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Research article

Comparison between Double Intramedullary Pinning and Veterinary Cuttable Plating Techniques in Repairing Induced Diaphyseal Femoral Fracture in Cats

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Abstract

Background: Diaphyseal fracture of the femur is one of the most common affections in cats. It has been treated using bone plating and intramedullary pinning (IMP) techniques. Veterinary cuttable plating (VCP) was highly recommended for most fractures, but it is not always applicable due to its expensive specific instruments and required surgical skills. Double IMP is believed to be a suitable technique for treating cat femoral fractures. **Aim:** This study aimed to compare the two techniques in treating induced femoral fractures in cats in terms of their efficacy, practicality and potential complications. **Methods:** Eleven healthy mature local cats were used. They were randomly divided into two groups; group A, double IMP treated (7 cats), and group B, VCP treated (4 cats). All cats were clinically, physically, and radiographically examined and prepared for surgery. Fracture of the right femur in each cat was induced using an ultrasonic oscillating saw. Internal fixation of femoral fractures in the group A was conducted using double IMP technique while in the group B was performed by VCP technique. Ability of weight bearing, grade of lameness and radiographic assessment were reported after operation. **Results:** Post-operative clinical examination of the cats showed no abnormality. Five cats in the group A and all cats in the group B were able to bear their complete weight on the operated limb on the 2nd day post-surgery. Grade of lameness was significantly higher in the group A in the first 15 days. However, on days 30 and 60, cats showed no more lameness in both groups except two cats in the group A. These two cases suffered from other complications due to their uncontrollable behavior. Other than these two cases, evidence of complete bone healing was seen in the 8th week post-operation in the group A but in the 12th week in the group B. **Conclusion:** Using double IMP technique in repairing femoral fractures in cats offered practical and reliable results, with less recovery time.

Keywords: cat, femur, fracture, surgery, fixation

Introduction

Femoral fractures are the most common fracture occurred in hindlimbs of cats, (Hill, 1977; Bookbinder and Flanders, 1992; Beale 2004, Libardoni *et al*., 2018, Roberts and Meeson, 2022). In a survey included 141 fractures, 63.64% out of almost 80% of the cases which occurred in the hind limbs showed femoral fractures (Cardoso *et al*., 2016). Furthermore, in another study conducted on 282 cats with long bone fractures, it was found that femoral fractures had the highest incidence (45%), followed by tibia (26%), radius and ulna (16%) and humerus (13%) (Harasen, 2003). Femoral fractures were seen in many regions of the femur such as the metaphyseal, diaphyseal, and/or epiphyseal parts of the bone (Tercanlioglu and Sarierler, 2009; Shiju *et al*., 2010). However, the majority of the fractures were found in the diaphyseal part of the femur (Phillips, 1979; Harasen 2003).

Diagnosis of fracture relies on physical and radiography examinations (Roberts and Meeson, 2022). Pre-operative clinical examination provides a primary diagnosis of fracture and its location. Specific clinical signs of fracture include non-weight bearing, soft tissue swelling, pain, crepitus and instability of the joints proximal and distal to the fracture (Kaur *et al*., 2015; Hossain, 2021). Nevertheless, the most reliable diagnostic tool used for evaluating fractures in all animal species is radiography (Thrall, 2017; Hossain, 2021; Scott *et al*., 2022). It is also very important in the preoperative decision to select the proper tool for treating the fracture (Scott *et al*., 2022).

The principal aim of fracture treatment is to return the fracture fragments to their normal anatomical position either by using the external coaptation or the open fixation via surgery (internal fixations). The external coaptation used bandages, casts, splints and slings (Fossum, 2018) while the surgical treatment was conducted by implanting intramedullary pins, bone plates, screws and wires. Although the external coaptation is simple, practical and cost effective, it cannot be applied for long bone fracture above elbow and stifle (Fossum, 2018) because of the surrounding thick muscular mass in these regions (Scott *et al*., 2022). Applying the external fixation in treating femoral diaphyseal fracture showed the highest percentage of bony complications post-fixation (Könning *et al*., 2013).

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Therefore, the internal fixation was the practical choice for treating bone fractures in such regions. This bone fixation technique includes various types of bone plates, screws, intramedullary pinning (IMP) and cerclage wire. All these devices are applied by different techniques either alone or combined. Selecting a particular device is made based on a number of factors such as nature of fracture, practicality, cost-effectiveness and availability (Tercanlioglu and Sarierler, 2009; Könning *et al*., 2013). Mid-diaphyseal fracture of the femur in cats has been treated using two main orthopedic devices, bone plates with screws and pins with wires (Fossum, 2018; Scott *et al*., 2022). The most common method for fracture treatment by the internal fixation is bone plating (Broos and Sermon, 2004). Plates are ideally suitable for transverse or short oblique fractures of the femur (Piermattei *et al*., 2006). Veterinary cuttable plate (VCP), which is one of the plates used for stabilizing diaphyseal femoral fracture in cats, was associated with good outcome without major complications (Vedrine and Gérard, 2018; Roberts and Meeson, 2022). However, although this technique was commonly used and highly recommended, it requires expensive particular instruments and special surgical skills (Scott, 2005; Sanchez and Perry, 2021; Zurita and Craig, 2022). This is in fact contrary to IMP techniques which use much less expensive and available instruments (Beale, 2004).

IMP or stacked pin application is used for fixing the simple diaphyseal femoral fractures in cats (Hach, 2000; Altunatmaz *et al*., 2017; Kumar Bishnoi *et al*., 2022). It acts primarily as an internal splint of the medullary canal of long bones that shares loading, maintains axial alignment of the fracture and resists bending forces applied to bones (Tarr and Wiss, 1986; Hach, 2000; Stiffler, 2004; Könning *et al*., 2013). There are different types of the intramedullary pinning such as Steinmann pins, Kirschner wires (K-wires) and Rush pins. Despite the differences in their material characters and methodology, using any of them in treating femoral diaphyseal fractures is practical and less difficult. This was due to the straight and uniform morphometry of the medullary canal (Chandler and Beale, 2002) which facilitated introducing and placing the pins much more easily into the medullary canal (Chandler and Beale, 2002). For a better stability, it was also recommended to use two (double pining technique) or multiple intramedullary pins (stack pining) to fill the diameter of the medullary canals (Gibson, 1991). However, complete filling of the canal with pins may lead to a disruption of medullary blood supply and increase risk of delayed union (Brinker *et al*., 2016) as well as pins migration (Schrader, 1991). Therefore, it is generally advised to use a pin that is 60% to 75% of the medullary canal (Chandler and Beale, 2002).

Locally, femoral fractures in cats are commonly observed (Ali, 2013, Bennour *et al*., 2014) and treating such fractures with traditional methods such as external coaptation have already showed unsuccessful results (Fossum, 2018). Using improper fixations might be because of financial issues, which preventing using the expensive plating techniques. It could also be related to the insufficient knowledge about the promising outcome of using the other less expensive internal fixation techniques such as IMP. Therefore, the high incidence of cat femoral fractures and the importance of presenting a cheaper, practical and reliable internal fixation technique to the local field have promoted conducting the current study. The present experiment was firstly aimed to compare the efficacy and practically of intramedullary pining in fixing diaphyseal femoral fracture in cats with the gold standard technique of internal bone fracture fixation (bone plate). Secondly, identifying the potential complications that may occur with applying these orthopedic devices for fracture repair, particularly infection, migration, bending and breakage of the devices.

Materials and methods

All experiments of this study were conducted at the Surgery and Diagnostic Imaging Unit of the Faculty of Veterinary Medicine at the University of Tripoli, Libya.

Animals

The present study was conducted on eleven healthy mature cats (n=11). They were local domestic shorthair cats (DSH). The mean of their ages was 12 ±0.40 months old and the mean of their weights was 3.47±0.12 kg. They were 7 males and 4 females.

The cats were randomly divided into group A (7 cats) and group B (4 cats). All cats were housed in a suitable place and in good environment for at least 10 days prior the experiments. Regular clinical examinations were conducted on each cat during this period.

Pre-operative radiography

All cats were clinically examined prior to conducting the surgical operation.

Two radiographic views (at right angle) were used, medio-lateral and cranio-caudal views, to ensure the eligibility of the cats for such experiment. The procedures of taking medio-lateral and cranio-caudal pre-operative radiographs of the right femur of each cat were performed based on the standard procedures described by Thrall (2017). A mobile x-ray machine (ACEM, Bologna, Italy) was used at 100 cm focal film distance. The x-ray films were processed using an automatic processer (Optimax, Protec Medizintechnik GmbH & Co., Germany).

Prophylactic antibiotic and anesthetic protocol

Systemic broad-spectrum antibiotic (Ceftriaxone sodium 10%, WG Critical Care LLC, Italy) at a dose of 25 mg/kg were injected intramuscularly (IM) few hours prior to the surgery (Piermattei and Flo, 2006; Albarellos *et al*. 2007).

A single dose of Meloxicam (Meloxicash, ashish, India) was administered subcutaneously (SC) at a dose of 0.2 mg/kg approximately few hours pre-operation (Slingsby and Watterman-Pearson, 2002; Tranquilli *et al*., 2007; Robertson, 2008; Gaynor and Muir Iii, 2014; Mathews *et al*., 2014).

Atropine 20 µg/kg body weight were administered SC, a total of 1 mg/kg xylazine (Xyla, Interchemie, Holland) were IM injected at approximately 20 min before the induction of general anesthesia (Muir *et al*., 2007; Tranquilli *et al*., 2007).

Induction of general anesthesia was performed using IM administration of Ketamine (Ketamin 10%, Bremer Pharma GmbH, Germany). The dosage was 15 mg/kg

body weight (Muir *et al*., 2007; Tranquilli *et al*., 2007; Clarke and Trim, 2013; El-shafey *et al*., 2022).

Surgical Protocol

Surgical site was prepared according to the standard procedures. A fenestrate drape was placed over the whole body except the right hind limb. The paw was covered by a cotton layer and an adhesive bandage.

Surgical approach and inducing diaphyseal fracture

The site of surgical incision was identified by an imaginary line which extended between the greater trochanter and the lateral epicondyle of the femur. Skin incision was made along the cranio-lateral border of the femur with the guidance of the imaginary line, which then undermined and reflected. Then, the superficial leaf of the tensor fascia lata was incised along the cranial border of the biceps femoris muscle that was reflected caudally. Finally, the incision continued through the septum of the vastus lateralis muscle which then reflected cranially and expose the femoral shaft (Piermattei and Flo, 2006; Fossum, 2013; Tobias and Johnston, 2013). In cats of group A, the shaft of the femur was only exposed at the middle part whereas in group B, the exposure included most of the shaft.

The fractures were induced using ultrasonic oscillating saw (Aesculap AG, Germany). The saw made a complete transverse fracture at the mid-diaphyseal part of the exposed femur (Figure 1).

Internal fixation techniques

Implantation of the internal fixation devices was performed as previously described (Brinker, 1998; Denny and Butterworth, 2000; Piermattei and Flo, 2006).

Double IMP technique

This technique was performed on cats in the group A using surgical stainless steel intramedullary pins "Kirschner wire" (PH Orthcom. Ltd., China) of 1.8 mm diameter. The procedure started by determining the required length and diameter of Kirschner wire (K-wire). A caliper was used to measure the width of the medullary cavity on the pre-operative radiographs. The double K-wire was inserted into the medullary cavity using an orthopedic drill. The insertion started from the fracture site in a retrograde fashion toward the proximal end of the femur until penetrating the trochanteric fossa to outside the bone (Figure 2). A second pin was then inserted following the same way. The two pins were withdrawn proximally until their other ends became within the medullary canal. The proximal and distal bone fragments were then aligned and the gap between them was reduced. After that, the first pin was introduced in a normograde manner to seat into the distal epiphyseal part of the femur followed by introducing the second pin in the same manner. The total size of both pins was 3.6 mm which represented approximately 72% of medullary cavity width, as previously recommended (Scott, 2005; Altunatmaz *et al*., 2017).

VCP technique

Plating technique was performed on cats in the group B using surgical stainless veterinary reconstruction cuttable plates (PH Orthcom. Ltd., China) of 2.4 mm size (Figure 3). Screws, 2.4 self-tapping cortical 2.4×1.4mm, were applied to fix the induced fracture. The steps of implanting the bone plate were started by measuring the required length of the plate, which was taken during surgical operation. The fracture was reduced and the bone ends anatomically aligned. The plate was placed on the lateral side of the femur and fixed over the fracture using plate holders. Drill bit was used to drill the first hole about 1 cm away from the fracture site using a sleeve guide. A depth gauge was used to measure the depth of the hole to select the appropriate screws. The first screw was fixed using a screwdriver. The next screw hole was then drilled on the other side of the fracture site and its screw was inserted. Another two screws were fixed on each side using the same procedure and away from the fracture site. The two holes of the plate, which were close to the fracture line, were left empty.

After the proper fracture fixation of all cases, the muscles and subcutaneous fascia were sutured in a simple continuous manner using absorbable surgical suture, Vicryl (DemeCRYL, DemeTECH, USA), with size 2/0 USP. Skin was then sutured in a simple interrupted pattern (Fossum, 2013) using non-absorbable silk (DemeSILK, DemeTECH, USA), with size 2/0 USP.

Post-operative care

Wounds were daily dressed and Meloxicam 0.1 mg/kg body weight was administrated SC for four days post-operation. Ceftriaxone injected IM twice a day at a dose of 25 mg/kg for 5 days post-surgery. Cats were housed in strict confinement for 6 weeks. The skin sutures were removed after 12-14 days post-operation, after the skin was completely healed. For group A, k-wires were routinely removed after confirming the complete bone healing in approximately 2 month post-surgery.

Clinical assessment was performed based on post-operative physical examinations such as body temperature, respiratory rate, pulse rate and mucus membrane status.

Gait analysis

This analysis was conducted using lameness scoring system (Ingwersen *et al*., 2012) in which its grade ranged from 0 to 4, based on the severity of the lameness. The analysis was carried out after cats made full recovery from anesthesia. It started from the 2nd day and continued until the 60th days post-surgery. Each cat was gently placed in standing position (on all four limbs) and allowed to walk for approximately 30 to 45 seconds (Ingwersen *et al*., 2012).

Post-operative radiographic assessment

For each cat, two post-operative radiographs, medio-lateral and cranio-caudal radiographs (Figure 4), were taken eight times after the surgical operation. The first time radiograph (0) was taken immediately after the operation in order to assess the alignment of the fractured bony ends as well as the proper placement of the fixation device. The other radiographs were taken in 2nd, 3th, 4th, 5th, 6th, 7th and 8th weeks postoperatively. At these times, the radiographic assessment was basically focused on the placement of the fixation devices and the signs of healing that included characters of callus formation (density/bridging, non- bridging) and the fracture line.

Functional outcome

Based on the periodical clinical examinations, the functional outcome of each cat was assessed on the 60th post-operative day and categorized as excellent, good, fair or poor (Clark, 1987).

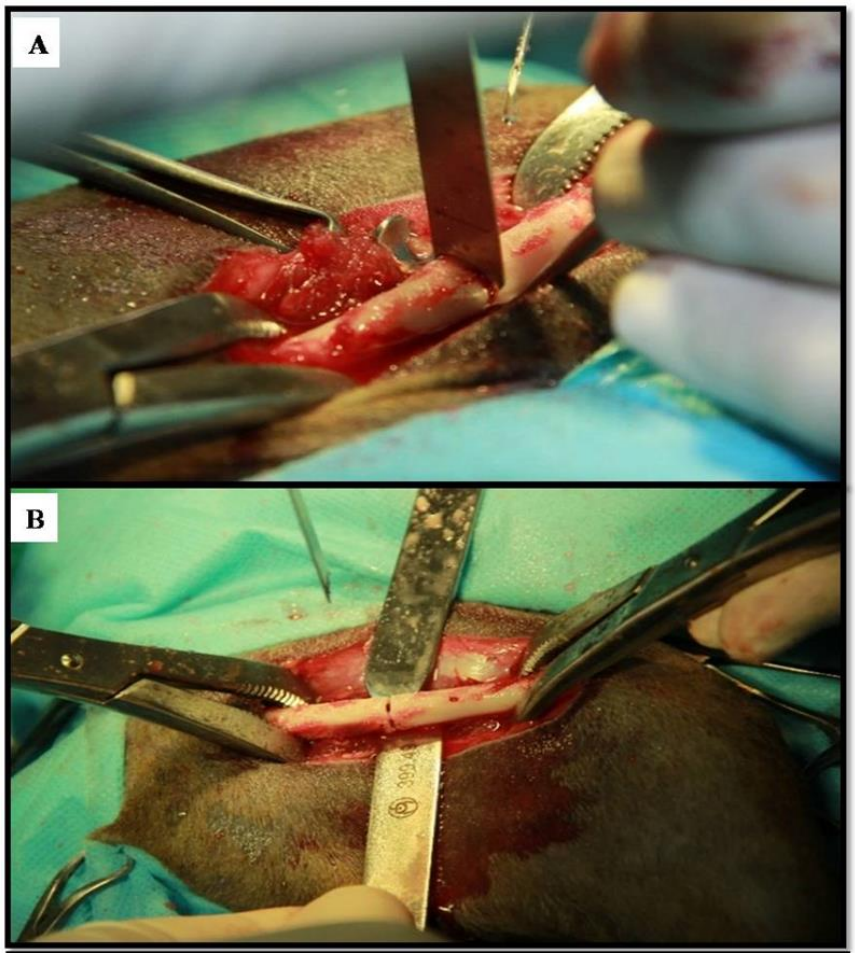


Figure 1. A. Inducing diaphyseal transverse fracture to a right femur of a cat using oscillating saw. **B**. Protecting the underline tissues using two Homman retractor.

At the end of the study, the cats were released and sent for adoption program after their full recovery.

Statistical analysis

The data of the degree of lameness which was score-type data and not normally distributed, was statistically analyzed using MANN-WHITNEY U test (Mendenhall, 1987).

Ethical approval

This study was approved by the Bioethics Committee at the Biotechnology Research Center, Tripoli, Libya (BEC-BTRC) and assigned the reference No. 349-9/2016.

Results

Pre-operative clinical and radiographic examinations revealed that all cats were clinically healthy and had no bone abnormalities in their femurs and the surrounding regions.

Post-operative examination showed that the surgical incisions in all cats healed completely within 10 to 14 days without any complications and the skin sutures were removed at this time.

Weight bearing post-operation

In the group A, five cats showed a complete ability to bear their weight on the operated limb on the 2nd day post-surgery. The other two cats started to carry the complete weight on the 5th and 6th day post-surgery.

In the group B, all the four cats were able to carry their complete weight on the 2nd day post-surgery.

Gait analysis

Evaluating the severity and degree of lameness of the cats based on lameness scoring system is shown in table 1. On the second day post-surgery, all cats in the group A suffered from the highest degree and severity of the lameness (grade 4, lameness mean 4.0 ± 0.00). Whereas, the grade of lameness at the same time in cats of the group B was slightly lower (mean 3.0 ± 0.00). On the day seven post-operation, the grade of lameness in five cats of the group A showed some kind of improvement from grade 4 to grade 3 (mean 3.29 ± 0.18). At the same time, all cats in the group B showed a grade of 2. This pattern of improvement was seen almost in the same way on the day 15, in which the mean of the lameness grade was 2.29 ± 0.18 in the group A and was 1.0 ± 0.00 in the group B. On both days 30 and 60, all the cats showed no more lameness (grade 0) except two cases in the group A. These two cats (A3 and A6) showed the slowest rate of the lameness improvement.

Statistical analysis

The statistical results of the lameness scoring system showed that the lameness grade was significantly lower (p-values 0.006) in cats of the group B than in the group A on the days 2, 7 and 15 post-surgery with large effect sizes (Table 1). However, on the days 30 and 60 there

Table 1. Grade of lameness in the cats in the group A, treated using double pining (7 cats) and in the group B, treated using plate fixation (4 cats).

Day PS	Group A cats								Group B cats					p-value	Effect size
	A1	A2	A3	A4	A5	A6	A7	*Mean ± SE*	B1	B2	B3	B4	*Mean ± SE*		
2	4	4	4	4	4	4	4	*4.0 ± 0.0*	3	3	3	3	*3.0 ± 0.0*	0.006*	0.95
7	3	3	4	3	3	4	3	*3.29 ± 0.18*	2	2	2	2	*2.0 ± 0.0*	0.006*	0.86
15	2	2	3	2	2	3	2	*2.29 ± 0.18*	1	1	1	1	*1.0 ± 0.0*	0.006*	0.86
30	0	0	2	0	0	3	0	*0.71 ± 0.47*	0	0	0	0	*0.0 ± 0.0*	0.53	0.34
60	0	0	2	0	0	2	0	*0.57 ± 0.36*	0	0	0	0	*0.0 ± 0.0*	0.53	0.34

PS: post-surgery, SE: standard error, *: significant

were no significant differences between the two groups in the lameness grade.

Radiographic assessment of fracture union

In the group A, five cats (A1, A2, A4, A5, and A7) showed excellent bone healing within the expected time frame. In the 3rd week, callus formation was notable with narrowing of the fracture line. In the 4th, 5th and 6th weeks bridging callus was noticed filling the gap of fracture with slight cortical union and visible fracture line. The radiographic evaluation on the 7th week revealed complete union with radio dense callus between fracture bone fragments and signs of bone remodeling. However, in the other two cats (A3, A6) there was a delay in the union with improper bone healing. Their bone healing showed some complications included non-bridging callus in the 4thweek, pins migration with large amount of callus formation at the fracture site in the 5th week and clear fracture line. One week later (6th week), more callus bridging with mild bending were noted.

In group B, there was no bone healing complication noted in any cat of this group.

Removing fixation devices

In the group A, the intramedullary pins in cases A1, A2, A4, A5 and A7 were removed under sedation and aseptic conditions after complete bone union in the 8th week. In the other two cats (A3, A6), the introduced k-wires were dropped in a retrograde manner due to the aggressive behavior of the cats and continuous shakings of the operated limbs in the 5th and the 6th week respectively. In the group B, the plates were removed surgically from all the cats in the 3rd month post-operation.

Functional outcome after removing the fixation device

Although the functional outcome of the operated limb was just fair in two cats in group A (A3, A6), it was excellent in all the other cats involved in this study.

Discussion

Although the double IMP is one of the surgical techniques used to treat mid-shaft femoral fractures in cats, it has not been extensively reported in the scientific literature. The current study compared between this technique and bone plating, in treating induced mid-diaphyseal femoral fractures in healthy mature cats.

Evaluating the successful use of both techniques was based on the post-operative outcome which included weight bearing, gait analysis and radiographic assessment. In general, the ability of cats to bear weight on the treated fractured femurs was reported to be gradually seen in the first couple of days post-surgery in both double IMP and VCP techniques (Kushwaha *et al*., 2011; Hossain *et al*., 2017; Canlı et *al*., 2024). This was also reported in the current study, in which all the treated cats began to bear weight on their operated limbs on the 2nd day post-surgery except in two cats in the group A. These two cats which were treated using the double IMP began to bear weight on the 5th and 6th day post-surgery. Identifying the reason of delaying weight-bearing in the two cats was difficult, but the probability of having some bone healing complications was obviously high. Later on, the lameness scoring system of these cats showed a mild degree of lameness during walking up to the 60th day post-treatment. This was clearly due to bone healing complications and the pins migration which were also reported in a number of previous studies (Prasad *et al*., 2011; Das *et al*., 2019). Radiographically, the bone healing complications were noted to appear as a non-bridging callus in the 4th week whereas the pins migration occurred in the 5th week. Unlike all the cats in this study, these two cats were slightly aggressive and showed uncontrollable behavior even with keeping them in cages for eight weeks. They were restless and frequently shook their operated limbs throughout the study. This repetitive movement might lead to retrograde migration of the fixation device, compromising bone

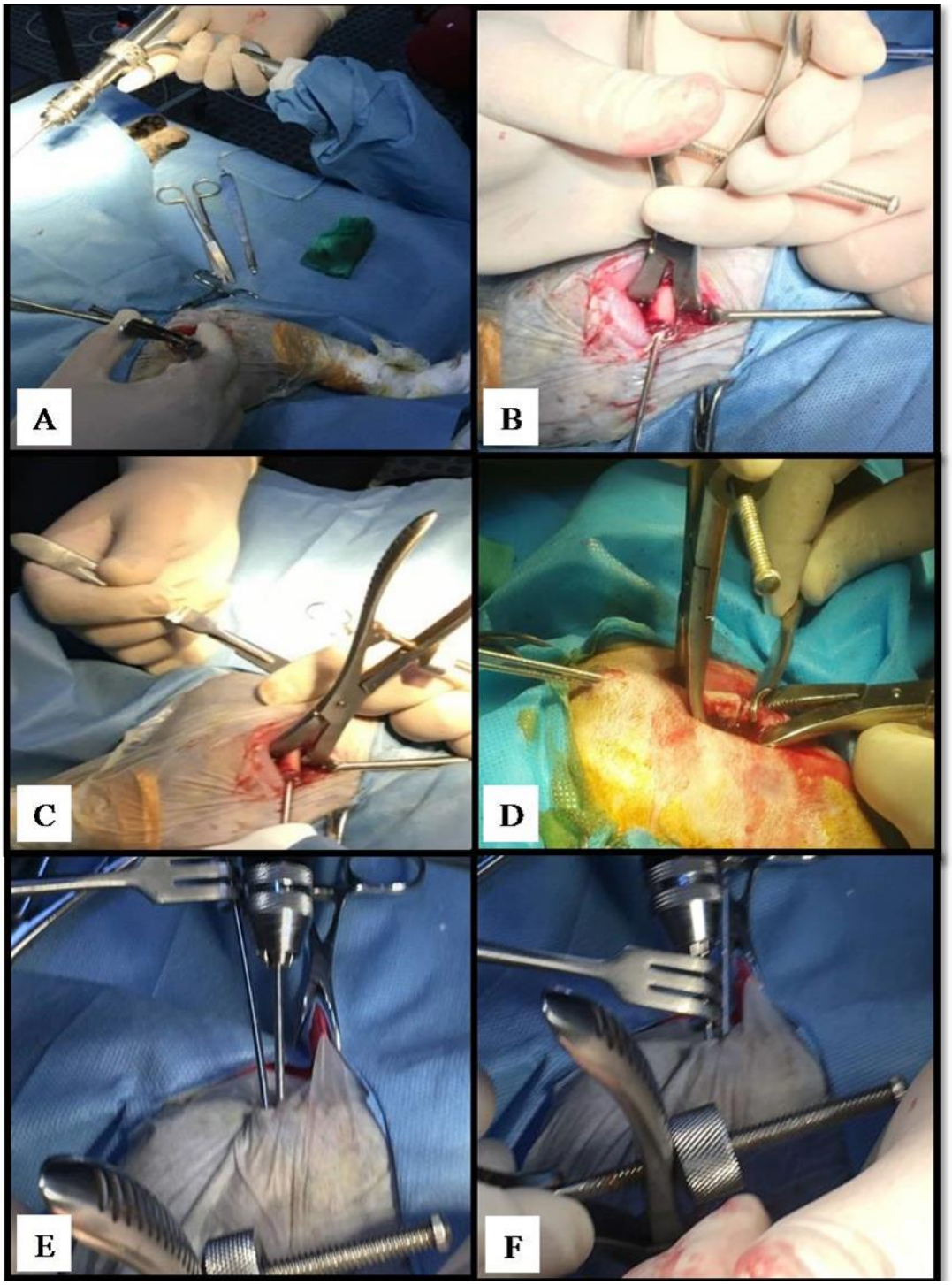


Figure 2. The steps of introducing intramedullary pin to repair the femoral induced fracture of a cat. **A.** Fixing the pin in the tip of drill. **B.** Introducing the pin into medullary cavity in retrograde manner. **C.** Making a skin incision over the tip of the pin which existed via trochantric fossa. **D.** Align the bone fragments prior to introduce the two pins into the medullary cavity of the distal fragment. **E.** Introducing the first pin in a normograde manner into the distal fragment. **F.** Introducing the second pin in a normograde manner into the distal fragment of the femur.

alignment and ultimately resulting in nonunion. Failing bone to heal due to mechanical issues despite sufficient blood supply to the fracture site is known as hypertrophic nonunion (Chung *et al*., 2021). In another word, the lack of fixation stability combined with uncontrolled movement during the postoperative period would end with bone nonunion (Sushyam and Biswas, 2020).

Hence, restricting the movement after surgery, especially in the early recovery phase, to prevent any forces that could displace the fixation device or delay the healing process should be highly considered.

In respect to the rest of the cats in the groups A and B, the results of the post-operative assessment of lameness scoring system showed a remarkable similarity between

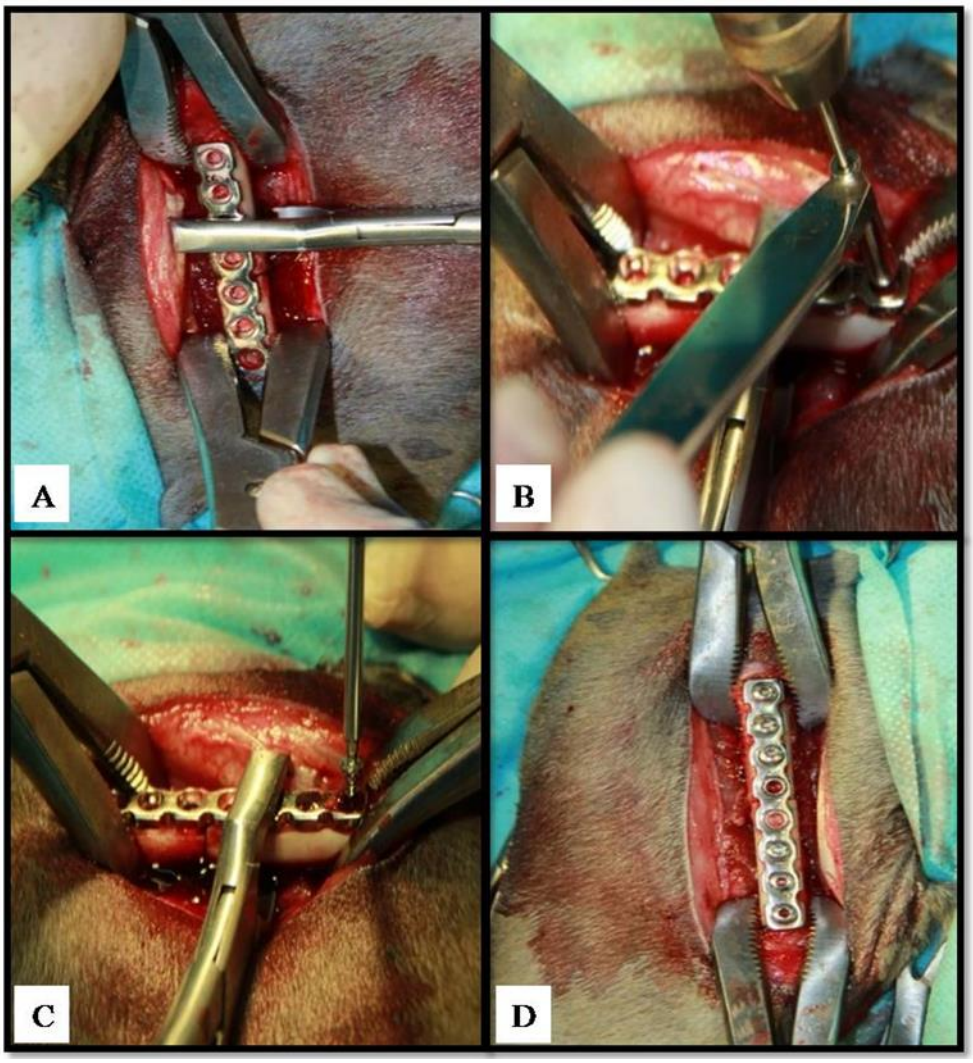


Figure 3. The steps of applying a bone plate to fix the induced mid-shaft femoral fracture in a cat. **A.** Aligning bone fragments and the bone plate. **B.** The drill guide to make a hole into the femur. **C.** Introducing self-taping bone screw. **D.** Placing the bone screw and fixing the plate.

the IMP and VCP techniques. The cats were able to walk without any signs of lameness in approximately 30 days post-surgery. This was almost in agreement with results that reported by Ober *et al*. (2017) and Erwin *et al*. (2018) who used the double IMP and VCP techniques respectively. However, in terms of radiographic evaluation which was based on characteristics of callus formation and visibility of fracture line, there was a priority in the time of complete bone healing with using the double IMP. The cats which were treated with this method showed complete bone healing in the 8th week and the fixation devices were then removed. Whereas the cats that were treated using VCP in the group B, their bone healing was completed in the 12th week post-operatively and the devices were then removed.

The longer required time for complete bone healing using plate fixation as seen in the current study and the others (Könning *et al*., 2013) would be explained as a result of the greater surgical exposure, vascular damage and consequently post-operative soft tissue reaction (Zhu *et al*., 2002). Therefore, it might be acceptable to agree that VCP are the best for treating most of fracture types, but there is no doubt that a four weeks extra time for the complete healing is a significant disadvantage.

There were some limitations in the current study due to financial issues which allowed using only eleven healthy cats. Consequently, the successful outcome of comparing the two techniques should be taken with some kind of consideration to verify the superiority of the double IMP method. Despite such small sample size, the achieved results might promote a more extensive clinical study using the same methodology.

Conclusion

The use of the double IMP technique to treat mid-diaphyseal femoral fractures in cats was a practical and reliable technique. It required less operational and recovery time comparing with the VCP technique, by which the cats needed four more weeks for the complete bone healing. Based on the promising presented findings, it is highly recommended to conduct a larger-scale study to assess the efficacy of the double IMP technique to treat cats' mid-diaphyseal fractures in the field.

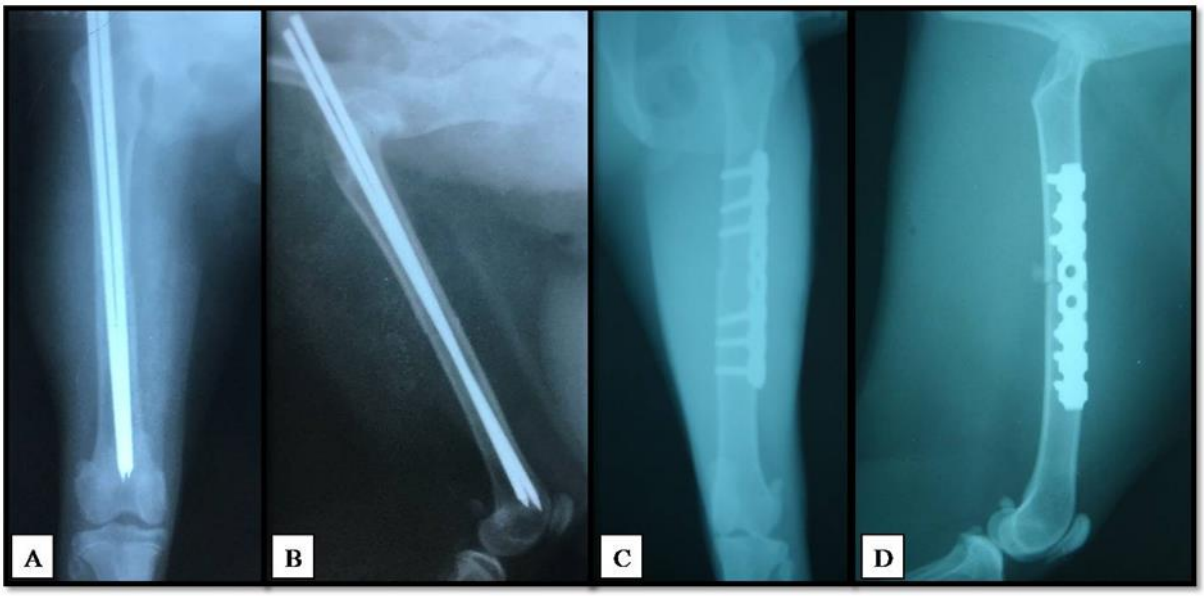


Figure 4. Radiographic views; cranio-caudal (**A&C**) and medio-lateral views (**B&D**), of right fractured femurs of two cats. **A**&**B.** Radiographs of a cat in the group A treated using double pining technique where the two intramedullary pins are well positioned in the medullary cavity of the femur. **C&D**. Radiographs of a cat in the group B treated using veterinary cuttable plate where the bone plate was properly positioned on the lateral aspect of the femur with three screws on each side of fracture line. All radiographs show proper reduction and alignment of bone fragments.

Author contributions

Nizar M. Khabuli performed the experimental work. Mohamed H. Abushhiwa and Lotfi M. Ben Ali designed the study, supervised the experimentation and drafted the manuscript. Aiman H. Oheida contributed in writing and approving the manuscript.

Conflict of interest

The authors declare no conflict of interest in relation to this work.

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