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Evaluating the Therapeutic Impact of Autologous Platelet-Rich Plasma (PRP) on Long Bone Fracture Healing in Cats*

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Abstract: This study evaluated the clinical and radiographic effects of platelet-rich plasma (PRP) on the healing of long bone fractures in cats. It featured a control group (n= 11) and a PRP group (n= 11). In the control group, only osteosynthesis was performed, whereas the PRP group received a combination of PRP and a gelatin sponge applied to the osteosynthesis site. Clinical and radiographic evaluations were conducted on days 0 (preoperative), 15 and 30 (postoperative). Clinical evaluation included lameness scoring, while radiographic assessment focused on fracture healing according to the Hammer scale, fracture reduction, intramedullary pin positioning, and callus formation. A significant reduction in lameness scores was observed on the 15th and 30th days in both groups. Although no statistically significant differences were detected between the groups on days 15, and 30, the PRP Group demonstrated lower scores. Based on the evaluations performed using the Hammer scale, no statistically significant differences were observed between the groups in callus formation and fracture line scores at the postoperative time points of days 15 and 30. However, upon examining the union score, the difference observed between the two time periods was found to be statistically significant, with a more pronounced effect in the PRP group on day 15. In conclusion, no complications were observed during the study. While PRP did not significantly alter the overall clinical healing process of long bone diaphyseal fractures in cats, it was found to accelerate early healing and reduce lameness.
Keywords: Callus, feline, growth factor, intramedullary pinning, platelets

Kedilerde Uzun Kemik Kırıklarının İyileşmesinde Otolog Trombositten Zengin Plazmanın (PRP) Terapötik Etkisinin Değerlendirilmesi

Öz: Bu çalışmada, kedilerde uzun kemik kırıklarının iyileşmesi üzerine trombositten zengin plazmanın (PRP) klinik ve radyografik etkileri değerlendirildi. Çalışma iki gruptan oluştu: kontrol grubu (n= 11) ve PRP grubu (n= 11). Kontrol grubunda sadece osteosentez yapılırken, PRP grubuna osteosentez bölgesine jelatin sünger ile kombine edilen PRP uygulandı. Klinik ve radyografik değerlendirmeler 0. (preoperatif), 15. ve 30. (postoperatif) günlerde yapıldı. Klinik değerlendirme topallık skorlamasını içerirken, radyografik değerlendirme Hammer skalasına göre kırık iyileşmesi, kırık redüksiyonu, intramedüller pin konumlandırması ve kallus oluşumuna odaklandı. Her iki grupta da 15. ve 30. günlerde topallık skorlamasında anlamlı bir azalma gözlendi. 15. ve 30. günlerde gruplar arasında istatistiksel olarak anlamlı bir fark saptanmasa da, PRP grubunda daha düşük skorlama elde edildi. Gruplar arasında postoperatif 15. ve 30. gün zaman noktalarında kallus oluşumu ve kırık hattı skorlarında istatistiksel olarak anlamlı bir fark gözlenmedi. Ancak, kaynama skoru incelendiğinde, iki zaman dilimi arasında gözlenen farkın istatistiksel olarak anlamlı olduğu, PRP grubunda 15. günde daha belirgin bir etki olduğu bulundu. Sonuç olarak, çalışma sırasında herhangi bir komplikasyon gözlenmedi. PRP'nin, kedilerde uzun kemik diafiz kırıklarının genel klinik iyileşme sürecini önemli ölçüde değiştirmezken, erken dönemde, yangı fazında iyileşmeyi hızlandırdığı ve topallamayı azalttığı görüldü.
Anahtar kelimeler: Büyüme faktörü, felin, intramedullar pin, kallus, trombosit

Introduction

Fracture healing is a complex and prolonged process influenced by various factors, including species-specific differences, age, sex, and medical and environmental conditions. To reduce the risk of complications such as non-union or delayed union in bone healing, treatment strategies should focus on enhancing and optimizing the healing process. Providing favourable conditions are established, bone tissue with disrupted integrity has the capacity for complete regeneration through the interplay between biomechanical and biological processes. These conditions may range from simple splint immobilization to fixation using implants such as intramedullary pins, plates and screws. In recent years, research has focused on exploring and utilizing materials with oste-

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ogenic and osteoinductive properties to enhance the biological environment of bone tissue (Giannoudis et al., 2007; Van Lieshout and Den Hartog, 2021).

Platelet-rich plasma (PRP) and its derivatives are widely utilized in various fields of veterinary medicine, particularly in the context of bone fracture healing, and remain an active area of investigation across diverse therapeutic models.

In the process of bone regeneration during fracture healing, particularly in the remodelling phase, the activity and proliferation of bone cells, alongside growth factors, play a crucial role in enhancing vascularization, which is critical for effective bone healing (Li et al., 2007). Platelet-derived growth factor, which facilitates the reconstruction and local angiogenesis of bone tissue, insulin-like growth factors, which are critical for bone mass acquisition, callus formation, and direct defect repair during the developmental period (Yuan et al., 2021; Reible et al., 2018), and transforming growth factor-beta, which promotes cartilage formation and contributes to callus development (Sarahrudi et al., 2011), represent some of the key growth factors present in platelet-rich plasma. Numerous clinical and experimental studies have demonstrated the efficacy of plasma-rich growth factors and PRP in promoting cartilage and bone healing in animals (Kasten et al., 2008; Thor et al., 2013).

The objective of this study is to clinically and radiographically evaluate the short-term effects of platelet-rich plasma on fracture healing in diaphyseal fractures of feline extremities, utilizing an absorbable gelatin sponge as a delivery material.

Materials and Methods

Ethical approval for the study was obtained from the Animal Experiments Local Ethics Committee of Ankara University (Approval no: 2020-5-34). Moreover, the owners of the animals were adequately informed about the study, and their consent was obtained prior to their participation in the research.

Animals

The study included 22 cats, aged between 1 and 6 years, that were admitted to Ankara University Faculty of Veterinary Medicine Animal Hospital with single-piece fractures along the diaphyseal region of the femur, humerus, or tibia. A detailed anamnesis obtained from the owners indicated that all cases had a history of trauma, such as falls from height or traffic accidents. Only cats with no prior trauma to the same region and no underlying conditions affecting bone metabolism were included in the study

The cats included in the study were randomly divided into two equal groups: The control group (n=11), which received a saline solution to the fracture site following surgery, and the PRP group (n=11), which received PRP-gelatin sponge application to the fracture site following surgery.

PRP Preparation

Approximately 3 mL of peripheral venous blood was aseptically collected from cats and transferred into citrate anticoagulant tubes (9nc 3.2%) (Oryan et al., 2016; Toyoda et al., 2018) and centrifuged (300g/5 minutes, Sirena, AFI Group). The blood sublayer was removed, and the platelet-rich fraction was subjected to a second centrifugation at 700g for 17 minutes. Following this step, the supernatant platelet-poor plasma (PPP) fraction was discarded. The resulting extract, enriched with PRP and growth factors, was carefully collected using a pipette. To activate the platelets, 0.1% calcium chloride was added prior to application, rendering the preparation suitable for local use at the fracture site (Oryan et al., 2016; Singh et al., 2017; Malhotra et al., 2014; Toyoda et al., 2018) (Figure 1).

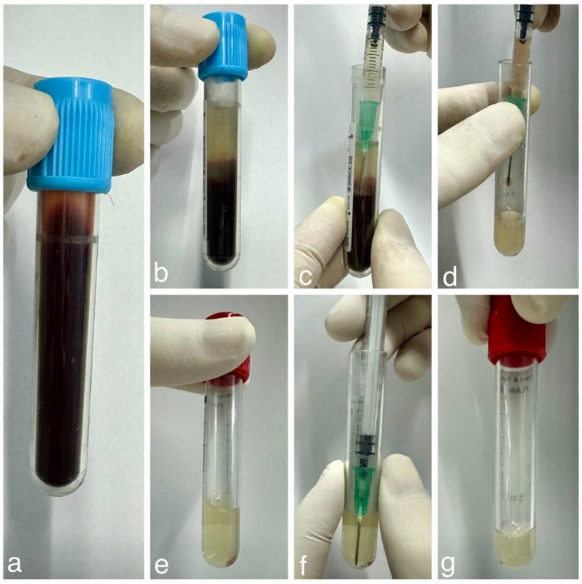

Figure 1. PRP preparation stages. **a.** Collection of blood from v. cephalica in a citrated tube **b.** The appearance of the platelet-rich and platelet-poor plasma mixture after the first centrifugation process (300g x 5min) **c.** Collection of the mixture with the help of a cannula **d.** The collected mixture is transferred to a new tube **e.** Appearance after the second centrifugation process **f.** Collection of PRP and PPP with the help of a cannula **g.** Appearance of the PRP ready for application in a sterile tube.

Operation Method and Application of PRP to the Fracture Area

General anaesthesia was provided with medetomidine HCl (80 µg/kg, intramuscularly, Domitor®, Finland) and ketamine HCl (5 mg/kg, intramuscularly, Keta-Control®, Türkiye). Isoflurane (with 100% oxygen, Isoflurane®, USA) was used for anaesthesia maintenance. Butorphanol tartrate (0.1mg/kg, subcutaneously, Butomidor®, Austria) was administered for analgesia. Balanced electrolyte solutions (Ringer's lactate, 50 ml/kg/h, intravenously) and cefazolin (25 mg/kg, intravenously, Eqizolin®, Türkiye) were administered intraoperatively.

In all cases, fracture reduction was successfully achieved using the retrograde intramedullary pinning technique. A lateral surgical approach was employed for diaphyseal fractures of the femur and humerus, while a medial approach was selected for tibial fractures. Following precise anatomical alignment, fracture stabilization was performed using a standard surgical technique. Depending on the diameter of the medullary cavity, a Steinmann pin was inserted via the retrograde technique, ensuring that the implant occupied approximately 70-80% of the medullary canal's diameter. To prevent rotational instability of the fracture ends, a cerclage wire was additionally applied.

In the PRP group, following completion of the fixation procedure, a 1×1×1 cm absorbable gelatin sponge (Surgifoam, Johnson & Johnson Ethicon, USA) was placed over the fracture line and preoperatively prepared autologous PRP was applied to the site using a sterile syringe.

During the postoperative period, the affected limb was supported with a bandage for one week to minimize movement, with bandages replaced during follow-up visits. Additionally, activity restriction was enforced throughout the recovery period to facilitate optimal healing. All animals received amoxicillin-clavulanic acid (25 mg/kg, orally) for one week postoperatively. For analgesia, meloxicam HCl (0.3 mg/kg, subcutaneously) was administered for five days.

Clinical Examination

Clinical evaluations of the animals in both groups were conducted on the 0th, 15th and 30th days. In the clinical evaluation of the cases, lameness scoring was used.

Grade 1. Low-grade lameness. Lameness is barely visible or no lameness is apparent, but the cat lifts its foot while sitting (thoracic limbs) or cannot jump (pelvic limbs).

Grade 2. Moderate lameness. Lameness is clearly visible, but the limb is used for most steps.

Grade 3. High-grade lameness. The cat only touches its toes or does not bear any weight (Voss and Steffen, 2009).

Radiographic Examination

Radiographic assessments were performed on the 0th, 15th, and 30th days by two independent experts in a blinded evaluation. Direct radiographs were obtained in mediolateral and anteroposterior positions for radiographic evaluations (Figure 2). The assessment included the examination of fracture healing, fracture reduction, intramedullary pin position, and callus formation.

Figure 2. Preoperative and postoperative day 0 mediolateral and anteroposterior radiographic images of the long oblique diaphyseal humeral fracture in Case 13. The preoperative images depict the extent and orientation of the fracture, while the postoperative images demonstrate fracture alignment and stabilization following surgical intervention.

Day 0 radiographs were chosen to determine the accuracy of the postoperative surgical method; day 15 radiographs were chosen to reveal the differences in healing in the soft callus process; and day 30 radiographs were chosen to compare and evaluate the clinical healing process (Marsh and Li, 1999). Postoperative radiographic scoring was performed based on the scale developed by Hammer et al. (1985) (Table 1).

Table 1. Hammer scale (1985)

Grade	Callus formation	Fracture Line	Union
1	Homogeneous bone structure	Obscured/eliminated	Successful
2	Massive bone trabeculae crossing the fracture line	Barely visible	Successful
3	Visible bridging at the fracture site	Visible	Indeterminate
4	No evidence of bridging at the fracture site	Separate/distinct	Unsuccessful
5	No callus formation	Separate/distinct	Unsuccessful

Statistical Analysis

Descriptive statistics were calculated and presented as the median (min-max) or arithmetic mean ± standard deviation, depending on the data distribution. The Student's t-test was used to analyse the distribution of age across groups, while the chi-square test was employed to evaluate the frequency distribution of gender between groups. A robust single-factor repeated two-way analysis of variance was conducted to assess changes in callus formation, fracture line, union, and lameness scores between groups and over time. Model estimation utilized the bootstrap method, median estimator, and Mahalanobis distance criteria. Data analysis was performed using R software (Version 4.2.1; R Core Team, 2021) and the WRS2 package (Mair and Wilcox, 2020). A criterion of $p<0.05$ was used for all statistical comparisons.

Results

The study included a total of 22 cats, consisting of 7 females and 4 males for the control group, and 2 females and 9 males for the PRP group. The average age of the cases was determined to be 1.41±0.38 years for the control group, 1.09±0.3 years for the PRP group. When examining the distribution of fractures in the study, tibial fractures were the most common (68.2%), followed by femoral (18.8%) and humeral (13.63%) fractures (Table 2).

Table 2. Signalement, fracture localization and type, treatment for control and PRP groups.

Groups	Cats	Breeds	Age	Gender	Fracture localization	Fracture type
Control Group	1	Van cat	1	M	H	Long oblique diaphyseal
	2	Crossbreed	1.5	M	F	Diaphyseal spiral
	3	Crossbreed	1	M	T/F	Short oblique diaphyseal
	4	Crossbreed	2	Fe	T/F	Mid-diaphyseal transverse
	5	Crossbreed	1.5	M	T	Distal-third oblique diaphyseal
	6	Crossbreed	1.5	Fe	T/F	Proximal-third oblique diaphyseal
	7	Crossbreed	1	M	T/F	Distal-third oblique diaphyseal
	8	Crossbreed	2	M	T/F	Proximal-third diaphyseal oblique
	9	Crossbreed	1.5	M	T	Short oblique diaphyseal
	10	Crossbreed	1.5	M	T	Short oblique diaphyseal
	11	Crossbreed	1	M	F	Long oblique diaphyseal
PRP Group	12	Crossbreed	1	Fe	F	Mid-diaphyseal transverse
	13	Crossbreed	2	M	H	Long oblique diaphyseal
	14	Crossbreed	1	M	F	Proximal-third oblique diaphyseal
	15	Crossbreed	1	M	T/F	Short oblique diaphyseal
	16	Crossbreed	1	Fe	T	Short oblique diaphyseal
	17	Crossbreed	1	Fe	T/F	Distal-third oblique diaphyseal
	18	Crossbreed	1	Fe	H	Long oblique diaphyseal
	19	Crossbreed	1	M	T	Short oblique diaphyseal
	20	Crossbreed	1	Fe	T	Short oblique diaphyseal
	21	Crossbreed	1	Fe	T	Short oblique diaphyseal
	22	Crossbreed	1	Fe	T/F	Long oblique diaphyseal

T: Tibia, H: Humerus, F: Femur; M: Male, Fe: Female

Clinical Examination Findings

A significant reduction in lameness scores was observed within each group on days 15 and 30 compared to day 0 (Figure 3). Although no statistically significant differences were detected between the groups on days 15 and 30 (p= 0.579), the PRP group exhibited lower lameness scores (Table 3). Additionally, qualitative assessment of pain responses indicated that animals in the PRP group displayed lower responsiveness during both evaluation periods.

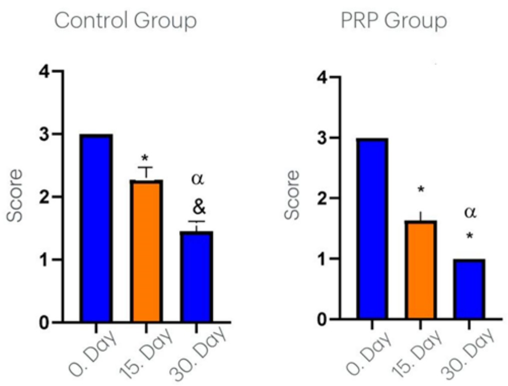

Figure 3. A significant reduction in lameness scores was observed on days 15 and 30 compared to day 0 within the group. A significant reduction in lameness scores was observed on the 15th and 30th days in both groups (Control group *p<0.01, α p<0.01, &p<0.0001; PRP group *p<0.0001, αp<0.0001).

Radiographic Examination Findings

Based on the evaluations performed using the Hammer scale, no statistically significant differences were observed between the groups in callus formation and fracture line scores at the postoperative time points of days 15 and 30 (p=0.509, p=0.155, respectively). However, upon examining the union score, the difference observed between the two time periods was found to be statistically significant, with a more pronounced effect in the PRP group on day 15 (p=0.007). When the scores for callus formation, fracture line, and union were examined within the groups, a statistically significant decrease was observed on both day 15 and day 30 compared to day 0 (p<0.001, p<0.012, p<0.001, respectively) (Table 3;Figure 4).

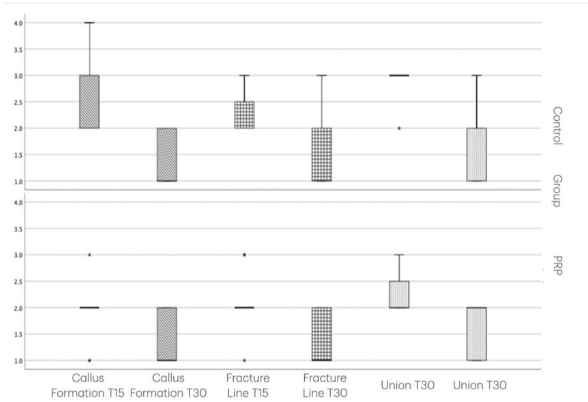

Figure 4. Radiographic evaluation of callus formation, fracture line visibility, and bone union in both groups on postoperative days 15 and 30. Comparative analysis illustrates the progression of healing and differences in callus development over time.

Table 3. Biostatistical analysis of lameness scoring, callus formation, fracture line and bone healing (union) score on the postoperative 15th and 30th days.

Lameness Score

		15th day	30th day	p		
Group	***n***	***Median (min-max)***	***Median (min-max)***	***Group***	***Time***	***Group*Time***
Control	11	2 (1 - 3)	1 (1 - 2)	0.579	<0.001	0.472
PRP	11	2 (1 - 2)	1 (1 - 1)			

Callus Formation

		15th day	30th day	p		
Group	***n***	***Median (min-max)***	***Median (min-max)***	***Group***	***Time***	***Group*Time***
Control	11	2 (2 - 4)	1 (1 - 2)	0.509	<0.001	0.462
PRP	11	2 (1 - 3)	1 (1 - 2)			

Fracture Line

		15th day	30th day	p		
Group	***n***	***Median (min-max)***	***Median (min-max)***	***Group***	***Time***	***Group*Time***
Control	11	2 (2 - 3)	2 (1 - 3)	0.155	0.012	0.182
PRP	11	2 (1 - 3)	1 (1 - 2)			

Bone Healing (Union) Score

		15th day	30th day	p		
Group	***n***	***Median (min-max)***	***Median (min-max)***	***Group***	***Time***	***Group*Time***
Control	11	3 (2 - 3)	2 (1 - 3)	0.007	0.028	0.058
PRP	11	2 (2 - 3)	2 (1 - 2)			

In all cases, anatomical reduction of the fracture site was successfully achieved on postoperative day 0, with the intramedullary pin properly positioned and appropriately sized. By postoperative day 15, the fracture edges had lost their sharpness and appeared rounded (Figure 5). A reduction in the gap between fracture fragments was observed, accompanied by clear evidence of periosteal callus formation surrounding the fracture site. By postoperative day 30, the fracture line had become indistinct, and the amount of callus tissue had increased, appearing more pronounced compared to the previous evaluation (Figure 6).

Figure 5. Postoperative day 15 mediolateral and anteroposterior radiographic images of diaphyseal oblique humeral fractures in Control and PRP Groups. Mediolateral (a) and anteroposterior (b) direct radiographic images of animal 1 in the control group on postoperative day 15 reveal a clearly defined fracture line, with a noticeable reduction in the sharpness of the fracture edges and the initiation of fibrous callus formation (white arrows). In contrast, mediolateral (c) and anteroposterior (d) radiographic images of animal 18 in the PRP group at the same time point demonstrate more advanced callus formation compared to the control group, with a marked reduction in the prominence of the fracture line (white arrows).

Figure 6. Postoperative day 30 mediolateral and anteroposterior radiographic images of diaphyseal oblique humeral fractures in Control and PRP Groups. Mediolateral (a) and anteroposterior (b) direct radiographic images of animal 1 in the control group on postoperative day 30 demonstrate a further reduction in the prominence of the fracture line compared to postoperative day 15, although it remains discernible (white arrows). Conversely, mediolateral (c) and anteroposterior (d) radiographic images of animal 18 in the PRP group at the same time point reveal that the fracture line has almost completely resolved (white arrows).

Discussion and Conclusion

A review of clinical and experimental studies indicates that PRP has been utilized in various fields, either alone or in combination with compatible materials (Zhang et al., 2018; Singh et al., 2017). In recent years, the use of autologous PRP as an alternative to promote fracture healing has gained increasing popularity. Although its definitive effects are still under investigation, platelet-derived growth factors in PRP have been shown to play a role in angiogenesis, osteoblast proliferation, mesenchymal cell activation, and chemotaxis during fracture healing (Garcia et al., 2012; Van Lieshout and Den Hartog, 2021). In this study, the effects of PRP were clinically and radiographically evaluated using scoring systems in diaphyseal fractures of feline extremities. PRP was found to be safe, cost-effective, and easy to apply, with satisfactory short-term outcomes in fracture healing. To the authors' knowledge, no clinical research has been published regarding the use of PRP in diaphyseal fractures of feline extremities.

Various techniques have been developed for the preparation and activation of PRP. Single or multiple centrifugation methods, cooled and uncooled centrifuge devices, and different anticoagulant agents can be employed to obtain PRP. Additionally, numerous commercial PRP preparation kits are available for researchers. Despite the variety of preparation methods, it is essential to emphasize that all procedures must adhere to aseptic and antiseptic principles. (Miguel-Pastor et al., 2022). In this study, sodium citrate was selected as the anticoagulant, and PRP was obtained through double centrifugation using a refrigerated centrifuge. Sodium citrate was chosen because it induces minimal to no alteration in platelet shape and volume, thereby preserving the normal discoid structure of platelets. Additionally, its low pH results in reduced pain at the application site (Oryan et al., 2016). Although double centrifugation increases processing time, it facilitates the separation of platelet-rich and platelet-poor plasma fractions from red blood cells and enhances platelet yield (Saqlain et al., 2023). As outlined in the study methodology, the lowest possible G-force was selected for both centrifugation steps. This choice was made primarily to preserve platelet membrane stability during centrifugation and to prevent premature platelet degranulation (Hanna et al., 2004). Consequently, the PRP preparation method used in this study was designed to optimize platelet yield.

PRP extract can be applied independently or in combination with various biomaterials, such as gelatin sponges, autografts, and allografts. Gelatin sponges are readily available, cost-effective compared to many other materials, biocompatible due to their non-toxic nature, and biodegradable, allowing them to gradually degrade in the body and facilitate the formation of new tissue. Their high porosity enables deep tissue penetration by cells and biomolecules (Xue et al., 2017). When used as a PRP carrier, gelatin sponges offer significant advantages, including accelerated wound healing, enhanced tissue regeneration, and increased cellular proliferation. The sponge facilitates the gradual and sustained release of growth factors from PRP, while simultaneously degrading within the body over time. This system provides an optimal method for the continuous release of growth factors and their efficient delivery to the site of injury (Rodriguez et al., 2014). Based on these properties, the application of PRP extract to the fracture line using a gelatin sponge was chosen in this study.

The effects of PRP on fracture healing in long bones remain a topic of debate regarding its contribution to healing speed. While some studies report that PRP has no significant impact on the healing process (Singh et al., 2017; Peerbooms et al., 2012; Ozak et al., 2010), others have demonstrated that PRP significantly accelerates fracture healing (Basdelioglu et al., 2020; Canbeyli et al., 2018). When examining the fracture healing process, three main phases can be identified: inflammation, repair, and remodelling. During the inflammation phase, platelets, along with growth factors, are transported to the site, initiating angiogenesis and cell proliferation, which are fundamental steps in the healing process (Haluzan et al., 2015). The initial two-week period, encompassing the inflammatory phase, represents the cellular basis of healing at the fracture site. The concentrated growth factors present in PRP can potentially stimulate early-stage healing (Singh et al., 2017; Maruyama et al., 2020). Upon reviewing the study data, it was observed that the preoperative and postoperative scores on days 15 and 30 showed significant positive changes in animals treated with PRP. Although a similar trend was noted in the control group, the PRP group exhibited greater radiographic consolidation, particularly during the two-week period corresponding to the inflammatory phase. Additionally, the statistically lower union score observed in the PRP group indicated that fracture line union was more successful during this period. By the 30th postoperative day-a key marker of clinical recovery-the effects of PRP continued to show positive progression. However, no significant difference was observed between the PRP and control groups in the earlier period. This finding suggests that PRP may offer more pronounced benefits in the early (cellular) phase of long bone fracture healing in cats. When lameness scoring, a key indicator of clinical recovery, was evaluated, no significant differences were detected between the two groups. However, the reduction in pain symptoms, particularly in the PRP group, was positively interpreted in terms of animal well-being. Although this improvement could not be demonstrated statistically, qualitative differences were noted based on the observed responses of the animals.

Various scoring systems are employed to evaluate fracture healing. Among these, Hammer scoring is widely preferred by researchers for radiographic assessments, as it allows for the separate evaluation of healing degrees in different extremities, fracture lines, callus formation, and the presence or absence of union (Hammer et al., 1985; Leow et al., 2016). In this study, Hammer scoring was applied during preoperative and postoperative radiographic evaluations. Clinical evaluation was supported by objective data obtained through lameness scoring. Due to the difficulty in observing more than a few steps at a time in cats, the 1-10 or 1-5 scale systems commonly used for dogs can be challenging to implement in this species. Therefore, based on previous studies, an alternative 1-3 grading scheme was utilized, as it proved to be more practical for clinical examinations (Voss and Steffen, 2009).

In conclusion, this study demonstrated that the application of an autologous PRP-gelatin sponge significantly enhanced healing during the first two weeks of radiographic evaluations, particularly during the inflammatory phase and soft callus stages of diaphyseal fractures in the extremities of cats. However, this significant difference diminished during the transition to the hard callus and remodelling phases, yielding results comparable to the control group. Although no statistically significant differences were observed in fracture healing as assessed by clinical lameness scoring, the more rapid resolution of pain in the PRP group could be considered a favourable outcome. Given the limited number of studies investigating the effects of PRP on bone healing in cats, future research incorporating various biomaterial combinations would help further elucidate its potential benefits.

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