articular cartilage in the dogs with OA condition (Scott and Taylor, 1997; Nganvongpanit, 2004). The pathogenesis of OA is still unclear and came from the multifactorial together (Soontornvipart et al., 2015), but there is a study indicate that the stimulant can affect the changing of structure of articular cartilage, which can be leaded by some cytokines include MMPs, Prostaglandin E_2 (PGE_2), Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Tumor necrosis factor (TNF), and some proteinases (Freitag et al., 2016) These cytokines leaded to chondrocyte proliferation and increased the level of water in synovial fluid. On the other hand, the reduction of proteoglycan was occurred with collagen type II, which caused the gap and the strip of the articular surface, which the partial of subchondral bone could be able to become a joint mice, and when the friction was occurred on the subchondral bone instead of articular surface, the heat was increased and leaded to subchondral bone sclerosis, osteophyte formation, and fibrous walled cyst, which caused dogs with OA condition were suffered from the pain and disturb their living standards (Burnett, et al., 2006; Pettitti and German, 2015; Chen, et al., 2018). One of the orthopedic joint-related disease with OA

condition is hip dysplasia (HD). HD is one of the OA condition on the coxofemoral with the prevalence of 20-40%. There were many factors that leaded to canine hip dysplasia (CHD), which could be the age, breed, and obesity, while the sex would not have much effect on it (Greene et al., 2013). The CHD could be found mostly in a large type of dog breed. There was also a report that 75% were found in Golden Retrievers and Rottweilers. There was some study found that the CHD was involved with the heredity from the candidate genes FBN2 and CTBP2 (CFA28). These genes leaded to delayed capital ossification, acetabular dysplasia, and joint capsule instability, which induced to the OA condition (Clark, 2015 ; Meeson et al., 2019).

The diagnosis of coxofemoral osteoarthritis can be detected by certain clinical common signs namely lameness, walking or palpation pain, or changes in daily behaviors. The superior standard for coxofemoral osteoarthritis diagnosis is radiography, while other clinical techniques are radiographic, arthroscope, CT, MRI, synovial fluid analysis, and ultrasound (Terjesen and Genderson, 2005). There are a variety of therapeutic choices for hip joint osteoarthritis such as medicals, surgical and physicals therapeutics, however, particular patients with older age or concurrent diseases can experience the increased level of risk for anesthesia.

Therefore, the incidence of OA in coxofemoral can be found in both humans and dogs, which is applicable to use them as instances of PRP treatment. The coxofemoral joint relates to another joint such as knee and lumbosacral joint because there is a study found that knee disease such as patellar luxation (PL), cranial cruciate ligament rupture (Crcl rupture) and knee OA were influenced by a function of a crucial muscle of coxofemoral, which are hamstring and quadriceps group. These muscles control the flexion and extension of the coxofemoral and knee (Hughes et al., 2007). There is a cadaveric study such that the excessive increment of quadriceps angle can lead to the medial patellar luxation, and also lead to Crcl rupture condition. (Reiman et al., 2009)

2.3 Coxofemoral osteoarthritis and Platelets Rich Plasma (PRP)

Over the past ten years, countless biological cell-based therapies for OA treatment have been discussed and studied, including PRP, mesenchymal stem cell (MSCs), autologous protein solution (APS), adipose-derived stromal vascular fraction, and autologous conditioned serum (Bogers, 2018). These therapies aim to inhibit inflammatory process and to enhance regeneration, cell differentiation, proliferation, and angiogenesis of articular joint (Alves and Grimalt, 2018). Of all the therapies mentioned above, the PRP is one of the most widely accepted treatments. There was a study of PRP and HA in 150 humans with OA knee conditions found that the value of Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain score and International Knee Documentation Knee (IKDC) score were significantly better in a group that injected PRP more than HA in the duration of 12 months (Bansal et al., 2021). In addition to the study of using PRP treatment in OA knee conditions, there was a study of injecting PRP in another joint by injecting PRP twice with 6 weeks waiting time after injecting in each injection to 100 humans with OA ankle condition. The results revealed that PHP could be able to relief the pain, increase ROM, and functional improvement, which the longest outcome measurement was 5 years (Paget et al., 2019). Furthermore, there was study in vitro of PRP to cytokines in synoviocytes under inflammatory joint disease with rheumatoid arthritis (RA). The results discovered that it could reduce the amount of TNF and IL-1, which was proinflammatory cytokines (Tohidnezhad et al., 2017). There are multiple solid evidences regarding the PRP usages in veterinary orthopedic field, for instance, the outcome concerning the PRP treatment in OA dog (DH, et al.,2017 and Arican et al., 2018), the effect of the PRP on OA from CrCL rupture in canine (Vilar et al., 2018), and the in vivo and in vitro studies of the beneficial effects of the PRP for RA in mice (Tong, et al., 2017). The example of the PRP experiment in terms of pathology was leaded to the OA condition on the knee joint of the rabbits, which were induced by the collagenase enzyme. After that, the PRP was injecting with a desire to improve a treatment. Then, the rabbits were euthanized and evaluate the characteristics in terms of pathology with Yoshimi's score. In gross

morphological examination and Mankin's score in histological examination have found that a group of PRP-injected has significant different in terms of pathology than a control group that was injected with the saline. The results of these studies found that the platelet secreted the cells-invoked substances could be able to reduce the inflammation at the damaged area (Arican et al., 2018).

Accordingly, these were the reasons why the PRP was considered to be one of the interesting cell-based therapies for OA patient. The ultimate purpose of coxofemoral osteoarthritis treatment is to mitigate pain and improve patient's level of well-being. Hence, early detection and management of the disease offer substantial advantage for patient's treatment and clinical outcomes.

2.4 Platelets Rich Plasma (PRP) preparation

There were multiple methods of preparing PRP with the purpose of generating 2-11 times platelet concentration to the platelet baseline, which was effective in terms of the clinical treatment (Cook and Smith, 2018). The differences of PRP preparation could be the centrifugation speed, the type of syringe, the anti-coagulant substance, and the blood preservation, which found that the wide variation of reported protocols for PRP preparation leaded to variable compositions such as concentration of platelet, white blood cell, red blood cell, and the parts of separated blood layers. These factors affect to the different biological response, which was platelet recovery, platelet integrity, and platelet viability. The previous studies have shown that the double centrifugation technique was more popular than a single centrifugation technique because it produced higher platelet concentration. The purpose of the blood centrifugation was to precipitate the components within the density gradients of blood, which the platelet would be on the top because it had the lowest density. The next level under the platelet would be WBC and RBC respectively, which had higher density (Brodniewicz-Proba, 1991). There was a study discovered that the suitable centrifugation of the PRP for dogs are required more power than the human, even though the centrifugation times were similar (Araki et al., 2011 and Amable et al., 2013) because the dog blood have mean corpuscular

volume, mean corpuscular hemoglobin value, and density lower than human blood, as well as the size of the blood cell (Protero, 2015). The PRP with high centrifugation power could be resulted in highest platelet recovery. In contrast, the platelet could overlap with the WBC layer, which was more difficult to differentiate the plasma layer (Shin et al., 2017). Furthermore, the study found that centrifuge the blood with 1,000 rpm 10 minutes and follow by 4,000 rpm 10 minutes could produce the platelet concentration 5 times higher than the platelet baseline. Moreover, this technique could promote a high platelet recovery for 70-80% without decreasing platelet viability and platelet integrity at all (Perez et al., 2014). There was also a study found that the centrifugation of blood with 1000 rpm in 5 minutes, follow by 1,500 rpm 15 mins respectively, would get the 5 times higher of the platelet compared to the platelet baseline with 80% of platelet recovery from the whole blood (Shin et al., 2017). In addition, there was a method to produce PRP by using Arthrex® double syringe with 1,500 rpm 5 minutes by a single centrifugation technique, which could produce the platelet concentration 1-3 times higher than the platelet baseline. This method had an advantage in PRP preparation because it used less than 10 minutes to prepare, which was not needed to use anti-coagulant. Additionally, it was a closed system, so it could reduce the contamination during the centrifugation and injection process (Cook and Smith, 2018) (Figure 1).

Figure 1 The preparation of PRP with Arthrex® double syringe (Cook and Smith, 2018)

2.5 Coxofemoral osteoarthritis and kinematics evaluation

Gait analysis was another parameter that could be able to describe and understand pathologic gait and orthopedics disease condition. The principle of analyzing the abnormality of gait was required to understand the normal locomotion in dog, which was divided into two main groups: symmetric and asymmetric. The example of symmetric gait was walking, trotting, and pacing, and the example of asymmetric gait was galloping. When considering gaits, one full cycle is referred to as a stride. Gait analysis composed of Kinetic and Kinematic gait analysis; Kinetic gait analysis was an explanation of ground reaction force on the weight bearing in each step such as peak vertical force (PVF) and vertical impulse (VI), which were popular parameter to detect lameness (Carr et al., 2016). Kinematic gait analysis was the parameter that used to describe rate and location of limb, which composed of stance phase, stride phase, angular velocities, range of motion (ROM), and displacement. Kinematic data could be analyzed by using the video from two or three dimensional and could be able to gather the data from both grass field and the treadmill. Nevertheless, the accuracy of the data depends on the equipment and the technique that were used to collect the data (Tobias and Johnston, 2012).

Kinematics evaluation There are a variety of kinematics techniques that could be considered, but the practical one that is commonly used across numerous scopes is a 2D-kinematics analysis. The advantages of this technique are particularly evident in its simple technical analysis, its economical, its ability to be portable and applied to real field situation, its no-obligation for prior experience, and its approachable free software, Kinovea® (Feeney, et al., 2007; Elrahim, et al., 2016 and Divi, et al., 2018). ROM was another parameter that could be able to analyze thew movement of joint, which the composition was muscle, tendon, ligament, and articular cartilage, by considering from maximum extension angle (MEA) and maximum flexion angle (MFA). Both parameters could be used to determine the flexibility of each joint for the further diagnosis. There were many studies found that ROM in dogs with orthopedic joint-related diseases was lower that the dogs in

normal condition. One of the studies reported that the greater reduction in peak vertical force and maximum extension angle of coxofemoral joint were found in dysplastic dogs compared with normal dogs (Souza, et al., 2019). Another literature also revealed the significant difference in maximum angle of coxofemoral joint and maximum angular velocity in coxofemoral dysplastic dogs compared to healthy dogs (Miqueleto, et al., 2012). The kinematic analysis is one of the techniques used to evaluate human movement. It can be applied in various fields including rehabilitation, sport training, clinical research, and orthopedics fields (Divi, et al., 2018). In veterinary orthopedic field, several researches demonstrated that changes in kinematics could result in some orthopedics disease.

CHAPTER III

MATERIALS AND METHODS

3.1 Animals

Phase 1

This study would incorporate 6 healthy dogs. The study was be performed at the Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University. First and foremost, all the dogs would be examined for orthopedics through physical examinations, blood collection before a deep sedative and blood collection. The 15 ml of blood of each syringe would be 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 must be filled and permitted by the owner to use their dogs prior to the participation in this study.

Inclusion criteria

1. Healthy dog between 1-5 years old.
2. Dog with weight between 20 – 50 kg.
3. Any breed and sex would be acceptable.
4. Each dog must not have orthopedics, neurological, or systemic concurrent diseases. Also, the patients should not have any operation during the last 12 months.
5. Dog with normal blood result of complete blood count and blood chemistry.
6. Each dog shall be engaged in weight control during study.

Exclusion criteria

1. Dog with orthopedics or systemic concurrent diseases, or the one who had a surgery throughout the last 12 months before the assessment date.
2. Dog during pregnancy and lactation periods.

Phase 2

This study would incorporate 9 dogs presented with coxofemoral OA. The research would be performed at the same location as the first phase. First and foremost, all dogs would be examined for orthopedics through physical examinations, blood collection, hips radiography in hip extended view and lateral

view, and lameness assessment score. The dogs' owners have to conduct the dog brief pain inventory (CBPI) and Liverpool Osteoarthritis in dogs (LOAD) before and after the study. Consent form must be filled and permitted by the owner to use their dogs prior to the participation in this study.

Inclusion criteria

1. Dog that does not exceed 10 years of age with diagnostic mild to severe grade coxofemoral OA of radiographic findings on at least one side coxofemoral joint, and has at least 1 month of consistent signs associated with the OA.
2. Dog with weight between 15 - 60 kg.
3. Any breed and sex will acceptable.
4. Each dog must not have other orthopedics, neurological, or systemic concurrent diseases. Also, the patients should not have any operation during the last 12 months.
5. Each dog must have a minimum 1 week of refrainment from steroid and non-steroidal anti-inflammation (NSAIDs) treatment prior to the initial assessment and throughout the study.
6. Dog with normal blood result of complete blood count and blood chemistry.
7. Each dog shall be engaged in weight control during study.
8. Each dog will be the moderate or severe grade of coxofemoral osteoarthritis.

Exclusion criteria

1. Dog with orthopedics or systemic concurrent diseases, or the one who had a surgery in the last 12 months before the assessment date.
2. Dog during pregnancy and lactation periods.
3. Patient with complications and adverse effects from arthrocentesis and injection of platelets rich plasma (PRP) treatment.

The study was approved by the Office of Animal Ethics Committee of Faculty of Veterinary Science, Chulalongkorn University. (no. 2031038)

3.2 Study designs

This study was separated into 2 phases with samples of 16 dogs. In the first phases, the study mainly focused on 6 healthy dogs that has 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 a single dog, then the blood from each dog would be centrifuged with a 10 different centrifugation speeds and time. After that, the platelet concentration, and the additional components from the plasma with platelet concentration would be collected. In the second phase, the study would be performed by diagnosing with coxofemoral OA, with the aim to compare before and after outcomes of PRP treatment for dog coxofemoral OA. The PRP preparation and treatment would be done under deep sedative condition with dexmedetomidine (3-5 mcg/kg) and morphine (0.3 mg/kg) with appropriate aseptic technique. All dogs would be 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 range of motion (ROM) would be 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 would be provided with antibiotics at the day of the injection. Area restriction would be imposed for 3 days after the PRP injection. After that, the owners would be able to walk the dogs as usual.

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 dog walk and trotting on treadmill	✓			
Collect kinematic data		✓		✓

3.3 Anesthetic protocols and post-treatment care

The platelet calculation and health status check should be done prior to general anesthesia. The patient should be withheld from food and water for a period of 12 and 6 hours before premedication, respectively. Dexmedetomidine (3-5 mcg/kg) and morphine sulphate (0.3 mg/kg) would be injected intramuscularly for deep sedation. Cephazolin (25 mg/kg) would be given for prophylaxis antibiotics before blood collection and PRP injection. Area restriction should be done for 3 days after PRP injection.

3.4 Platelet rich plasma (PRP) preparation

Phase 1

One-hundred- and fifty-ml of whole blood would sample be collected from jugular vein from healthy dog, under deep sedation and asepsis by 18 gauge 1 ½ -inch needle, which connected with a CPDA-1 single blood bag 150 ml, and it would be separated into 15 ml double syringe system (Arthrex®, Device innovation Co.,Ltd., Naples, Florida, USA)(Figure 2). Afterwards, the sample would be centrifuged by a centrifuge machine (Hettich Zentrifugen, Rotofix32A) (Figure 3) with centrifugation speed (rpm) and time (minute) (Table 2) After the centrifuge process

was completed, the centrifuged blood would be separated in 3 layers as follows: Firstly, the upper contained a plasma, which was concentrated platelets call Platelet Rich Plasma (PRP). Secondly, the middle layer is buffy coats contained white blood cell (WBC). Lastly, the bottom layer was red blood cell (Figure 4). In the end of the process, a 1 ml sample of plasma (upper layer) would be sent to Vet Clinical Center (VCC) Laboratory for platelet count with Wright-Giemsa stained technique with 100x magnification under light microscope (Figure 5) and using the formula number of platelets x 10 field x 2 x 10^3 / μL to collect platelet count and fluid analysis for further PRP treatment in dog coxofemoral osteoarthritis in phase 2.

Figure 2 Double syringe system
(Arthrex®, Device innovation Co.,Ltd., Naples, Florida, USA)

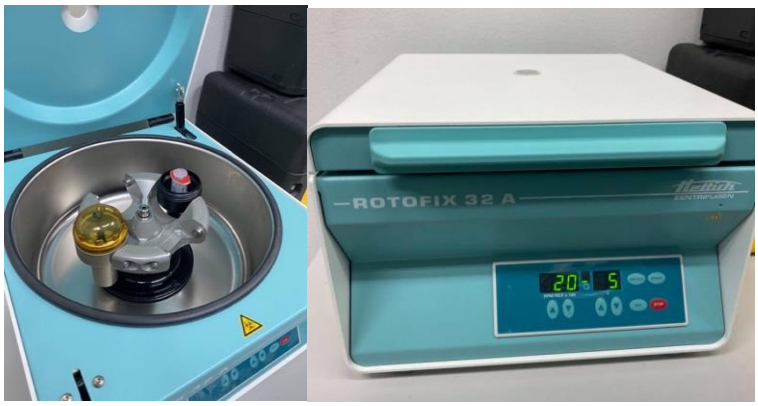


Figure 3 Centrifuge machine (Hettich Zentrifugen, Rotofix32A)

Table 2 The different method of centrifugation speed (rpm) and time (minute) for PRP

Centrifugation speed (rpm)	Time (minute)
1500	3
1500	5
2000	3
2000	5
2500	3
2500	5
3000	3
3000	5
3500	3

3500 5

Figure 4 Separation of blood to 3 layers in Arthrex® double syringe after centrifuge.

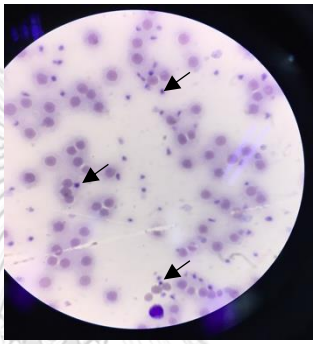


Figure 5 Cytology of blood smear and platelet in the Wright-giemsa stained technique slide (black arrow)

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) would be separated into three fractions: first, a 0.25 ml. EDTA tube which would be sent to the Vet Clinical Center (VCC) for platelets count and cytology, the second part 0.25 ml would be 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 would be injected to the affected coxofemoral joint (Figure 6).

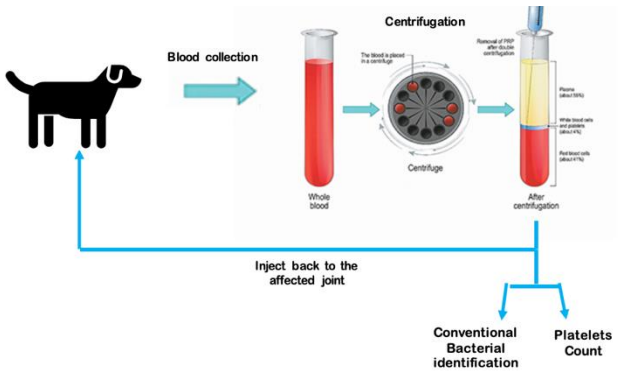


Figure 6 Diagram of Platelet Rich Plasma preparation

3.5 Clinical evaluation

3.5.1 Radiographic OA scoring

All the dog's hips would be diagnosed by radiography in hip extended view. Grading of OA of coxofemoral joint would be evaluated by a veterinary radiologist at the first visit and at 8 weeks after PRP injection. According to osteoarthritis grading (modified from Kellgren and Lawrence classification, 1957 and Wessely, et al., 2017), dogs were chosen in criteria of mild and severe grade of OA in this study (Table 3) and the radiographic OA scoring in Figure 7.

Table 3 Osteoarthritis grading and corresponding radiographic changes modified from Kellgren and Lawrence classification, 1957 and Wessely et al., 2017

Grade	Radiographic findings
1 (No OA)	Possible narrowing of joint space medially and not found osteophytes around femoral head
2 (Mild OA)	Definite narrowing of the joint space inferiorly, definite osteophytes and slight sclerosis
3 (Moderate OA)	Marked narrowing of the joint space, slightly osteophytes, some sclerosis and cyst formation and deformity of femoral head and acetabulum
4 (Severe OA)	Gross loss of joint space with sclerosis and cysts, marked deformity of the femoral head and acetabulum and large osteophytes

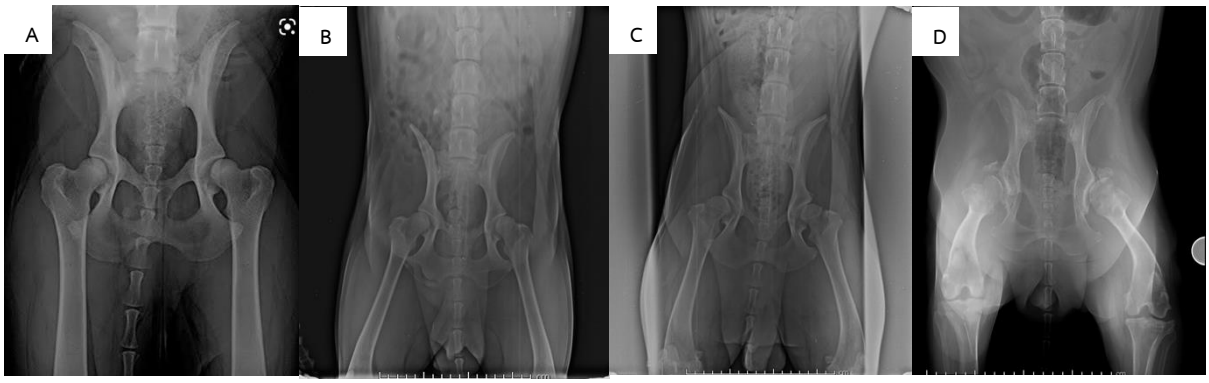


Figure 7 Osteoarthritis grading and corresponding radiographic changes of coxofemoral joint ; A = grade 1 (no OA) , B = grade 2 (mild OA), C = grade 3 (moderate OA) and D= grade 4 (severe OA)

3.5.2 Lameness score

Lameness score in this study would be confirmed to the Lameness score criteria (modified from Impellizeri, et al., 2000 and Hazewinkel, et al., 2008)(table 4). It would be evaluated by 3 veterinarians at first visit and 8 weeks after PRP injection via 2 planes video record (left and right view).

Table 4 Lameness score criteria modified from Impellizeri et al.,2000 and Hazewinkel et al.,2008

Score	Signs
0	Normal gait , not found lameness when walking and trotting
1	Mild lameness ; when walking and normal gait when trotting
2	Mild lameness ; Consistent lameness when walking and pronounced lameness when trotting
3	Severe lameness ; Non-weight bearing when walking and algetic gait when trotting
4	Non-weight bearing when walking and trotting

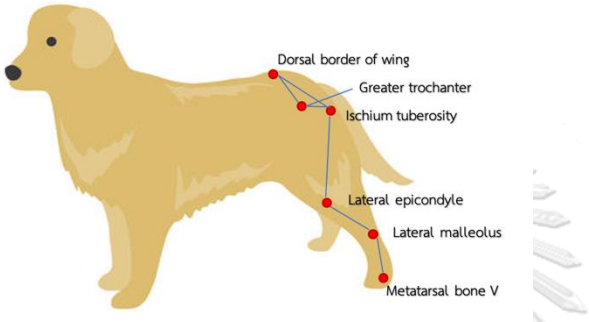
3.5.3 Kinematics data analysis

To precede the initial kinematic analysis by Kinovea software at one week before PRP injection and 8 weeks after PRP injection, the dogs should be trained to walk and trot on a treadmill. They would be trained at a frequency of three to nine sessions per day, for approximately two or three repetitions of 3 – 5 mins per session (Miqueleto, 2012) throughout the week before the study.

For the preparation of video-based kinematic data collection, each dog should be shaved at the tagged point with 12 retroflected 18-mm diameter spherical markers with adhesive tape (Miqueleto, et al. ,2012)(Figure 8). The marker would be 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. (Souza, et al., 2018 and Miqueleto, et al., 2012) (Figure 9).

Figure 8 18-mm diameter spherical markers with adhesive tape



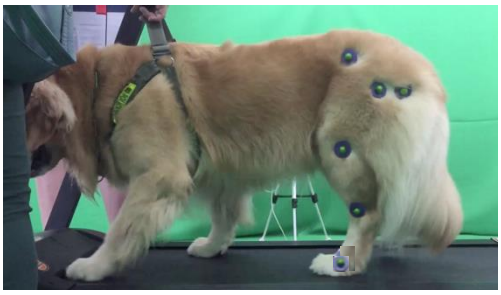


Figure 9 Anatomical landmarks for marker replacement

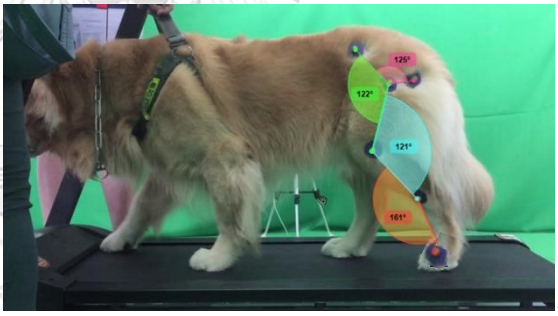


Figure 10 Degree of coxofemoral joint, stifle joint and tarsal joint via Kinovea® software

To setup the video-based kinematic data collection, the iPhone 6 with a camera setting of 60 frame-per-second (fps) and a resolution of 1920x1080 would be used. The kinematic data would be 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 should be calibrated and marked the distance from treadmill and camera. Subsequently, the treadmill would commence 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, 2012). 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 (Figure 10) at one week before the PRP injection and 8 weeks after PRP injection.

Figure 11 Position of treadmill and cameras

3.5.4 Owner Questionnaires

The owner would be assessed the modified questionnaire to Thai version of canine brief pain inventory (CBPI) by University of Pennsylvania which created by Dr. Dorothy Cimino Brown and Liverpool Osteoarthritis in dogs (LOAD) by University of Liverpool at Day 0 and 8 weeks after PRP injection in this study.

3.6 Data presentation and 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 statistic. 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.

CHAPTER IV

RESULTS

4.1 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 body-condition score (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 the hydration status less than 5%. The hematology and the blood chemistry of all dogs were normal.

Table 5 The characteristics of healthy dogs (n=6) in phase 1 were presented as mean and standard deviation of ages and weight, excepted the gender was presented as number with percentage and body condition score (BCS) was presented as number with median.

Parameters	Value
Ages (Months)	29.53 ± 22.16
Sex [number (%)]	
Male	4 (66.7%)
Female	2 (33.3%)
Weight (kg)	32.67 ± 9.85
BCS (5-point score)	3 (3.0 – 3.5)
Hydration status (%)	<5

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 10 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 – 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 the hydration status less than 5%. The hematology and the blood chemistry of all dogs were normal.

Table 6 The characteristics of dogs coxofemoral OA (n=9) in phase 2 were presented as mean and standard deviation of ages and weight, excepted the gender was presented as number with percentage and body condition score (BCS) was presented as number with median.

Parameters	Value
Ages (Months)	46.2 ± 23.22
Sex [number (%)]	
Male	8 (77.78%)
Female	2 (22.22%)
Weight (kg)	34.93 ± 12.38
BCS (5-point score)	3.5 (3 – 3.5)
Hydration status (%)	<5

4.2 Platelet Rich Plasma (PRP) preparation

Phase 1

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 (minute) 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 (Figure 12).

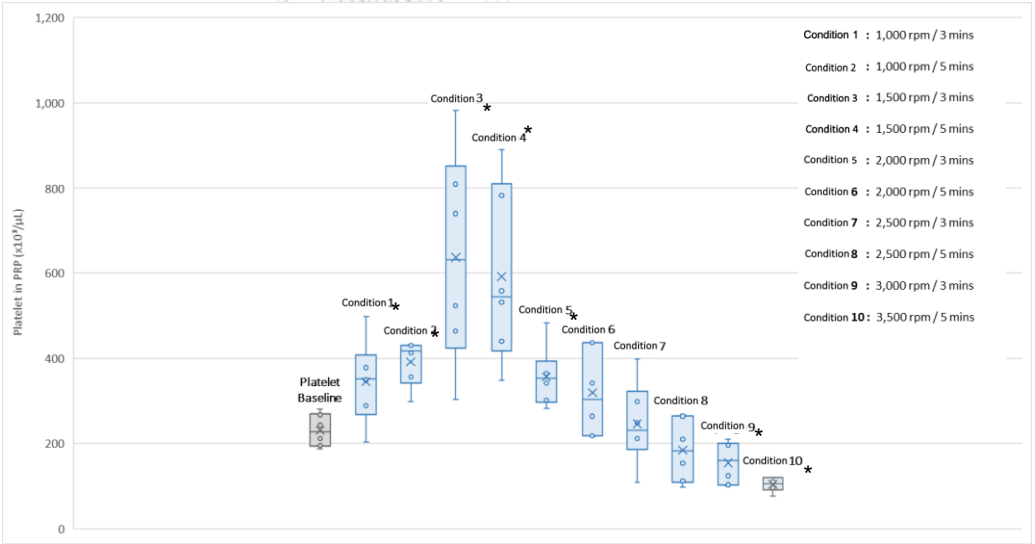


Figure 12 The box plot showed median and interval (IQR) compared between concentration platelet in PRP and platelet baseline with different centrifugation speed (rpm) and time (minute) in 10 techniques in 6 dogs.

* p-value < 0.05 compared platelet concentration between baseline and after centrifugation, using Wilcoxon Signed Ranks test.

Phase 2

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 (Figure 13). The total of platelet concentration after centrifugation was in 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 hours after incubation.

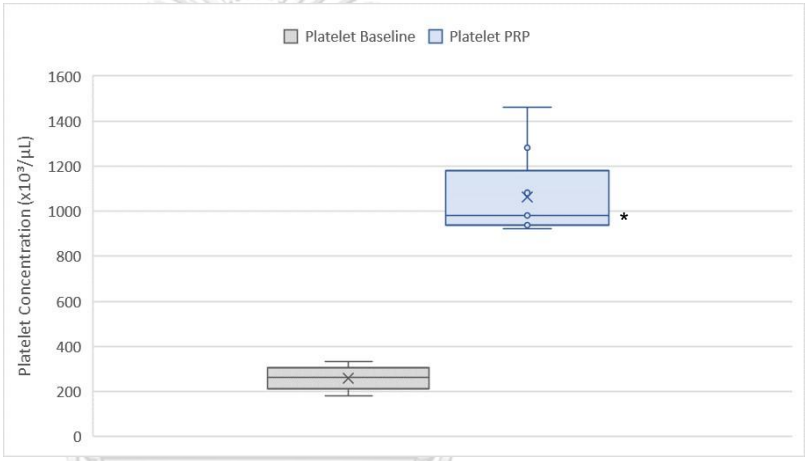


Figure 13 The box plot showed median and interval (IQR) of platelet concentration in PRP of 9 coxofemoral OA dogs.
* p-value < 0.05 compared platelet concentration between baseline and after centrifugation, using Wilcoxon Signed Ranks test.

The median and interval (IQR) of platelet concentration of 9 OA dogs compared between platelet baseline, hematocrit (Hct) and white blood cell (WBC) , found there was correlation between platelet concentration of baseline and PRP. Whereas, there was negative correlation between Hct and platelet concentration in PRP (Figure 14).

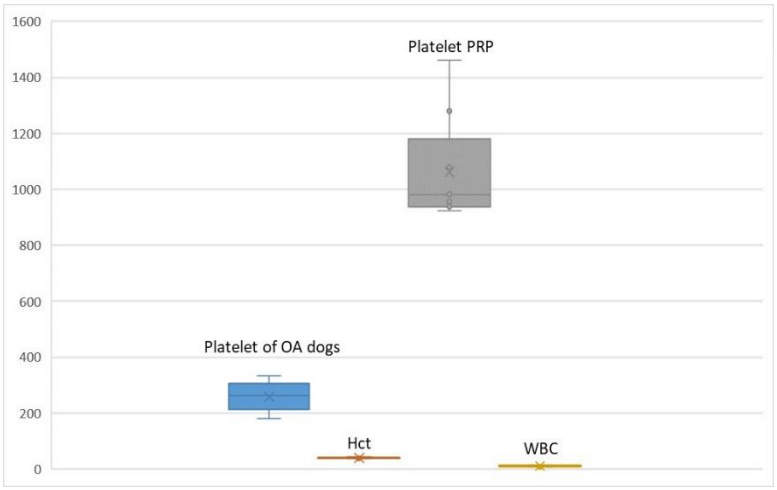


Figure 14 The box plot presented median and interval (IQR) of platelet concentration in PRP compared between platelet baseline, hematocrit (Hct) and white blood cell (WBC) of 9 coxofemoral OA dogs.

4.3 Clinical evaluation

4.3.1 Radiographic OA scoring

18 coxofemoral joint would be diagnosed by radiography in hip extended view. Score would be evaluated at the first visit and at 8 weeks after PRP injection. The bar graph showed no statistically change ($p > 0.05$) between day 0 and 8 weeks after PRP injection (Figure 15).

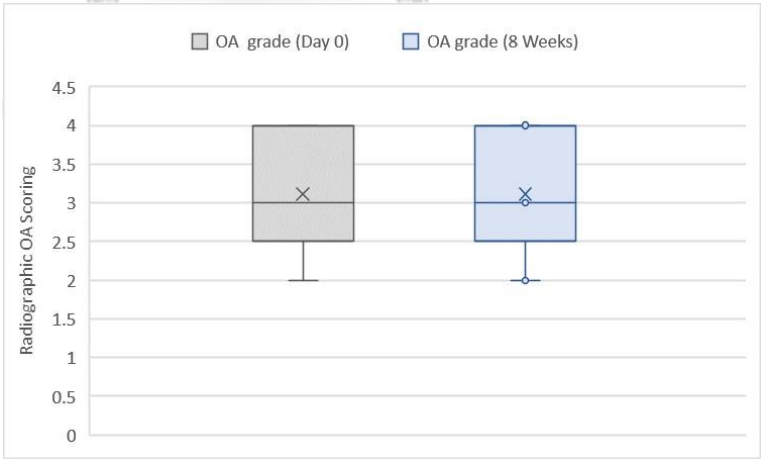


Figure 15 The box plot showed median and interval (IQR) of radiographic grading of both coxofemoral joint using Wilcoxon Signed Ranks test.

* p-value < 0.05 compared radiographic examination at the first visit and

at 8 weeks after PRP injection, using Wilcoxon Signed Ranks test.

4.3.2 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 showed significant difference ($p < 0.05$) at 8 weeks. Median and range (IQR) of lameness score in 9 dogs (Figure 16).

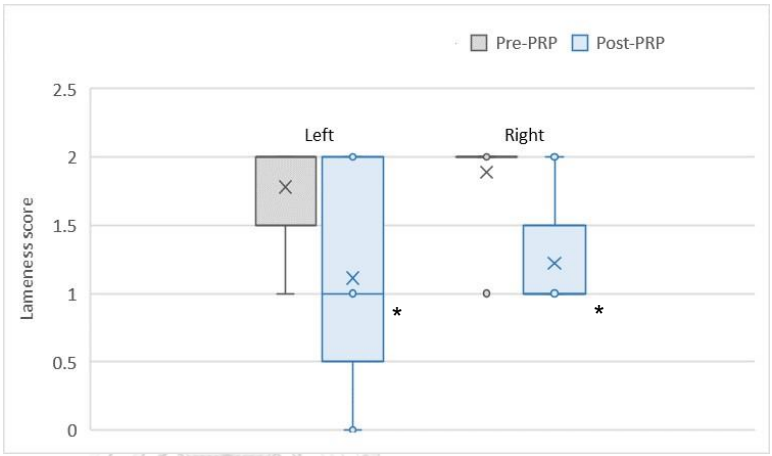


Figure 16 The box plot showed median and range(IQR) of lameness score by 3 blinded evaluation veterinarians at first visit and 8 weeks.
* p-value < 0.05 compared lameness score , using Wilcoxon Signed Ranks test.

4.3.3 Kinematics data analysis

The kinematic data of 9 dogs would be collected in the sagittal plan 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 grouping by comparing Maximum extension angle (MEA), Maximum flexion angle (MFA), pre-PRP injection, and post-PRP injection. The results of coxofemoral joint indicated that there was a significant difference ($p < 0.05$) between before the PRP injection and at 8 weeks after injection on all coxofemoral joints (Figure 17 and Table 7). On the other hand, there was no significantly difference in the results of stifle and tarsal joints (Figure 18 , Figure 19, Table 8 and Table 9).

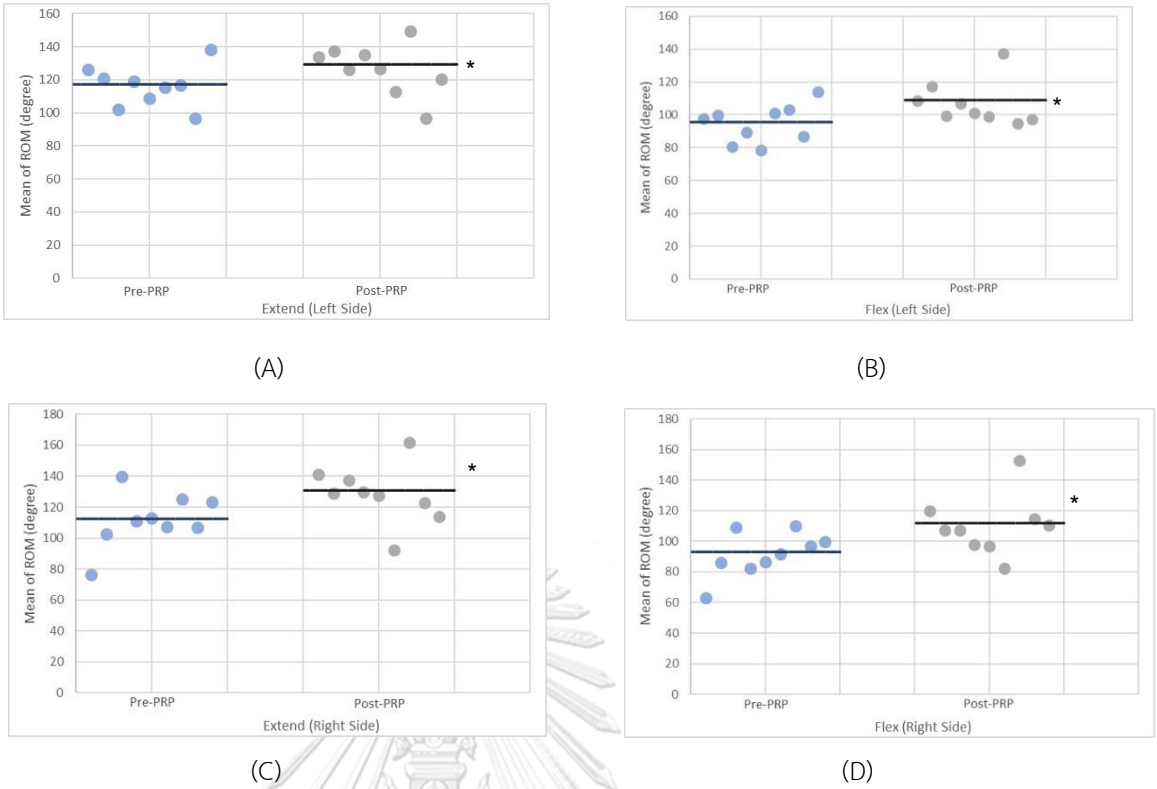


Figure 17 Scatter dot plot showed mean of MEA and MFA angle of 9 coxofemoral OA dogs. (A) described the mean of MEA angle of left coxofemoral joint in pre-PRP and post-PRP treatment , (B) described the mean of MFA angle of left coxofemoral joint in pre-PRP and post-PRP treatment , (C) described the mean of MEA angle of right coxofemoral joint in pre-PRP and post-PRP treatment, (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 7 Comparison of the active range of motion (AROM) of 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.

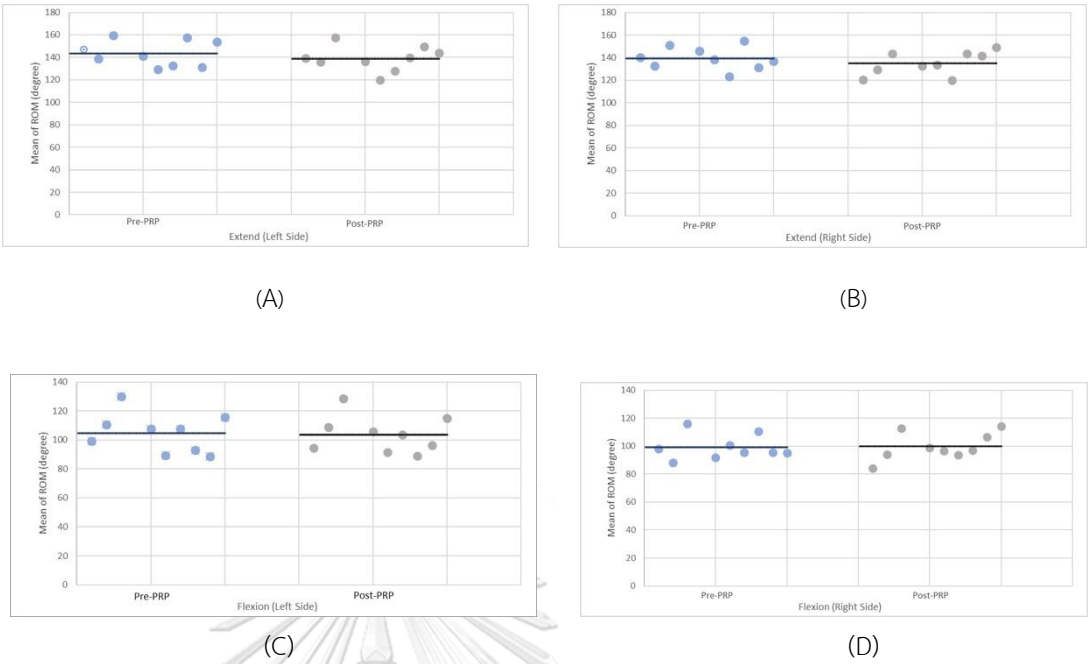


Figure 18 Scatter dot plot showed mean of MEA and MFA angle of 9 stifle OA dogs.

(A) described the mean of MEA angle of left stifle joint in pre-PRP and post-PRP treatment , (B) described the mean of MFA angle of left stifle joint in pre-PRP and post-PRP treatment , (C) described the mean of MEA angle of right stifle joint in pre-PRP and post-PRP treatment, (D)described the mean of MFA angle of right stifle 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 8 Comparison of the active range of motion (AROM) of 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.

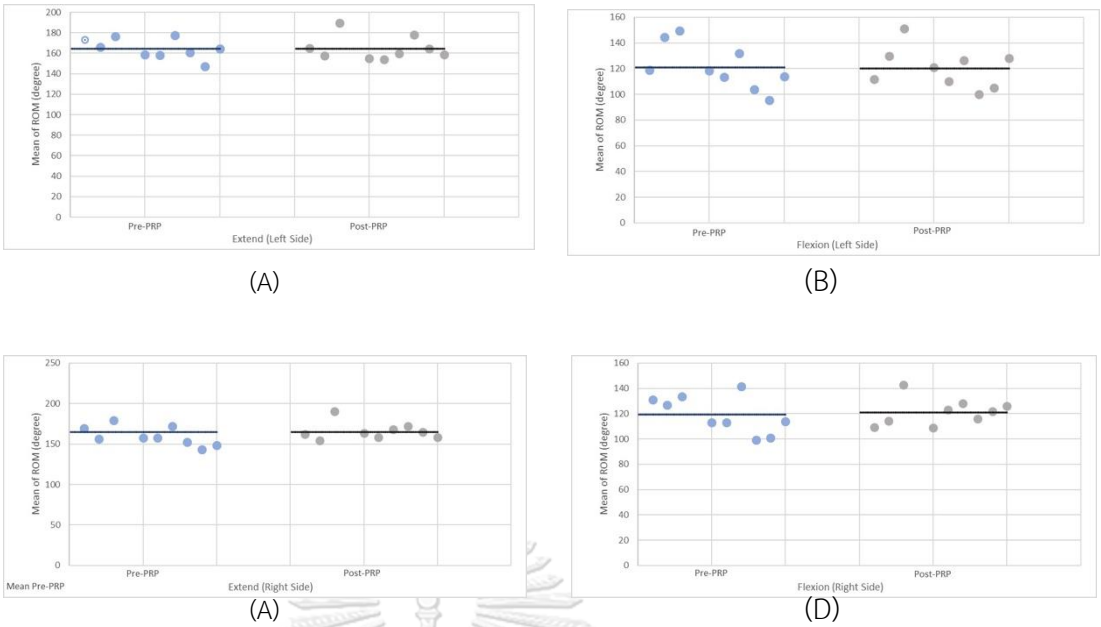


Figure 19 Scatter dot plot showed mean of MEA and MFA angle of 9 tarsal OA dogs. (A) described the mean of MEA angle of left tarsal joint in pre-PRP and post-PRP treatment , (B) described the mean of MFA angle of left tarsal joint in pre-PRP and post-PRP treatment , (C) described the mean of MEA angle of right tarsal joint in pre-PRP and post-PRP treatment, (D) described the mean of MFA angle of right tarsal 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 9 Comparison of the active range of motion (AROM) of tarsal joint before and after PRP treatment with mean±SD.

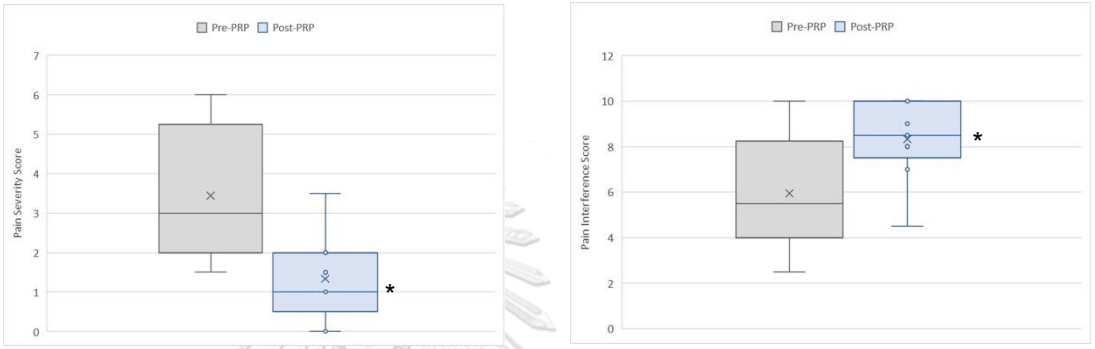
Weeks	Right tarsal joint		Left tarsal joint	
	Extension	Flexion	Extension	Flexion
0	159.49 ± 12.66	119.14 ± 16.34	164.68 ± 11.76	121.09 ± 18.41
8	165.7 ± 11.16	121.14 ± 12.49	164.69 ± 12.66	120.33 ± 15.95

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

4.3.4 Owner Questionnaires

The owner of 9 dogs would assess the modified questionnaire to Thai version of CBPI at Day 0 and 8 weeks after PRP injection in this study. The first four questions for evaluating pain severity score (PSS), and the last six questions

evaluated pain interference score (PIS). Although all the questions were using the same answer metric, which was 0 to 10 score, the last six questions were evaluated the answers by reversing the score of the respond from the owner, which was from 10 to 0. The result revealed that the degree of pain was significantly improve ($p < 0.05$), as the same as the life quality.



(A) (B)

Figure 20 This box plot displayed the result median and range (IQR) the degree of PSS and PIS from the CBPI questionnaire between a day before PRP injection and 8 weeks after PRP injection.

(A) The pain severity score result showed the level of dogs' pain by the owner.

(B) The pain interference score result showed the dog's life quality by the owner.

** p-value < 0.05 compared degree of pain and life quality by the owner, using Wilcoxon Signed Ranks test.

Table 10 Median and range (IQR) of the PSS and PIS, that evaluated by CBPI questionnaire from the owner at D0 and 8 weeks

Weeks	Pain Severity Score	Pain Interference Score
0	3.125 (2 – 5.125)	5.75 (4 – 8. 25)
8	1.25 (0.75 – 2)	7.5 (7.5 – 10)

*p-value < 0.05 compared between pre-PRP treatment and post-PRP treatment, using Wilcoxon Signed Ranks test.

CHAPTER V

DISCUSSION

Nowadays, a treatment in terms of orthopedics field by using (Platelet Rich Plasma) PRP are immensely popular in both human and animals. Mostly diseases with a reason from a degeneration condition uses PRP as a supportive treatment, and a famous one is Osteoarthritis (OA) because the platelet is the first cell that is going to firstly arrive at the damaged position before any other cells. The studies explained that the platelet in PRP had 2-11 times of platelet concentration higher than the platelet baseline with the significant clinical treatment results. The platelet concentration releases cytokines (Alves and Grimalt, 2018; Calvo, et al., 2019), which also releases platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), insulin-like growth factor-2 (IGF-2), epidermal growth factor (EGF), hepatocyte growth factor (HGF), and basic fibroblastic growth factor (bFGF). All of these cytokines are responsible for decreasing catabolism, improving anabolism, as well as promote chondral remodeling (Dhillon, et al., 2017).

The optimized technique of PRP preparation in this research was the single centrifugation (Arthrex® double syringe centrifugation) as it was closed system syringe during the PRP centrifugation process, which reduced a contamination in plasma that would be injected into samples. Besides, the single centrifugation technique could be able to reduce the possibility of allergy and a side effect reaction from anticoagulant substance because it used a short period of time in centrifugation process to produce the PRP before the blood clotting was occurred (Cook and Smith, 2018). In order to prepare the PRP for human it was required to use 1,500 round per minute (rpm) for 5 minutes in centrifugation process, which was found that there was a high chance of blood to be clotted if there was no anticoagulant substance due to a long process from the blood collection process to the centrifugation process, so the first phase of our study

was performed without using any anticoagulant substance, which found that the most suitable centrifugation speed and a duration of time with the highest platelet concentration was 1,500 rpm for 3 minutes. Additionally, the platelet concentration of the PRP was 2-6 times higher than the platelet baseline with a low amount of white blood cells in plasma as well. There was a report stated that too many amounts of white blood cells in plasma caused the catabolic effect due to the releasing of cytokines such as matrix metalloproteinase (MMPs) and interleukin-B, which leaded to the inflammation conditions later (Park et al., 2004). In contrast, studies found that the white blood cells in plasma could be able to prevent the infection of *Escherichia coli* and *Staphylococcus aureus* in vitro experiment (Moojen et al., 2008). It was revealed that the super platelet concentration could be able to restrict the osteoblast activity, which leaded to the apoptosis condition, as well as detained the functionalities of growth factor (Haynesworth et al., 2002; Hall et al., 2009; Yin et al., 2016). Conversely, the overpowered centrifugation would be resulted in the amount of platelet concentration of PRP output lower than the platelet baseline because the overlapping of the layer in platelet and red blood cells, which was difficult to extract the PRP from it.

Our study revealed that the rising of hematocrit (Hct) value in whole blood caused the inclination downward to platelet in PRP that significantly difference, which correlate with the study of hematology in whole blood with the amount of platelet in PRP, and additional studies indicated that the high Hct value caused the increment of viscosity of blood and increase adhesion of platelet in the area of vascular wall (Spann et al., 2015). Additionally, there was the experiment of increasing red blood cells in mice, which caused the rising of Hct value, and also found that the platelet in PRP after increasing red blood cells were lower than the platelet baseline (Walton et al., 2017). The relationship between platelet in whole blood and platelet in PRP were a positive correlation such that increasing of whole blood also increasing the platelet in PRP, while the

white blood cells and platelet poor plasma have no impact on the platelet concentration in PRP (Andrade et al., 2008).

The coxofemoral osteoarthritis (OA) condition in dogs could be categorized into 2 categories, which are a Primary OA, which was idiopathic, but it would have some factor that could lead to this state such as aging and obesity. Next category was a Secondary OA from the injury, bone diseases, the infection, and it could be a result of the Canine Hip Dysplasia (CHD) condition as well. These factors triggered the inflammation stimulus substances on the coxofemoral, which were MMPs, PGE_2, IL-1, IL-6, IL-8, TNF, and some proteinases (Freitag et al., 2016). These substances could result in the damage to proteoglycan and collagen type II, which were the crucial components of articular cartilage. The gross morphological and histological characteristics of articular cartilage from the induction leaded to the OA condition with an enzyme collagenase, which found a cartilage erosion characteristic to the level of subchondral bone. A severity of OA condition also based on the force that interact with joint.

In our study, there was no significant difference in the demographic data of the 9 OA dogs during the 8 weeks period of the study. The study revealed that all 9 dogs have a period of adult lifespan, which was 2-6 years (Meeson et al., 2019), which the results from the study of the age factor with OA was still uncleared because there was a study reported that the age has the direct variation to the risk of OA condition (Nilsson et al., 2018). However, in some studies explored that the OA condition could be occurred in young age due to the other factors that could coexist such as laxity of hip, which was brought to the structure abnormality since the juvenile (Witsberger et al., 2008 ; Hou et al., 2013 ; Taylor – Brown et. al, 2015). This study also observed that there was the OA condition in the male dogs more than a female (7/2 dogs), which consistent to the incident of OA in 64 dogs' study (Lamani et al., 2019) that the OA condition were more affected to the male dogs (64.06%) than the female (35.93%). On the other hand, there was study claimed that the sex was not the factor of OA

condition. The body weight and BCS of the OA dogs in this study indicated that they have slightly higher than the ideal BCS value, which consistent with most of the researches that the obesity affected to increase the risk of being the OA condition with a comparison study to the clinical signs of lameness between the groups were fed ad libitum and others were fed less, and the studies found that the group that provided ad libitum had prevalence of the OA condition more than another group (Impellizeri et al., 2000 ; Smith et al., 2006).

For the dog breed in this study, the Golden Retriever was the most common breed (6/9 dogs), then the Chow Chow, Shetland sheepdog, Alaskan Malamute, and a mixed breed, respectively. There was a study revealed that the OA condition occurred the most on the large dog breed, and also the Golden Retriever was the most dog breed that was found the OA condition (Scholar et al., 2019; Anderson et al., 2020). In addition, there was studies (Rinnovati et al., 2016 ; Borgman et al., 2021) about relationship between hydration status and the platelet concentration, which found that the animal with dehydration condition would have the platelet concentration higher than normal, which affected to higher platelet concentration in PRP as well. Conversely, the too many platelet concentrations could lead to the thrombosis condition. In this study found that all the sample dogs have a normal dehydration status, which could be able to reduce the variability on the amount of platelet concentration before and after the centrifugation. Regarding the radiographic OA scoring with the hip extended view, there was 4 dogs with the severe OA condition, 3 and 2 dogs with moderate OA condition and mild OA condition, respectively. There was no significant statistically difference in terms of radiographic OA score in the comparison between the pre-PRP injection and the post-PRP injection within the duration of 8 weeks, which similar to the study of Arican et al. (2018) on the dogs with OA condition on the stifle joint, which were categorized into controlled group and the PRP injected group. The result of this study with the duration of 12 weeks indicated that there was no difference and progression of radiographic signs similar to the other studies as well (Fahie et al., 2013; Silva et al., 2013). Nevertheless,

whether the PRP could be able to detain the progression of OA in terms of radiographic or not, it was needed a further study with longer study duration.

The lameness score of the OA dogs in this study was evaluated by the blinded evaluation from a total of 3 veterinarians in both pre-PRP injection and post-PRP injection, was resulted in 2/4 lameness score in 7 dogs, and 1/4 lameness score in another 2 dogs at the time of pre-PRP injection. When compared to the radiographic signs, it was found that the 4 dogs with severe OA condition were in a group of 2/4 lameness score in both left and right sides of the coxofemoral joint, which consistent to the characteristic of hindlimb lameness condition in dogs, which pelvic limb weight bearing would rebalance the weight to the front legs, and the forelimbs would be placed in caudally position more than normal, and also found the 'hip hike' characteristic. Furthermore, there was a peak vertical force (PVF) value and vertical impulse (VI) of pelvic limb, which was the most commonly used indices to detect lameness lower in the dogs with OA condition when compared to the dogs with normal coxofemoral joint condition (Vilar aet al., 2016; Carr et al., 2016; Souza et al., 2019). After the blinded evaluation by 3 veterinarians after the 8-weeks after the PRP injection, it was explored that the trends of lameness score were decreased on both left and right side, which similar to the study of Fahie et al. (2013) stated that the PRP injection in each joint of dogs with OA condition for 12 weeks revealed that the percentage of median score were reduced when compared to the score before the PRP injection. Besides, this study also found that there was no correlation between lameness score and radiographic OA scoring, which was not significant difference due to the lameness score have a better result with unchanged radiographic OA scoring.

From the previous studies, the time that were used by trotting on the treadmill during the video recording was approximately 3 minutes, which did not lead to fatigue of the dogs. However, the dog's behavior, the marker placement, the acceleration of the dogs, and the plain in the video could affect the variation of the analyzed results of the kinematic data analysis. The questionnaire from the

owner with Canine Brief Pain Inventory (CBPI) is accepted subjective method of assessing as lameness score and radiographic OA scoring. CBPI questionnaire would be categorized into 2 parts, which were pain severity score (PSS) in question number 1 to 4, and pain interference score (PIS) in question number 5 to 10. In this study, the score of PSS and PIS of 10 OA dogs were tended to increase at 8 weeks after the PRP injection because the dog's daily activity and life quality were better when they were felt less pain, which similar to the results of other studies at the time after injecting PRP (Fahie et al., 2013; Aminkov et al., 2020). Liverpool Osteoarthritis in Dogs (LOAD) was one another questionnaire that focusing on the level of dog and ability to exercise, which CBPI and LOAD were correlated to each other. Moreover, there was a study between the 2 questionnaires, which were subjective assessment, and PVF and VI value, which were an objective assessment between carprofen compared with placebo treated dog by Brown et al. (2013), and the results shown that there were no correlation among these assessments because both questionnaires were easily biased by the owner. In addition, only using a questionnaire as a single result indicator may not be sufficient because there was a bias from the owner if they knew what their dog received and what would affect to them. Thus, there should be an objective indicator in the assessment as well.

According to Kinematics data analysis, there was 2 phases of dog trotting, which was a swing phase, and a stance phase. The range of motion (ROM) in each phase and each joint would be different in each period of time. The relationship between coxofemoral, stifle, and tarsal joint of pelvic limb was an inverse dynamic. To be specific, when maximum extension of hip in the late portion of stance phase, the maximun flexion value of stifle and tarsal joint were low at the same time, which could be found between the stance phase and the swing phase (Bockstahler et al., 2007). The data that were used to analyze in this study were Active Range of Motion (AROM) by separated into Maximum Extension Angle (MEA) and Maximum Flexion Angle (MFA) in both left and right sides, before PRP injection (pre-PRP), and after PRP injection (post-PRP). The information of 9 OA

dogs were a large dog breed and most of them were Golden Retriever (60%). It has shown that in the pre-PRP experiment, an average of MEA and MFA for the left hip was 116 ± 13.33 degrees and 94.42 ± 12.47 degrees, respectively, while an average of MEA and MFA for the right hip was 111.75 ± 18 degrees and 91.69 ± 14.37 degrees respectively. When compare these values with mean value measurement in healthy Retrievers (MEA: 162 degrees; MFA: 50 degrees) by a study from Thomas et al. (2006) indicated that the average of MEA on both left and right in pre-PRP experiment was lower than the average from the study. In the other hand, the average of MFA was higher than the average from the study, which there was a research revealed that the dogs with OA condition have MEA , VI and PVF values lower than an average when compared to a normal dog (Souza et al., 2018). Additionally, there was a study by Greene et al, (2013) about ROM specifically in Labrador Retrievers with 60 samples dogs with a hip dysplasia condition, and the results of this study found that MEA on the hip joint would be decreased 1 degree every year, which was related to the lameness score and radiographic OA scoring that found osteophyte was increased 1 mm in every 3 years of ages.

For the ROM in the 8 weeks post-PRP experiment, this study found that an average of MEA and MFA for the left hip was 126.55 ± 15.59 degrees and 106.87 ± 13.17 degrees respectively, while an average of MEA and MFA for the right hip was 128.49 ± 18.59 degrees and 110.04 ± 18.67 degrees respectively. The result of studying MEA and MFA of stifle and tarsal joint revealed that the MEA lower than an average as the same as coxofemoral joint. On the other hand, when compare with the average after 8 weeks of PRP injection, they were discovered that there was no significant difference in terms of statistics. In contrast, these results of coxofemoral joint indicated that MEA and MFA were significantly increased when compared to the values on the pre-PRP experiment, but it still lower than the average MEA and MFA of healthy dogs, which consistent with the research by Hilty et al., (2017) that injected PRP to a total of 8 dogs with OA conditions and discovered that there was a rising in ROM evaluation score after 15 days of PRP injection, the lameness score also reduced, but after 30 days of PRP injection it was

found that ROM were gradually decreased but still more than the value before the PRP injection. Furthermore, there was a study that injected PRP for a single time to 75 humans with OA knee condition. The results shown that the trend of stiffness score was lower after 2 weeks of PRP injection and back to the same value before PRP injection at 12 months after PRP injection (Bansal et al.,2021). In addition, it was discovered that PRP could be able to increase ROM, which relief the pain of the dogs with secondary OA from cranial cruciate rupture as well (Vilar et al., 2018; Lee et al., 2019). To sum up, the duration of effectiveness of PRP depends on many factors. For example, the preparation, the platelet concentration, the characteristics, and the severity of the disease, which require a further study.

The limitation of this study was the number of sample dogs, which was too small and would not represent the overall results. There was also a struggle to bring the dog to do trotting on the treadmill as some of them was rejected to do. The only objective data in this study was a range of motion value, which there should be more parameters and additional objective data to analyze together such as PFV and VI, which was required a specific equipment such as force plate gait analysis. Moreover, the period of time in this study was relatively short, which could not reflect the long-term result of PRP.

In conclusion, the PRP preparation with double syringe system was the fast sterile technique with a result of an appropriate amount of platelet concentration in plasma. The result of PRP injection to the dogs with OA condition in this study was clinically effectiveness as the lameness score was lower, the increment of AROM on the hip joint, the decrement of pain that leaded to more activity in their daily life from the owner point of view, and there was no side effect found from intra-articular PRP injection. On the other hand, there was no different in terms of radiographic OA scoring at 8 weeks after the PRP injection. The purpose of reducing pain to the dogs with OA conditions is to make them can be able to do the physical therapy and perform an exercise to generate the muscle mass to support the hip joint. For instance, the sit-to-stand exercise, trotting on treadmill, and walking up and down

the hill. These exercises can be able to reduce the pain and increase the ROM on the hip joint, and also improve the long-term quality of life of the dogs. Furthermore, the diagnosis of OA might need to use the further imaging system such as CT scan, or the other OA biomarkers for the better accuracy of data. The further study suggestion of PRP on the OA might need a study focus on the in vitro as well.

APPENDIX

Appendix 1 Consent Form

ใบยินยอมเข้าร่วมโครงการวิจัย (Consent Form)

วันที่.....เดือน.....พ.ศ.....

ข้าพเจ้า.....
อยู่บ้านเลขที่.....หมู่.....ถนน.....
แขวง/ตำบล.....เขต/อำเภอ.....จังหวัด.....
รหัสไปรษณีย์.....โทรศัพท์.....
สุนัขชื่อ.....พันธุ์.....เพศ.....อายุ.....

ข้าพเจ้าได้ลงลายมือชื่อในใบยินยอมเข้าร่วมโครงการวิจัยเพื่อเป็นหลักฐานแสดงว่า

ข้อ 1. ข้าพเจ้า ได้รับทราบโครงวิจัยของ สัตวแพทย์หญิง ปฐมา อรุณรัตนากุล เรื่อง ประสิทธิภาพและผลทางคลินิกของการใช้พลาสมาที่มีเกล็ดเลือดเข้มข้นต่อภาวะข้อสะโพกเสื่อมในสุนัข

ข้อ 2. ข้าพเจ้า ได้รับการอธิบายจากผู้วิจัยเกี่ยวกับวัตถุประสงค์ของการวิจัย วิธีการวิจัย ประสิทธิภาพ ความปลอดภัย อาการหรืออันตรายที่อาจเกิดขึ้น รวมทั้งแนวทางป้องกัน และแก้ไข ค่าใช้จ่ายที่ข้าพเจ้าจะต้องรับผิดชอบจ่ายเอง อีกทั้งยังได้รับคำอธิบายและตอบข้อสงสัยจากผู้วิจัยเป็นที่เรียบร้อยแล้ว

ข้อ 3. ข้าพเจ้า ได้รับการรับรองจากผู้วิจัยว่าจะเก็บข้อมูลส่วนตัวเกี่ยวกับสัตว์เลี้ยงของข้าพเจ้าเป็นความลับ จะเปิดเผยเฉพาะสรุปผลการวิจัยเท่านั้น นอกจากนี้การเปิดเผยข้อมูลเกี่ยวกับสัตว์เลี้ยงของข้าพเจ้าต่อ หน่วยงานต่างๆที่เกี่ยวข้องต้องได้รับอนุญาตจากข้าพเจ้าและจะกระทำได้เฉพาะกรณีจำเป็นด้วยเหตุผลทางวิชาการเท่านั้น

ข้อ 4. ข้าพเจ้า ได้รับทราบแล้วว่าข้าพเจ้ามีสิทธิ์จะบอกเลิกการร่วมโครงการวิจัยนี้ และการบอกเลิกการร่วมโครงการวิจัยจะไม่มีผลกระทบต่อการดูแลรักษาโรคของสัตว์เลี้ยงของข้าพเจ้า

ข้อ 5. ข้าพเจ้า ได้อ่านและเข้าใจข้อความข้างต้นแล้ว จึงได้ลงนามในใบยินยอมนี้ด้วยความสมัครใจ โดยมิได้มีการบังคับขู่เข็ญ หลอกลวงแต่ประการใด และจะให้ความร่วมมือในการวิจัยทุกประการ

ลงนาม.....ผู้ยินยอม
(.....)

ลงนาม.....พยาน
(.....)

ลงนาม.....ผู้ทำวิจัย
(.....)

Appendix 2 Modified questionnaire Canine Brief Pain Inventory (CBPL) by University of Pennsylvania which created by Dr. Dorothy Cimino Brown in Thai version

แบบประเมินสัตว์ป่วย ฉบับภาษาไทย

Modified from Canine Brief Pain Inventory (CBPI)

- **ประเมินความเจ็บปวดของสัตว์เลี้ยง**

  ให้คะแนนความเจ็บปวดของสุนัขโดยกา X ในช่องสี่เหลี่ยมที่ตัวเลข เพียงตัวเลขเดียว ลำดับจากไม่เจ็บปวด ถึง เจ็บปวดมาก (0 = ไม่เจ็บปวด และ 10 = เจ็บปวดมาก)

1. **ภายใน7วันที่ผ่านมา ช่วงที่มีอาการเจ็บปวดมากที่สุด สัตว์เลี้ยงของท่านมีระดับความเจ็บปวดมากที่สุด กี่คะแนน**

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

ไม่เจ็บปวด เจ็บปวดมาก

2. **ภายใน7วันที่ผ่านมา ช่วงที่มีอาการเจ็บปวดน้อยที่สุด สัตว์เลี้ยงของท่านมีระดับความเจ็บปวดน้อยที่สุด กี่คะแนน**

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

ไม่เจ็บปวด เจ็บปวดมาก

3. **ภายใน7วันที่ผ่านมา สัตว์เลี้ยงของท่านมีระดับความเจ็บปวดเฉลี่ย กี่คะแนน**

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

ไม่เจ็บปวด เจ็บปวดมาก

4. **ณ เวลานี้ สัตว์เลี้ยงของท่านมีระดับความเจ็บปวด กี่คะแนน**

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

ไม่เจ็บปวด เจ็บปวดมาก

- **ประเมินการใช้ชีวิตขณะอยู่บ้าน**

  ภายใน7วันที่ผ่านมา ความเจ็บปวดมีผลรบกวนการใช้ชีวิตของสัตว์เลี้ยงของท่านอย่างไร

  ให้คะแนนผลของการรบกวนการใช้ชีวิตจากความเจ็บปวดของสัตว์เลี้ยงโดยกา X ในช่องสี่เหลี่ยมที่ตัวเลข เพียงตัวเลขเดียว ลำดับจากไม่รบกวน ถึง รบกวนการใช้ชีวิตมาก (0 = ไม่รบกวน และ 10 = รบกวนการใช้ชีวิตมาก)

5. **กิจกรรมทั่วไป เช่น กิน นอน ขับถ่าย ความเจ็บปวดรบกวนสิ่งเหล่านี้ กี่คะแนน**

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

ไม่รบกวน รบกวนมาก

6. **ความสนุกสนานในชีวิต เช่น การเล่นกับเจ้าของ เล่นกับของเล่น เล่นกับตัวอื่นที่บ้าน ความเจ็บปวดรบกวนสิ่งเหล่านี้ กี่คะแนน**

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

ไม่รบกวน รบกวนมาก

7. **ความสามารถในการลุกยืน จากการนอนราบบนพื้น ความเจ็บปวดรบกวนสิ่งเหล่านี้ กี่คะแนน**

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

ไม่รบกวน รบกวนมาก

8. **ความสามารถในการเดิน ความเจ็บปวดรบกวนสิ่งเหล่านี้ กี่คะแนน**

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

ไม่รบกวน รบกวนมาก

9. **ความสามารถในการวิ่ง ความเจ็บปวดรบกวนสิ่งเหล่านี้ กี่คะแนน**

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

ไม่รบกวน รบกวนมาก

10. **ความสามารถในการปีนป่าย เช่น การขึ้นบันได ความเจ็บปวดรบกวนสิ่งเหล่านี้ กี่คะแนน**

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

ไม่รบกวน รบกวนมาก

- **ความคิดเห็นโดยรวม. ในขณะนี้คุณภาพชีวิตของสัตว์เลี้ยงของท่าน เป็นอย่างไร**

☐ แย่มาก ☐ เฉยๆ ☐ ปานกลาง ☐ ดี ☐ ดีมาก

Appendix 3 Modified questionnaire Liverpool Osteoarthritis in dogs (LOAD) by University of Liverpool in Thai version

แบบสอบถามเจ้าของสำหรับสุนัขที่มีปัญหาการเคลื่อนไหว (ฉบับภาษาไทย)
Modified from Liverpool Osteoarthritis in dogs (LOAD) (ต่อ)

ข้อมูลพื้นฐาน

1. **สุนัขของคุณมีปัญหาในการเคลื่อนไหวมานานเท่าไหร่?**

☐ น้อยกว่า 6 เดือน ☐ 6-12 เดือน ☐ 12-24 เดือน ☐ 24-36 เดือน ☐ มากกว่า 36 เดือน

2. **สุนัขของคุณได้รับการวินิจฉัยว่ามีปัญหาอื่นนอกเหนือจากโรคทางกระดูกหรือไม่?**

☐ ไม่ ☐ ใช่ โปรดระบุ

3. **โปรดระบุรายชื่อยาที่สุนัขของคุณได้รับตั้งแต่ได้รับมา (เท่าที่สามารถระบุได้)**

ข้อมูลการใช้ชีวิตทั่วไป

1. **ใน 1 สัปดาห์ที่ผ่านมา สุนัขของคุณมีระยะทางการเดินประมาณเท่าไหร่ต่อวันโดยเฉลี่ย?**

☐ 0-1 กิโลเมตร ☐ 1-2 กิโลเมตร ☐ 2-3 กิโลเมตร ☐ 3-4 กิโลเมตร ☐ มากกว่า 4 กิโลเมตร

2. **ใน 1 สัปดาห์ที่ผ่านมา สุนัขของคุณมีการเดินกี่ครั้งต่อวันโดยเฉลี่ย?**

☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ มากกว่า 4

3. **ประเภทการออกกำลังกายของสุนัขคุณคืออะไร?**

☐ อยู่กับสายจูงตลอดเวลา ☐ อยู่กับสายจูงโดยส่วนมาก ☐ ถอดสายจูงโดยส่วนมาก ☐ ถอดสายจูงตลอดเวลา

4. **วันใดใน 1 สัปดาห์ที่สุนัขของคุณมีการออกกำลังกายมากกว่าปกติ? (ตอบได้มากกว่า 1 ข้อ)**

☐ จันทร์ ☐ อังคาร ☐ พุธ ☐ พฤหัสบดี ☐ ศุกร์ ☐ เสาร์ ☐ อาทิตย์

5. พื้นผิวประเภทใดที่สุนัขของคุณใช้ในการออกกำลัง?

☐ สนามหญ้า ☐ พื้นดิน ☐ พื้นถนน ☐ พื้นผิวขรุขระ

6. เวลาสุนัขออกกำลังมีการควบคุมอย่างไร?

☐ อยู่กับสายจูง ☐ ถอดสายจูง ☐ วิ่งเหยาะๆ ด้วยสายจูง ☐ ปล่อยวิ่งอิสระ

7. ใครเป็นตัวกำหนดการออกกำลังกายของสุนัข?

☐ เจ้าของ ☐ ตัวสุนัข

ข้อมูลเรื่องการเคลื่อนไหว

<u>การใช้ชีวิตทั่วไป</u>

1. สุนัขของคุณมีการเคลื่อนไหวอย่างไรในสภาวะปกติ?

☐ ดีมาก ☐ ดี ☐ ปานกลาง ☐ แย่ ☐ แย่มาก

2. อาการเจ็บขาของสุนัขมีผลกระทบต่อการเคลื่อนไหวมากน้อยแค่ไหน?

☐ ไม่มีเลย ☐ มีเล็กน้อย ☐ มีปานกลาง ☐ มีผลกระทบมาก ☐ มีผลกระทบมากที่สุด

3. สุนัขของคุณมีความคล่องแคล่วแค่ไหน?

☐ คล่องแคล่วมากที่สุด ☐ คล่องแคล่วมาก ☐ คล่องแคล่วปานกลาง ☐ คล่องแคล่วน้อย ☐ ไม่คล่องแคล่ว

4. สภาพอากาศมีผลต่ออาการเจ็บขาของสุนัขของคุณมากน้อยแค่ไหน?

☐ ไม่มีเลย ☐ มีเล็กน้อย ☐ มีปานกลาง ☐ มีผลกระทบมาก ☐ มีผลกระทบมากที่สุด

5. สุนัขของคุณมีระดับความตึงของข้อต่อมากแค่ไหนหลังจากนอนลงบนพื้น?

☐ ไม่เลย ☐ เล็กน้อย ☐ ปานกลาง ☐ มาก ☐ มากที่สุด

ขณะออกกำลังกาย

6. สุนัขของคุณมีความคล่องแคล่วมากน้อยแค่ไหนขณะออกกำลัง?

☐	☐	☐	☐	☐
คล่องแคล่วมากที่สุด	คล่องแคล่วมาก	คล่องแคล่วปานกลาง	คล่องแคล่วน้อย	ไม่คล่องแคล่ว

7. สุนัขของคุณมีความกระตือรือร้นมากน้อยแค่ไหนขณะออกกำลัง?

☐	☐	☐	☐	☐
กระตือรือร้นมากที่สุด	กระตือรือร้นมาก	กระตือรือร้นปานกลาง	กระตือรือร้นน้อย	กระตือรือร้นน้อยที่สุด

8. คุณให้คะแนนสุนัขของคุณในเรื่องความสามารถในการออกกำลังกายมากน้อยแค่ไหน?

☐	☐	☐	☐	☐
ดีมาก	ดี	ปานกลาง	แย่	แย่มาก

9. การออกกำลังกายมีผลต่อภาวะเจ็บขาของสุนัขของคุณมากน้อยแค่ไหน?

☐	☐	☐	☐	☐
ไม่มีเลย	มีเล็กน้อย	มีปานกลาง	มีผลกระทบมาก	มีผลกระทบมากที่สุด

10. สุนัขของคุณมีความถี่ในการพัก (หยุดหรือนั่งพัก) ขณะออกกำลังกายมากน้อยแค่ไหน?

☐	☐	☐	☐	☐
ไม่มีเลย	มีเล็กน้อย	มีปานกลาง	ค่อนข้างบ่อย	บ่อยมาก

11. สภาพอากาศมีผลต่อการออกกำลังกายของสุนัขของคุณมากน้อยแค่ไหน?

☐	☐	☐	☐	☐
ไม่มีเลย	มีเล็กน้อย	มีปานกลาง	มีผลกระทบมาก	มีผลกระทบมากที่สุด

12. สุนัขของคุณแสดงอาการตึงขาหลังจากออกกำลังมากน้อยแค่ไหน?

☐	☐	☐	☐	☐
ไม่เลย	เล็กน้อย	ปานกลาง	มาก	มากที่สุด

13. ภาวะเจ็บขามีผลกระทบต่อการออกกำลังกายของสุนัขของคุณมากน้อยแค่ไหน?

☐	☐	☐	☐	☐
ไม่มีเลย	มีเล็กน้อย	มีปานกลาง	มีผลกระทบมาก	มีผลกระทบมากที่สุด

Appendix 4 Demographic data of dogs in phase 1

	Dog	Breed	Sex	Age (month)	Weight (Kg.)	BCS	Hydration status(%)
Phase 1 group (N = 6)	1	Alaskan Malamute	Male	23.4	35.8	3	< 5
	2	Mixed breed	Female	14.1	20.6	3	< 5
	3	Siberian Husky	Male	13.2	29.3	3.5	< 5
	4	Rottweiler	Male	34.3	43.2	3	< 5
	5	Labrador	Female	10.2	43.7	3.5	< 5
	6	Siberian Husky	Male	72	23.4	3	< 5

Appendix 5 Demographic data of dogs in phase 2

	Dog	Breed	Sex	Age (month)	Weight (Kg.)	BCS	Hydration status(%)
Phase 2 group (N = 9)	1	Golden Retriever	Male	64.8	38.2	3	< 5
	2	Golden Retriever	Male	54	42.3	3.5	< 5
	3	Chow Chow	Female	25.2	18.5	3	< 5
	4	Golden Retriever	Male	67.2	38	3.5	< 5
	5	Shetland sheepdog	Female	49.2	14.8	3	< 5
	6	Golden Retriever	Male	72	50.5	4.5	< 5
	7	Alaskan Malamute	Male	24	48.4	3.5	< 5
	8	Golden Retriever	Male	15.6	33	3.5	< 5
	9	Golden Retriever	Male	18	42.6	3.5	< 5

Appendix 6 Blood profile of Dogs in phase 1 and 2

Parameter	Normal range	Dogs in phase 1 (N = 6)	Dogs in phase 2 (N = 9)
RBC ($10^6/\mu L$)	4.8 - 9.3	6.74±0.68	6.69±0.86
Hemoglobin (g/dl)	11.9 – 18.9	13.68±1.24	14.37±1.67
Hematocrit (%)	35 - 57	40.3±3.7	40.11±2.02
Platelet ($x10^3/\mu L$)	200 - 500	230.66±38.47	274.9±69.97
WBC ($x10^3/\mu L$)	6.00 – 17.00	9.89±3.03	11.19±2.41
Neutrophils ($x10^3/\mu L$)	58 – 85	75±2.82	77.5±2.91
Eosinophils ($x10^3/\mu L$)	0 - 9	2.16±1.47	1.7±1.16
Basophils ($x10^3/\mu L$)	0 – 1	0	0
Lymphocytes ($x10^3/\mu L$)	8 – 21	19.33±1.03	17.7±2.11
Monocytes ($x10^3/\mu L$)	2 – 10	2.5±0.54	3.8±1.87
BUN(mg/dl)	8 – 28	21.11±3.62	20.9±4.99
Creatinine(mg/dl)	0.5 – 1.7	1.12±0.25	1.3±0.23
ALT(SGPT)(U/L)	10 - 108	43.1±22.69	68±18.9
ALP(U/L)	10 - 150	43.33±13.18	62±22.91

Appendix 7 Data of hematocrit, white blood cell , platelet in whole blood and platelet in PRP of dogs in phase 2 (N = 10)

Parameter	Normal range	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6	Dog 7	Dog 8	Dog 9
RBC (10^6/μL)	4.8 - 9.3	7.6	5.26	5.3	6.32	7.5	6.98	6.93	7.45	6.34
Hemoglobin (g/dl)	11.9 – 18.9	14.4	11.8	12.4	13.1	15.6	14.2	15.2	16.5	13.7
Hematocrit (%)	35 - 57	36.8	40.2	37.4	39.7	42.5	40.8	39.9	41.2	39.3
Platelet (x10^3/μL)	200 - 500	262	181	332	216	304	211	421	310	264
WBC (x10^3/μL)	6.00 – 17.00	8.67	10.9	13.21	14.52	11.3	12.5	7.86	8.43	10.27
Neutrophils (x10^3/μL)	58 – 85	72	76	82	78	78	79	80	77	79
Eosinophils (x10^3/μL)	0 - 9	0	1	3	2	1	2	3	3	2
Basophils (x10^3/μL)	0 – 1	0	0	0	0	0	0	0	0	0
Lymphocytes (x10^3/μL)	8 – 21	14	19	20	18	17	20	19	19	16
Monocytes (x10^3/μL)	2 – 10	2	1	4	6	7	3	4	4	2
Platelet in PRP(x10^3/μL)	200 - 500	922	1080	956	938	982	938	1280	1462	998
Times (PLT in PRP compared to PLT in baseline)		3.52	5.97	2.88	4.34	3.96	4.45	4.21	4.72	3.78
TP (g%)	5.8 – 7.9	6.4	7	6.8	7.1	6.5	6.2	7.2	7	6.9
Bacterial identification/ sensitivity test of PRP		No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth

Appendix 8 Average of Maximum extension angle (MEA) and Maximum flexion angle (MFA) of coxofemoral joint in OA dogs in Day 0 and 8 weeks

Dog	MEA of left hip		MFA of left hip		MEA of right hip		MFA of right hip	
	D0	8 wks	D0	8 wks	D0	8 wks	D0	8 wks
1	126.2 ± 4.49	76.4 ± 7.82	97.5 ± 3.27	63.1 ± 7.05	76.4 ± 7.82	133.9 ± 3.51	63.1 ± 7.05	108.7 ± 3.92
2	120.9 ± 5.49	102.4 ± 3.6	99.6 ± 2.37	86.2 ± 3.16	102.4 ± 3.6	137.3 ± 2.31	86.2 ± 3.16	117.4 ± 1.51
3	101.9 ± 2.6	139.7 ± 3.8	80.4 ± 4.01	108.9 ± 2.23	139.7 ± 3.8	126.3 ± 4.42	108.9 ± 2.23	99.4 ± 3.17
4	127.6 ± 3.86	120.6 ± 3.75	108.2 ± 2.66	104.4 ± 2.95	120.6 ± 3.75	155.8 ± 3.97	104.4 ± 2.95	129.9 ± 3.11
5	119.1 ± 3.25	111.3 ± 3.27	89.4 ± 4.45	82.3 ± 3.23	111.3 ± 3.27	135.3 ± 2.11	82.3 ± 3.23	106.8 ± 1.69
6	115.3 ± 1.89	107.5 ± 3.6	100.9 ± 4.84	91.8 ± 1.69	107.5 ± 3.6	112.6 ± 4.77	91.8 ± 1.69	99 ± 4.37
7	116.9 ± 3.87	125.2 ± 13.68	103 ± 2.16	109.9 ± 5.2	125.2 ± 13.68	149.6 ± 5.56	109.9 ± 5.2	137.5 ± 2.92
8	96.7 ± 13.59	106.7 ± 11.34	86.8 ± 11.11	97 ± 6.82	106.7 ± 11.34	96.9 ± 3.35	97 ± 6.82	94.6 ± 3.03
9	138.2 ± 8.79	123.5 ± 3.27	113.8 ± 10.82	99.7 ± 3.71	123.5 ± 3.27	120.5 ± 13.21	99.7 ± 3.71	97.4 ± 5.42

Appendix 9 Average of Maximum extension angle (MEA) and Maximum flexion angle (MFA) of stifle joint in OA dogs in Day 0 and 8 weeks

Dog	MEA of left stifle		MFA of left stifle		MEA of right stifle		MFA of right stifle	
	D0	8 wks	D0	8 wks	D0	8 wks	D0	8 wks
1	147.1 ± 3.78	139.3 ± 5.7	99.2 ± 6.01	94.3 ± 9.43	140.4 ± 4.25	120.4 ± 6.69	98.1 ± 7.55	84.3 ± 8.35
2	138.8 ± 4.34	135.8 ± 6.76	110.6 ± 1.78	108.7 ± 5.4	132.8 ± 3.94	129.5 ± 4.77	88.2 ± 4.37	93.9 ± 3.9
3	159.4 ± 4.99	157.5 ± 6.8	130.1 ± 5.43	128.6 ± 3.1	151 ± 6.73	143.7 ± 4.97	116.2 ± 6.07	112.6 ± 8.41
4	145.2 ± 6.66	140.2 ± 5.22	107.3 ± 10.48	99.3 ± 4.99	144.3 ± 7.01	125 ± 8.98	107.4 ± 11.19	101.9 ± 7.71
5	141 ± 4.69	136.4 ± 8.54	107.5 ± 7.76	105.7 ± 11.37	146.1 ± 7.31	132.6 ± 11.65	91.7 ± 30.31	98.7 ± 10.26
6	132.5 ± 1.9	128 ± 8.62	107.5 ± 5.89	103.7 ± 6.04	123.3 ± 7.85	119.8 ± 9.81	95.5 ± 4.03	93.8 ± 5.22
7	157.6 ± 8.4	139.5 ± 9.16	92.9 ± 4.04	88.8 ± 5.71	154.9 ± 9.06	143.4 ± 14.74	110.6 ± 7.18	97.1 ± 10.32
8	131.1 ± 7.53	149.6 ± 3.47	88.7 ± 5.06	96.4 ± 3.2	131.3 ± 14.03	141.5 ± 5.68	95.5 ± 14.14	106.4 ± 10.51
9	153.9 ± 5.02	143.9 ± 5.13	115.5 ± 4.14	115 ± 5.5	137 ± 4.94	149.4 ± 3.13	95.1 ± 10.18	114.2 ± 4.87

Appendix 10 Average of Maximum extension angle (MEA) and Maximum flexion angle (MFA) of tarsal joint in OA dogs in Day 0 and 8 weeks

Dog	MEA of left stifle		MFA of left stifle		MEA of right stifle		MFA of right stifle	
	D0	8 wks	D0	8 wks	D0	8 wks	D0	8 wks
1	147.1 ± 3.78	139.3 ± 5.7	99.2 ± 6.01	94.3 ± 9.43	140.4 ± 4.25	120.4 ± 6.69	98.1 ± 7.55	84.3 ± 8.35
2	138.8 ± 4.34	135.8 ± 6.76	110.6 ± 1.78	108.7 ± 5.4	132.8 ± 3.94	129.5 ± 4.77	88.2 ± 4.37	93.9 ± 3.9
3	159.4 ± 4.99	157.5 ± 6.8	130.1 ± 5.43	128.6 ± 3.1	151 ± 6.73	143.7 ± 4.97	116.2 ± 6.07	112.6 ± 8.41
4	145.2 ± 6.66	140.2 ± 5.22	107.3 ± 10.48	99.3 ± 4.99	144.3 ± 7.01	125 ± 8.98	107.4 ± 11.19	101.9 ± 7.71
5	141 ± 4.69	136.4 ± 8.54	107.5 ± 7.76	105.7 ± 11.37	146.1 ± 7.31	132.6 ± 11.65	91.7 ± 30.31	98.7 ± 10.26
6	132.5 ± 1.9	128 ± 8.62	107.5 ± 5.89	103.7 ± 6.04	123.3 ± 7.85	119.8 ± 9.81	95.5 ± 4.03	93.8 ± 5.22
7	157.6 ± 8.4	139.5 ± 9.16	92.9 ± 4.04	88.8 ± 5.71	154.9 ± 9.06	143.4 ± 14.74	110.6 ± 7.18	97.1 ± 10.32
8	131.1 ± 7.53	149.6 ± 3.47	88.7 ± 5.06	96.4 ± 3.2	131.3 ± 14.03	141.5 ± 5.68	95.5 ± 14.14	106.4 ± 10.51
9	153.9 ± 5.02	143.9 ± 5.13	115.5 ± 4.14	115 ± 5.5	137 ± 4.94	149.4 ± 3.13	95.1 ± 10.18	114.2 ± 4.87

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