



## Radiological Assessment of Critically Sized Femur Fracture Healing Using a Combination of Intramedullary Pin with Polymethylmethacrylate and Calcium Phosphate in Dog Model

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### ABSTRACT

This study aimed to investigate hypotheses related to critical-size bone defect reconstruction and compare the radiological parameters of Polymethylmethacrylate (PMMA) and calcium phosphate (CaP) bone materials. The research involved 36 healthy dogs divided into three groups (A, B, and C) with 12 animals in each group. Group A was treated with an intramedullary pin (IM-pin), group B was treated with PMMA in combination with an IM-pin, and group C was treated with CaP based bone graft material in combination with an IM-pin. The study assessed various endpoints at 14<sup>th</sup>, 28<sup>th</sup>, 45<sup>th</sup>, and 60<sup>th</sup> days post-surgery. The results indicate that regular radiological assessments were conducted in all three groups to evaluate fracture alignment and bone healing. On day 14<sup>th</sup> post-surgery, all groups showed appropriate fracture alignment, with varying degrees of gap reduction. Group A (IM pin only) had minimal gap reduction, while groups B (IM pin + PMMA) and C (CaP + IM pin) showed slight gap reduction. By day 28<sup>th</sup>, all groups demonstrated evidence of bone healing and callus formation, with groups B and C showing clearer callus formation in radiographs. In contrast, group A had considerable gap reduction due to increased immature callus formation and hyperplasia of surrounding tissues. On day 45<sup>th</sup>, substantial bone healing and callus formation were observed in all groups. Groups B and C exhibited prominent callus formation, indicating rapid healing and gap filling, while in group A, radiographs showed growth on both sides but an unfilled gap. By day 60<sup>th</sup>, fracture union was evident in groups B and C, while animals in group A treated with the IM pin only showed randomized luxation and an increased gap. It was concluded from the present study that radiographic results show the IM-Pin PMMA has proved to be the ideal implant material for femur bone fracture in dogs.

**Key words:** Femur, Fracture, Intramedullary pin, Polymethylmethacrylate, Calcium phosphate.

### INTRODUCTION

Fractures of long bones stand as a prominent challenge within orthopedics, with dogs experiencing a significant incidence of fractured bones. Studies by Wangchuk et al. (2021) reveal that 37.5% of reported long-bone fractures in dogs are attributed to this issue, marking a substantial concern in veterinary care. Additionally, fractures of the femur, a common occurrence in pets such as dogs and cats, constitute 20-25% of cases. Notably, the diaphysis of the femur

emerges as a focal point for fractures, particularly in dogs, where it accounts for a substantial 46%, as elucidated (Decamp et al. 2015; Alimov and Turaev 2023).

The clinical treatment of fractures due to bone defects is still a great challenge. Currently, orthopedic problems are treated with a variety of surgical and non-surgical techniques. Various options exist for addressing fractures, ranging from external to internal fixation. In extreme cases, considerations extend to cage rest and even amputation as last-resort choices. The appropriateness of fixation methods hinges on factors such as the fracture

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type, its severity, the available facilities, and the agreement of the pet owners (Muhammad et al. 2021; Foruria 2023). Intramedullary pins, CaP ceramics and PMMA are commonly used implants to treat bone fractures in veterinary patients (Zhu et al. 2017; Frydoni and Esmaeilnejad 2020; Singh et al. 2022).

However, fractures are diagnosed through radiography using at least two views exposure. In case of any anatomical doubt, it is suggested the expose of opposite radiograph for the proper comparison of both sides (Katsanos et al. 2011; Gaetke-Udager and Yablon 2019) to compare the diagnosis of the fracture, the line of radio-opacity indicates the break in the bone, whereas, the line of radiolucency indicates the fragments are distracted, furthermore, the line of radio-opacity indicates the fragments are overlapped or compressed (Olisemeke et al. 2014; Garcia et al. 2020).

Radiological analysis serves as the fundamental pillar for evaluating fracture union. While X-rays offer preliminary insights into the macrostructure, quantity, and integrity of the bone, they also indicate the extent of healing (Garnon et al. 2020). Furthermore, X-rays enable the visualization of periosteal reactions and provide valuable information on callus formation (Goyal et al. 2020; Zhao et al. 2021).

In the field of veterinary practices, veterinary radiology was initiated in 1886, when S. S. Lisovsky first introduced X-rays to show a dog. After this experiment lot of other researchers also used x-ray of different animals of different parts (Biduchak et al. 2019; Hoffmeyer et al. 2019).

It is suggested that radiographs should be taken before the repair of the fracture, post-surgery intervals of the radiographs are very helpful for proper monitoring of the patient's healing progress (Narrasingi 2019). Ideally, in every case, exposure settings and the position of the patient should be kept properly for all the x-ray films, this makes easier to compare the density of bone, position of the implantation and fracture healing (Paparoidamis et al. 2021). The intervals of follow-up radiographs are depending on the severity of the fracture, the age of the patient. However, radiographic films should be taken on a weekly basis or at intervals of every two, to three weeks, and follow-up radiographs of mature cases should be taken at intervals of every four to six weeks (Shah et al. 2020). Ultimately, in severe concerned cases, the radiographs should be taken at early intervals. Moreover, complications can be corrected sooner if they are detected earlier (Langley-Hobbs 2003; Steinmetz et al. 2020).

Key considerations regarding radiographic information and limitations encompass several aspects. Firstly, the potential of missing the fracture site arises when the X-ray beam is not positioned perpendicular to the gap. Secondly, the challenge exists in the early detection of incomplete or stress fractures (Tarrant and Balogh 2020). Lastly, limitations extend to insufficient knowledge about damage to cartilage and soft tissues. For a comprehensive assessment, radiographs should be scrutinized in front of the patient, considering the patient's case history and conducting a thorough physical examination, which includes an orthopedic evaluation. This approach proves beneficial for a holistic understanding of the case. Considering both present and

past studies, it is evident that radiology plays a significant role in the comprehensive assessment of fractures from various perspectives (Langley-Hobbs 2003).

The current study aims to find out the radiological assessment of the critically sized femur fracture treated with IM-Pin only and with the combination of PMMA and CaP bone material on different days.

## MATERIALS AND METHODS

### Ethical approval

The protocols applied in this study were approved Research Ethics Committee of the Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Pakistan, Approval No DAS-525-2022.

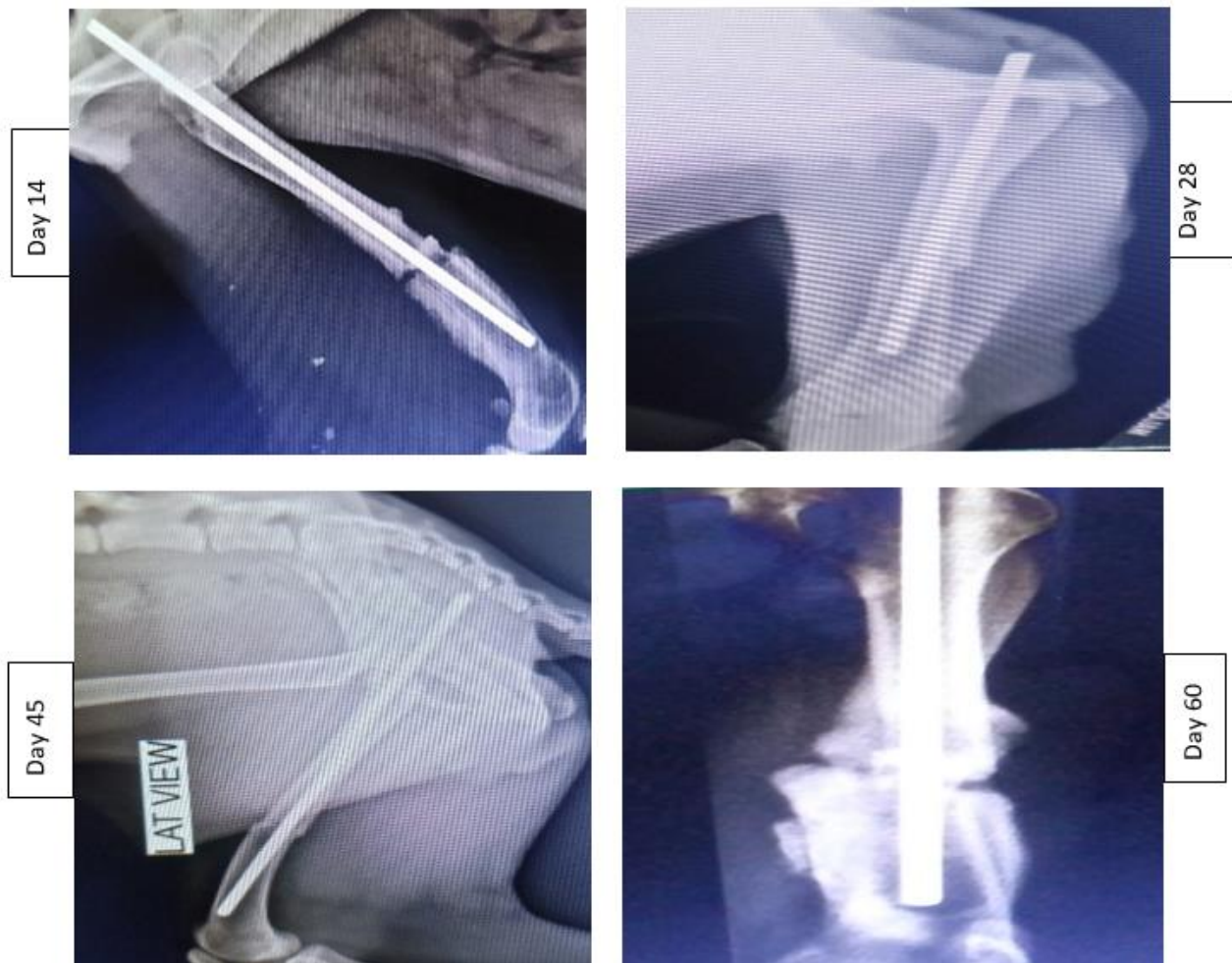
### Management of animals and experimental design

The radiological assessment was conducted on dogs to evaluate findings. The dogs were trapped two weeks earlier from the experiment as they can be aware of the environment. They were examined thoroughly before placing in the patient ward under standard environmental conditions (18 to 21°C, 30 to 70% humidity). Deworming of the dogs were done by using the tablet Caniverm 0.7g (Bioveta, Czech Republic) and vaccinated with Biocan-LR (Bioveta, Czech Republic) to remain protected against deadly diseases during research.

The study was made on open fracture management of the femur in the dog model using two different bone graft materials with the combination of intramedullary pin (IM-pin), the bone graft materials are PMMA, CP based bone graft material. The study was conducted on 36 healthy dogs and they were divided into three groups (A, B, and C), i.e., 12 dogs were kept in each group. In A group fractured dogs were managed with the intramedullary pin (IM-pin). In group B fractured dogs were managed by using PMMA with the metallic combination of intramedullary pin (IM-pin) and in group C fractured dogs were managed using CaP based bone graft material with the combination of intramedullary pin (IM-pin) (Fig. 1). However, to evaluate the results of the operated dogs, radiological assessment was taken after surgery on the 14<sup>th</sup>, 28<sup>th</sup>, 45<sup>th</sup> and 60<sup>th</sup> day (Table 1).

### Pre-operative preparation

Each dog was examined thoroughly before anesthesia and surgery. On the day of surgery, usual feed was withdrawn to avoid anesthetic complications which is helpful for speedy recovery after surgery. Experiments were conducted at the postgraduate research surgery room Department of Surgery and Obstetrics early in the morning. Intravenous catheter 20G×1 1/4 were placed into the cephalic vein and a T-connection canula was also attached to it for the induction of intravenous anesthesia and fluid therapy. Clipping of hair was performed using Oster hair clipper (Jarden Consumer Solution United States) blade no 40 at the site of the incision and the skin was aseptically prepared by using topical antiseptic povidone iodine solution. The drape sheet was put on at the operative site. Injection of Amoxycillin 5mg/kg was given preoperatively for the prevention from bacterial infection.



**Fig. 1:** Radiographs of Group A IM-Pin alignment.

**Table 1:** Experimental design (Radiological observations) in dogs

Groups	Animals	Technique	Radiological end points (Day)
A	12	Fracture fix with intramedullary pin (IM-pin).	14 <sup>th</sup> , 28 <sup>th</sup> , 45 <sup>th</sup> and 60 <sup>th</sup> .
B	12	Polymethylmethacrylate (PMMA) combination with intramedullary pin (IM-pin)	14 <sup>th</sup> , 28 <sup>th</sup> , 45 <sup>th</sup> and 60 <sup>th</sup> .
C	12	Calcium phosphate (CaP) combination with intramedullary pin (IM-pin).	14 <sup>th</sup> , 28 <sup>th</sup> , 45 <sup>th</sup> and 60 <sup>th</sup> .

### Pre-medication

All the dogs were off feed 8 to 12 hours before surgery. The dogs were premedicated 10 minutes before induction with Butorphanol 0.2mg/kg body weight intramuscularly, Atropine Sulphate 0.02 mg/kg body weight with combination of Xylazine 0.5mg/kg body weight.

### Anesthesia

The induction of anesthesia was performed by a combination of Ketamine Hydrochloride (HCL) 5mg/kg with Diazepam 0.25mg/kg intravenous, then endotracheal intubation was passed after loss of swallowing reflex and anesthesia was maintained with 1-2.5% Isoflurane mix with oxygen using small animal anesthesia machine (Surgi Vet Germany).

### Post-operative care

After closure and application of sterile dressing, the hind legs were hobbled proximal to the hock by using elastic veterinary wrap in a figure-eight pattern to prevent

limb abduction and formation of pressure ulcer. After surgery, the analgesic drugs were administered routinely to control pain buprenorphine (0.05mg/kg twice daily) for 3 days and antibiotic intramuscular Amoxicillin (15mg/kg once daily) for 10 days. Dogs were provided with a warm soft place after surgery as they can feel stressless and painless. Sutures were checked daily to sure wound healing, if in the case of chewing the E-collar was used for 3 to 5 days. After recovery from anesthesia maximum water was offered to dogs as they need much water post operatively, as a well-hydrated animal can recover quickly.

### Radiographic findings

Serial radiographs were taken before the surgery to ensure any abnormality. However, to compare the groups, different parameters were recorded after the surgery i.e., fragment alignment, fracture gap, post-surgical site gas, callus formation, pin position, periosteal reaction, time to healing and complications were also assessed. Radiographs were taken after surgery on the 14<sup>th</sup>, 28<sup>th</sup>, 45<sup>th</sup> and 60<sup>th</sup> day (Table 2).

**Table 2:** Radiological findings of fracture healing in dogs

Days	Fragment alignment	Post-surgical site gas	Callus formation	Pin position	Periosteal reaction	Time to healing	complication
14	(Reduction size) mm	(Considerable slight or none)	Callus type (regular or, homogenous growth or amorphous growth)	Greater trochanter, trochanteric fossa	Days	Days	Yes or No
28	(Reduction size) mm	(Considerable slight or none)	Callus type (regular or, homogenous growth or amorphous growth)	Greater trochanter, trochanteric fossa	Days	Days	Yes or No
45	(Reduction size) mm	(Considerable slight or none)	Callus type (regular or, homogenous growth or amorphous growth)	Greater trochanter, trochanteric fossa	Days	Days	Yes or No
60	(Reduction size) mm	(Considerable slight or none)	Callus type (regular or, homogenous growth or amorphous growth)	Greater trochanter, trochanteric fossa	Days	Days	Yes or No

## RESULTS

### Radiographic findings treated with IM-Pin alignment

Radiographs were taken before surgery as control. Visible fracture was seen on radiograph i.e., fragment alignment, fracture gap, post-surgical site gas, callus formation, pin position, periosteal reaction, time to healing and complications were assessed. Radiograph was taken after surgery on 14<sup>th</sup>, 28<sup>th</sup>, 45<sup>th</sup> and 60<sup>th</sup> day. Fig. 1 shows the different radiographs of different animals taken on different days treated with IM pin only in group A. These radiographs have clearly indicated positive and negative attributes of dogs treated with IM pin in critically sized femur fractures. The radiograph A was taken on day 14<sup>th</sup> after surgery indicates right fracture alignment but there was no bone growth achieved. Day-28<sup>th</sup> radiographs (B) of different animals demonstrated a good response to bone healing. While on 45<sup>th</sup> days, callus formation was not clear as indistinct, fluffy, and immature callus formation was detected at both interfacing edges of bone as shown in radiographs C of different animals. A well-defined distinction between 28<sup>th</sup> and 45<sup>th</sup> days of surgery was bridge formation in the latter radiographs in most of the animals. Moreover, after two months of surgery (i.e., on day 60) radiographs of different animals indicated prominent callus surrounding fracture sites. But exceptions of fracture luxation and periosteal reactions were also present as shown in radiograph D (Table 3).

### Radiographic findings treated with IM-pin + PMMA alignment

Fig. 2 shows radiographs of different animals taken on different days (14<sup>th</sup>, 28<sup>th</sup>, 45<sup>th</sup>, and 60<sup>th</sup>) treated with combinations of IM pin and PMMA. Fourteen days post-surgery radiograph (A) indicated a fracture line and mild blurring demonstrating reduced inflammation in different animals. Whereas indistinct and immature callus formation was detected at both interfacing edges of bone in a radiograph (B) taken after 28<sup>th</sup> days in different animals of the same treatment. Radiographs of different animals taken on day 45 indicated a good response to bone healing and fracture union as shown in radiograph (C).

However complete union of the fractured edges was achieved after two months of surgery in radiographs of almost all animals as shown in radiograph D with prominent callus surrounding. Periosteal reactions in this treatment could not be exhibited in all animals throughout

the research period of observation. The results of the mechanical stability analysis were comparatively good to other treatment groups in different animals (Table 4).

### Radiographic findings treated with Calcium phosphate + IM-Pin alignment

Fig. 3 shows radiographs of critically sized femur fractures of different animals taken on different days (14, 28, 45, and 60) treated with combinations of IM pin and CaP. On day 14 of surgery radiograph (A) indicated the clear gap between the fracture edges of different animals. The bone alignment indicated in radiographs of different animals was appropriate with no callus formation at all in all animals. While radiograph (B) taken on the 28<sup>th</sup> day of surgery in different animals showed callus formation but was unlikely to PMMA callus growth recorded slowly. After 45 (Radiograph C) days of surgery gap was filled by the surrounding callus but yet not united the opposite edges (Plate 4.3). While radiographs of day 60 (D) of different animals demonstrated that the union was about to complete but the surrounding callus formation was not as eminent as previously recorded in PMMA (Table 5).

### Radiological evaluation of operated animals

Radiological assessment of fracture alignment and evaluation of bone healing was done regularly in all three groups. Although results of different days mentioned here were taken from all animals in all three groups. However, radiographs presented above were selected from different groups at different time intervals.

Results of day 14<sup>th</sup> after surgery in all three groups demonstrated the appropriate fracture alignment in animals of all groups. Furthermore, the extent of reduction in the gap in all three groups was different. In group A (IM pin only) gap reduction was not considerable. But in groups B (IM pin+PMMA) and C (CaP + IM pin) slight reduction in the gap was seen. Reduction in gaps in later groups might be due to compression as bone growth and cellular changes were not found on close examination. Periosteal reaction on this day indicated mild inflammation in animals of group A but groups B and C did not show any signs.

On day 28<sup>th</sup>, reduction in gaps evidenced bone healing in all groups. But the clear demonstration of callus formation was indicated in radiographs of group B and C animals. While in group A compared to day 14<sup>th</sup> gap was reduced considerably due to increased formation of immature callus and hyperplasia of surrounding tissues.

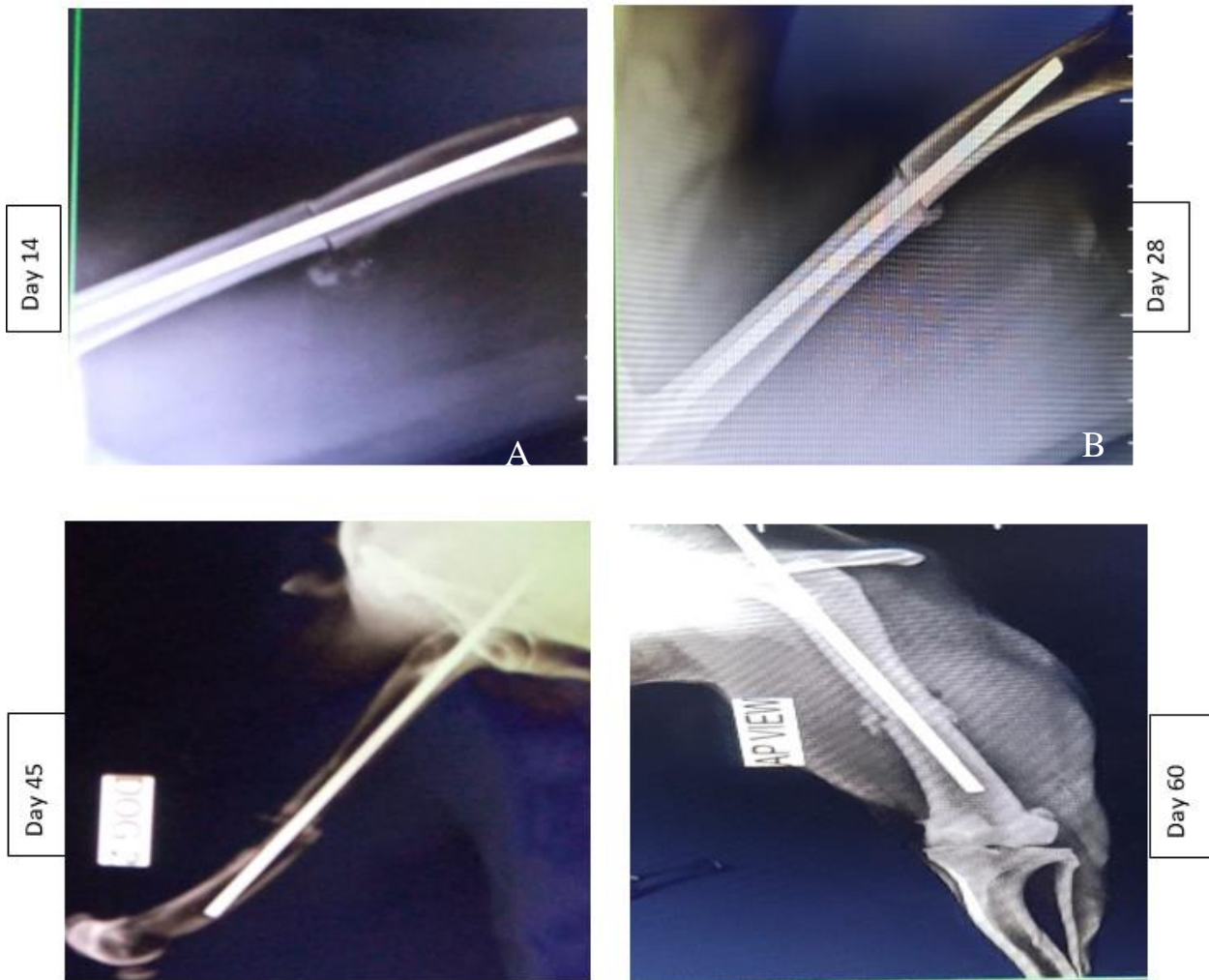


Fig. 2: Radiographs of Group B IM-pin + PMMA alignment.

Table 3: Radiographic findings of Group (A) IM-Pin

Day	Fragment alignment	Fracture gap	Post-surgical site gas	Callus formation	Pin position	Periosteal reaction	Time to healing	Complication
14	Good	Present	Absent	No callus	Greater trochanter	No	Not detected	No
28	Good	Present	Absent	No callus	Greater trochanter	No	At initial	No
45	Good	Absent	Absent	Amorphous	Greater trochanter	No	Prominent	No
60	Good	Present	Absent	Amorphous	Greater trochanter	Present	Diverse	Yes

Table 4: Radiological findings of Group B IM-pin + PMMA

Day	Fragment alignment	Fracture gap	Post-surgical site gas	Callus formation	Pin position	Periosteal reaction	Time to healing	complication
14	Good	Present	Absent	No callus	Greater trochanter	No	Not detected	No
28	Good	Started reduction	Absent	Regular	Greater trochanter	No	Detected growth	No
45	Good	Absent	Absent	Regular	Greater trochanter	No	Prominent	No
60	Good	Absent	Absent	Regular	Greater trochanter	No	United	No

Periosteal reactions were eliminated on this day as previously recorded in some animals of group A. Eminent callus formation in group B animals was recorded but still, fracture union not achieved.

Radiographs of day 45<sup>th</sup> showed remarkable growth of bone healing and formed Calli surrounding fracture edges in all groups of animals. Prominent callus formation in groups B and C animals demonstrated rapid healing by filling the gap but in group A radiographs presented growth on both sides but the gap was yet to be filled. Fracture union in group B was at the initial phase but not

detected in group A and C animals. Periosteal reactions were significantly present in some animals of group C as shown in Fig. 4.

Radiographs taken on day 60<sup>th</sup> of different animals of all three groups manifested fracture union in group B and C animals. Animals treated with IM pin only in animals of group A showed randomized luxation and increased gap. But the callus surrounding the fracture site was prominent in group A animals aligned correctly. Mild to moderate periosteal reaction was randomly present in animals of groups A and C, respectively.



**Fig. 3:** Radiographs of Group - C Calcium phosphate + IM-Pin alignment.

**Table 5:** Radiological findings of Group - C Calcium phosphate + IM-Pin

Day	Fragment alignment	Fracture gap	Post-surgical site gas	Callus formation	Pin position	Periosteal reaction	Time to healing	complication
14	Good	Present	Absent	No callus	Greater trochanter	No	Not detected	No
28	Good	Present	Absent	No callus	Greater trochanter	No	Detected	No
45	Good	Reduced	Absent	Amorphous	Greater trochanter	Present	Considerable	Yes
60	Good	Absent	Absent	Amorphous	Greater trochanter	Present	Gap was filled	Yes

**DISCUSSION**

All the animals selected were assessed but only presented significant differences in the results by comparing all the groups treated with IM-Pin only (Group A), IM-Pin+PMMA (Group B), IM-Pin+CaP (Group C), the radiological parameters were recorded on day14, 28, 45 and 60. Similarly, Liska., (2004) reported that the analysis of bone healing after surgery should be continued for 60 days.

In present study IM-Pin alone and with the combination of PMMA and CaP. Similarly, The use if IM-M-Pins in fracture may provide axial alignment and resist bending forces (Fossum 2007). However, PMMA and CaP provide stability and help fast bone healing with osteoconductive abilities, Turner et al. (2008) indicated the effectiveness of CaP and PMMA for restoration in orthopedic surgery. Varieties of results were found on different days during radiological comparison in dogs. Although, there is only limited work that has been done using PMMA or CaP with IM-Pin in dogs. The

concluding remarks provided by Ayouba et al. (2020) underscore the efficacy of employing an IM-Pin in conjunction with cement for femur fixation, emphasizing its noteworthy contributions to orthopedics. Notably, the authors highlight the innovative modification of introducing cement alongside IM-Pin usage for enhancing femur stability, marking a pioneering approach that was introduced for the first time in human subjects. This pioneering technique not only demonstrates the adaptability of orthopedic practices but also hints at the potential for advancements in the field of human femur stabilization. The study thus sheds light on a novel and promising avenue in orthopedic research and practice (Tayag and Villamin 2020).

The findings on the day, 14 post-surgery in all three groups demonstrated appropriate fracture alignment in animals of all groups, similarly, Singh et al. (2017) also recorded the appropriate fracture alignment on day 21 using IM-Pin in dogs. Furthermore, the extent of reduction in the gap in all three groups was different. In group A (IM pin only) gap reduction was not

considerable. However, in groups, B (IM pin+PMMA) and C (CaP + IM pin) slight reduction in the gap was seen. Reduction in gaps in later groups might be due to compression as bone growth and cellular changes were not found on close examination. Periosteal reaction on day 21 indicated mild inflammation in animals of group A but groups B and C did not show any of the signs. Furthermore, Ayouba et al. (2020) observed the consistent formation of membranes with a positive association with the periosteum when employing cement in human subjects. This finding underscores the potential benefits of using cement in orthopedic applications, suggesting a favorable interaction with the periosteum that could contribute to enhanced healing and stability. The establishment of continuous membranes, as reported in the study, adds an intriguing dimension to the discussion, warranting further exploration into the mechanistic aspects and clinical implications of this phenomenon (Shah et al. 2020).

On day 28<sup>th</sup>, reduction in gaps evidenced bone healing in all groups. But the clear demonstration of callus formation was indicated in radiographs of group B and C animals including less bone reaction. However, Turner et al. (2008) indicated deposition of PMMA and CaP in the space of vertebrae in his radiographs, similarly, the radiographs of present study also indicate the presence of cement material besides the fracture site. While in group A compared to day 14<sup>th</sup> gap was reduced considerably due to increased formation of immature callus and hyperplasia of surrounding tissues. Periosteal reactions were eliminated on this day as previously recorded in some animals of group A. Eminent callus formation in group B animals was recorded but still, fracture union was not achieved.

Radiographs of day 45<sup>th</sup> showed remarkable growth of bone healing and formed Calli surrounding fracture edges in all groups of animals. Prominent callus formation in groups B and C animals demonstrated rapid healing by filling the gap but in group A using IM-Pin only radiographs presented growth on both sides but the gap was yet to be filled, similarly Dubey et al. (1993) reported the absence of callus using IM-Pin. Fracture union in group B was at the initial phase but not detected in group A and C animals. Periosteal reactions were significantly present in some animals of group C as shown in Fig. 3 (C) (of group C).

Radiographs taken on day 60<sup>th</sup> of different animals of all three groups manifested fracture union in group B and C animals. Animals treated with IM pin only in animals of group A showed randomized luxation and increased gap. However, group B, treated with pin showed satisfactory results, the long-term findings by Ayouba et al. (2020) indicated positive results using PMMA with the combination of IM-Pin. But the callus surrounding the fracture site was prominent in group A animals aligned correctly. Mild to moderate periosteal reaction was randomly present in animals of groups A and C respectively. Animals treated solely with IM-Pin exhibited a concerning trend of IM-Pin displacement, evident in the current radiographs. This aligns with findings from prior studies, including those by Chandy et al. (2007), which also documented instances of non-union and delayed union attributed to IM-Pin pin-slippage. The recurrent

observation of IM-Pin instability underscores a critical challenge associated with this treatment approach. The documented evidence, both from the present study and previous research, highlights the need for a comprehensive reassessment of the efficacy and reliability of IM-Pin as a standalone intervention. These collective findings urge further exploration of alternative or complementary strategies to address the observed issues and enhance the overall success rate of femur fixation in animal orthopedics.

### Conclusion

Radiological assessment in order to investigate the reduction in the size of fracture gap on different days, the fragment alignment, fracture gap, post-surgical site gas, callus formation, pin position, periosteal reaction and time to healing was recorded comparatively better in the group (IM-Pin+PMMA) than IM-Pin and IM-Pin+CaP groups on 14<sup>th</sup>, 28<sup>th</sup>, 45<sup>th</sup> and 60<sup>th</sup> day post-surgery

### Declarations

The authors declare that they have no competing interests.

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### Authors' Contributions

Conceptualization, Supervision and Interpretation of data: Muhammad Ghiasuddin Shah, Akeel Ahmed Memon, Ahmed Nawaz Tunio, Atique Ahmed Behan and Muhammad Bilawal Arain. Methodology, Investigation, Review and Manuscript writing and editing: Abdul Salam Khoso, Ahmed Nawaz Tunio, Muhammad Bilawal Arain and Atique Ahmed Behan. All authors have seen the final version of the manuscript and approved for publication.

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