RESEARCH PAPER | Microbiology | Volume : 3 | Issue : 9 | Sept 2013 | ISSN - 2249-555X

Efficiency of Omental Transposition on the Healing of Tibial Fracture in Dogs

KEYWORDS: Tibia, Fracture, Omentum, Radiology, histology

AL-Timmemi HA	Ali A. Ajeel	Kalid K. Kadhim
Surgary Department, Faculty of Veterinary Medicine, Baghdad University, Iraq	Surgary Department, Faculty of Veterinary Medicine, Baghdad University, Iraq	Anatomy Department Faculty of Veterinary Medicine, Baghdad University, Iraq

Al-Jashamy	Karim
Pathology Department, Faculty of Medicine SEGI University, Malaysia	Pathology Department, Faculty of Medicine SEGI University, Malaysia

ABSTRACT *The tibia is one of the slowest-healing bones in the body especially at the distal diaphyseal fracture, so the objective of this study, was to investigate the efficacy of omentum transposition on the healing of tibial fracture of the dogs. Ten healthy mongrel dogs were used in this experiment. Tibial distal third fractures were induced and immobilized by intramedullary pins. The animals divided into two equal group (n=5). The first group as a control groups (CG) and the second groups used omental transposition as treated groups (TG). Comprehensive studies were including lameness grade, functional outcome, radiographic evaluation and histopathological examination. The clinical result showed that the lameness grade was improved on immediate postoperative day in all TG animals and the functional outcome was graded as excellent in three cases (60.0%) and good in two cases (40%). Radiographs and histopathological sample have been taken for each group. On day 56 post operations, the radiological results of the treated group showed complete disappeared of fracture line, remodeling of fracture site and clinical union was evidenced by closure of fracture. Histological result showed that the treated group had highly vascularized, remodeling of trabecular bone, good appositional surfaces and active of endochondral ossification. The conclusion of study, it is appeared the omental pedicle enhancing healing of the distal third tibial fracture.*

Introduction

Tibial fractures account for the third most common type of fracture after femur, radius and ulna (Seaman and Simpson, 2004), comprise 21.0 per cent (Unger et al., 1990) of all long bone fractures. Tibial diaphyseal fractures account for 75.0% to 81.0% of all tibial fractures (Boone et al., 1986). The goal of any fracture treatment is to restore the anatomical shape of the fractured bone to promote stability of fracture with suitable implants and enable the limb to early ambulation. The fracture repair techniques using bone plates, external fixators, interlocking nails, intramedullary pins and external coaptation are currently practiced in the tibial fracture management (Glyde and Arnett, 2006). During fracture healing, new blood vessels sprout from existing blood vessels to restore blood supply and to facilitate bone regeneration. Inadequate bone vascularity is associated with decreased bone formation and bone mass (Carano and Filvaroff, 2003). The tibia is one of the slowest-healing bones in the body, probably because it seldom has a good hematoma from which to form a callus. It is commonly that the distal diaphyseal tibial fracture was healing more slowly (Piermatti and Flo, 1997), because the blood circulation to this part of the bone is scanty (Leonard, 1971).

The omentum is a serious membrane made up of a lattice of blood vessels and fat. It is composed of two mesothelial sheets. It is ventrally attached to the greater curvature of the stomach to the spleen and to the left lobe of the pancreas The omentum releases polypeptide growth factor and activated macrophages, which results in capillary ingrowth into surrounding tissue (Liebermann., 2000), because of the omentum's antimicrobial defense mechanisms and it has ability to stimulate the neovascularization (Beelen, 1991), lymphatic and angiogenic capacities (Singh et al., 2008). Vascular endothelial growth factor (VEGF)-A of omentum stimulates angiogenesis because it's binding to VEGF receptors that promotes endothelial cell migration and proliferation, which is required for the development of new blood vessels (Ferrara, 2002). VEGF-A increases vascular permeability that may contribute to angiogenesis. According to the authors' knowledge, this is the first experimental research, in which the greater omentum transposition has been used in fracture bone healing. The objective of this study, was to investigate the efficacy of omentum transposition on the healing of tibial bone fracture of dogs

Materials and Methods

Experimental Animals Design

Ten healthy mongrel adult dogs (2-3years old), weighing 12-18 kg were used in this study. Dogs were housed in individual cages, fed with commercial food and given water ad libitum. The animals were kept in their respective cages for 15 days for acclimatization before experimentation. Broad-spectrum antibiotic injection of 22000IU pencillin and 20mg streptomycine oxytetracycline (Cipla, India, 200 mg), 1 ml/kg was given IM daily for five days. Antihelmintic injection of 0.4 mg/kg Ivermectin (Biomectine, Vetoquinol Ltd. Lure cedex, France, 10mg) at 0.4 ml/kg concentration subcutaneously (SC) was given on the first day and day 14 of acclimatization.

The experimental protocols, animal ethics and animal welfare were approved by the Animal Care and Use Committee (VETBAG/12.2.013/Surg 7), College of Veterinary Medicine, Baghdad University. Dogs were randomly divided into two groups (n=5). The tibial bone of the left side in all animals was fractured. In the first group, referred as the positive control group (CG), the tibial induced fracture was reduction and fixation with intramedullary pin. In the second group, the tibial induced fracture was treated with omental transposition group (TG) All animals of both groups were euthanized on days 56 post operations (PO).

Control Group (CG)

The tibial fracture was immediately reduced and fixation using the standard normagrade intramedullary pinning technique, which described below. Radiological assessment data

Volume : 3 | Issue : 9 | Sept 2013 | ISSN - 2249-555X

were recorded at the end of the study on day 56 PO. The animals were then euthanized and the splinting bone was collected for histopathology.

Anesthesia
The dogs were fasted for two hours prior to the anesthesia. Induction of anesthesia was achieved by intramuscular injection of a mixture of 15mg/kg Ketamine hydrochloride (Bioketan, Vetoquinol Biowet, Sp. Zo.O, France), 5mg/kg of Xylazine hydrochloride (ILIUM XYLAZIL-20, Australia) and Acepromazine maleate 1 mg/kg (Calmivet. Vetoquinol. Ltd. Lure cedex, France).

Modified Surgical Protocol
Hair on the skin of the dog was clipped from the lateral and caudal aspect of the left hind limb up to the level of the wing of the ilium dorsally to the level of the sacrum caudally and the tarsal joint distally. The skin was disinfected with Chlorohexidine gluconate (hibiscrub, 4% w/v Durham, UK), 70% Isopropyl alcohol (Jaya Pelita Pharma. SDN. BHD) and disinfected with 1.8% tinctured Iodine (Jaya Pelita Pharma SDN. BHD).

The paw was excluded from tarsal joint to the end of the limb and from the surgical area, by placing a latex glove over the distal extremity and securing it to the limb with a tape. The glove was covered with sterile skin towel and secured to the limb with towel clips. The animal was placed on the right lateral recumbency. The left hind limb was draped with the aperture of the fenestrated drape located at the intended operation site. The stifles joint was palpated and tarsal joint used as landmarks. The skin was curvilinear on the craniomedial crus from stifle to tarsal joint, using a scalpel blade ≠ 21. Subcutaneous tissue was incised on the same line using scalpel blade size 15, the muscle separated by blunt dissection using Mayo scissors to expose the tibial bone. A hand saw was used to induce fracture at the distal third of tibia.

The fracture of tibial bone was immediately fixed using normograde intramedullary pinning. To insert a Steinmann pin in the tibia, the pin is placed through the proximal fragment entering just medial and caudal to the tibial tuberosity of the medial side of the patellar ligament. Following introduction of the pin in the proximal fragment, the fracture is reduced and the pin is seated normally (Fig. 1).

A simple continuous suture was applied on the superficial fascia using 3-0 Vicryl (Biovek, Dynek Pty Ltd, Australia) with simple continuous suture and the skin was closed with using a 3-0 Vicryl subcuticular suture pattern. All animals were given postoperative analgesia of 10 mg/kg Tramadol hydrochloride (Domadol® India, 50 mg) at 0.2 ml/kg intramuscular by administered at 12-hour intervals for three consecutive days. Coaptation Meson Meta splint is applied to the planter side of the limb over padding and then taped into place for one week as supporting external splint.

Omental Pedicle Transposition (TG)
Hair on the midline abdominal wall was clipped off and disinfected from the xiphoid cartilage to the pubic region. Following splinting of tibial fracture bone as described in the control group, the position of the animal was changed to dorsal recumbency to create the omental pedicle. The abdominal wall was incised at the ventral midline 5-cm from xiphoid cartilage to umbilical region with scalpel blade size 20. Subcutaneous tissue and fascia was incised using scalpel blade size 15. The omentum was extended by releasing the dorsal leaf of omentum by blunt dissection from the duodenum and descending colon, which was then incised inversion L-shaped to provide double length of the omental pedicle (Fig. 2).

The omental pedicle was extended caudally on the peritoneal surfaces of the abdominal wall, at the level of the femoral bone. A separation between the semimembranous and adductor muscles was created by blunt dissection using curved Kelly forceps. The upper part of the abdominal wall was then perforated and the omental pedicle gently retracted using curved Kelly forceps. The animal's position was then changed to the right lateral position. Grasping the omental pedicle with curved Kelly forceps, the former was draw to the site of fracture bone through the tunnel under the skin. The omentum was wrapped around the fracture bone area and fixed with long digital extensor muscle by two stitches of 3.0 vicryl simple interrupted suturing (Fig. 3).

The midline incision was closed using 3.0 vicryl simple continuous sutures and subcutaneous tissue was closed using 3.0 vicryl, modified Cushing sutures. The skin was closed using 3.0 vicryl subcuticular sutures. Muscles and subcutaneous tissue of the crus were closed using 3.0 vicryl simple continuous sutures and lastly the skin was closed using 2.0 vicryl subcuticular sutures.

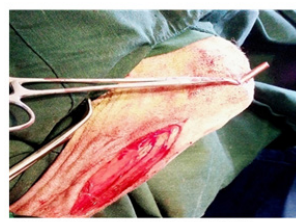
Figure (1): Photograph showing Steinmann pin normograde inserted in tibial fixation.

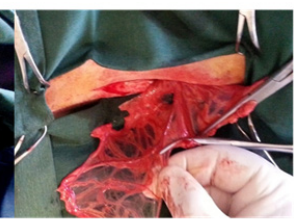
Figure (2): Photograph showing created omental pedicle.

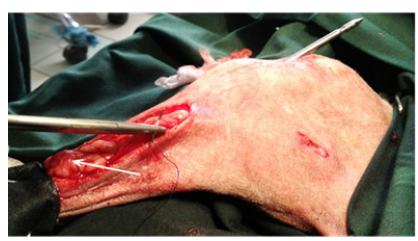
Figure (3): Photograph showing omental pedicle (arrow) wrapped around fracture site.

Radiography
Radiographs (craniocaudal, mediolateral) of the tibia were taken on immediate postoperative time based on 'four A's (apposition, alignment and angulation and apparatus position) and then at 8 weeks (56 days PO). On follow-up radiographs, apposition and alignment of fractured fragments with adequate cortical contact between fractured fragments were maintained at immediate postoperative day radiographic evaluation of activity, clinical union was noticed at 56th postoperative day in all treated cases and secondary bone healing was evidenced by formation of periosteal callus. Clinical union was evidenced by closure of fracture gap and soft tissue swelling at fracture site was noticed

Histological Examination
The animals were euthanized on 56 day PO after radiographical assessment. All the soft tissues were removed around the bones and the osteotomy site were studied grossly. A 2 cm segment of the bone symmetrically encompassing the repair site was harvested. The samples were fixed in phosphate-buffered formalin, decalcified in 10% nitric acid followed by rinsing in tap water for 5 hours and air-drying, then put in formalin for one more week. The samples were then dehydrated, and embedded in paraffin wax. Five-micrometer serial sections of the midsubstance of the bone were cut. The sections were stained with hematoxylin and eosin, and examined in a blinded fashion. Histological sections were evaluated for evidence of bone union and the relative amounts of bone, cartilage, and fibrous connective tissue and periosteal callus surrounding the osteotomy site.

Result
Lameness Grade
A lameness grade was assigned on the basis of severity of clinical signs on operatively at 1st, 7th, 14th, 30th and 56th

Volume : 3 | Issue : 9 | Sept 2013 | ISSN - 2249-555X

postoperative day to assess the response to treatment. Weight bearing was graded as followed by Vasseur et al. (1995). The lameness grade was 5 on operative day. Dogs started to bear weight on the operated limb on 7th to 10th postoperative day and walked normally without any signs of pain or limping 15 to 20 days after the operation. The lameness grading is represented in Table 1.

Functional Outcome

Functional outcome was evaluated on the 56th postoperative day and categorized as excellent, good, fair and poor in all animals (Clark, 1986). The assessment was subjective and based on individual evaluation. The functional limb outcome of the treated group was graded as excellent in three cases (60.0%) and good in two cases (40%), rather than control group was graded as good in two (40%), fair in two (40%) and poor in one case (20%)(Table 1).

Postoperative Complications

Seroma formation was observed in two case of control group at 1st postoperative day. Abduction rotation of operated limb was noticed at 7th postoperative day in one case of treated group

Table 1: Lameness grades and Functional outcome

Case No	Lameness grade					Functional outcome			
Cont group	Day 1	Day7	Day14	Day28	Day56	Excellent	Good	Fair	Poor
1	5	4	3	3	1	-	+	-	-
2	5	4	4	3	2	-	-	+	-
3	5	4	3	3	2	-	-	+	-
4	5	4	3	2	1	-	+	-	-
5	5	4	3	3	2	-	-	-	+
Treat group									
1	5	3	1	1	1	+	-	-	-
2	5	3	1	1	1	+	-	-	-
3	5	3	1	1	1	-	+	-	-
4	5	4	2	2	1	-	+	-	-
5	5	3	1	1	1	+	-	-	-

Radiology

Radiography of control group on day 56 post operation revealed still fracture line, little periosteal callus formation at the osteotomy site, soft tissue swelling at fracture site, inadequate cortical contact between fractured fragments, radiolucent at the site of fracture, periosteal reaction is not appeared and no clinical union (Fig. 4). Radiography of treated group on day 56 PO showed complete disappeared of fracture line, characteristic periosteal reaction, good remodulation of osteotomy site, radiopaque at the site of fracture, complete radiographic union and clinical union was evidenced by closure of fracture gap (Fig. 5).

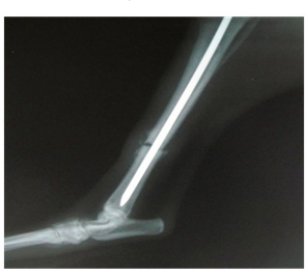

Figure (4): Radiograph of control group on day 56 PO showing fracture line, little periosteal callus.

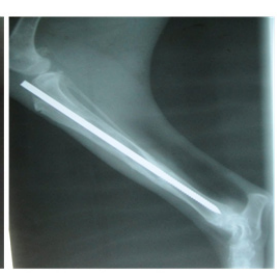

Figure (5): Radiograph of treated group on day 56 PO showing complete disappearance of fracture line, remodeling of callus formation and complete radiographic union

Histopathology

The results of histopatholoy on day 56th PO showed the greater omentum changed to a dense tissue attached firmly to the periosteal bone (Fig. 6). The trabicular bone completely bridged the bone defect in treatment group, highly vascular at the periosteal site of newly trabicular bone and scanty of endochondral site of ossification for replacement tissue formed during the remodeling of bone matrix (Fig. 7) and corticule bone is then laid down to reconstruct the shaft walls of the fracture bone (Fig. 8). Whereas, in control group the fracture line was only partially bridged by mixed soft callus and fibrocartilaginous tissue and osteoblasts begin to form spongy bone, which bulges from the fracture site and slowly calcifies (Fig. 9).

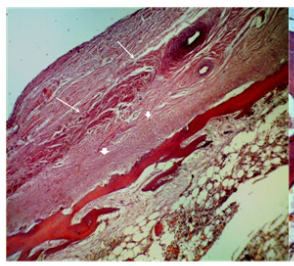

Figure (6): Micrograph of the treated group showing omental pedicle (arrows) adhered with periosteal site (arrows head). H&E X 200

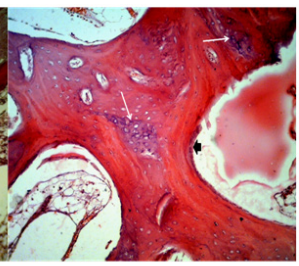

Figure (7): Micrograph of the treated group showing endochondrol ossification (arrows) and good osteoblastic appositional surfaces (arrow head). H&E X 200

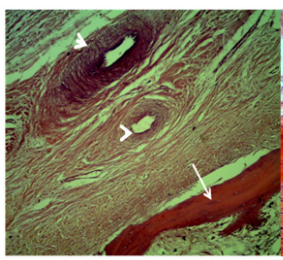

Figure (8): Micrograph of the treated group showing highly vascularized of periosteal site (arrows head) and remodeling of trabecular bone to compact bone (arrow). H&E X 200

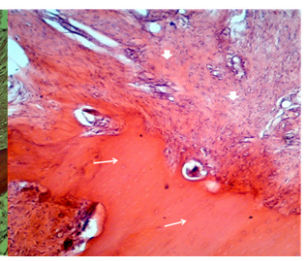

Figure (9): Micrograph of the control group showing fibrocartilaginous tissue (arrow head) bulges from the fracture site (arrows) and slowly calcifies. H&E X 100

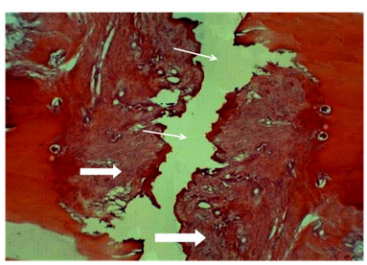

Figure (10): Micrograph of the control group showing fractureline (thin arrows) and fibrocartilaginous tissue bulges from the bone fragments (thick arrows). H&E X 200

Discussion

The results of this study showed that the omental pedicle promote healing of distal third tibial bone fracture. The radiological results of this study showed that the treated group (omental transposition) complete disappeared of fracture line with remodeling of callus formation and complete radiographic union, while the control group still revealed fracture line and few periosteal callus formation. Hispathological examination of treated group demonstrated remodeling of trabecular bone, good osteoblastic appositional surfaces and active of ossification processes. This result is in agreement with previous studies which reported that healing of tibeal defect treated with omental graft can influence bone healing through augmentation of vasculogenesis, as an essential element for proper bone healing (Oloumi et al., 2006). It is to be noted that this is the first study undertaken where the omental pedicle were used in fracture of long bone.

Normal weight bearing on all limbs at rest and when walking which was graded as 1 and this was attributed to omentum might secrete analgesic substances such as opioids, Beta-endophrin, Met-enkephalin, neurotransmitter including gamma aminobutyric acid (GABA), norepinephrine and other monoamines which play a role in the modulation of pain. Agner et al. (2001) reported the putative role of omentum analgesic substance in the mechanism of modulation of pain. Dogs started to bear weight on the operated limb on 7th to 10th postoperative day and walked normally without any signs of pain or limping 15 to 20 days after the operation. In the present study, all the dogs were evaluated for functional out-

Volume : 3 | Issue : 9 | Sept 2013 | ISSN - 2249-555X

come at 56th postoperative day, 3 cases (60%) had a successful return to function in 30 days of tibial fractures treated by omentum pedicle. The full functional limb of control group was with an average of 49 days.

Bone fracture results in disruption of the marrow architecture and blood vessels within and around the fracture site. During bone repair, the three components of the normal bone blood supply, medulary, periosteal, and osseus can be enhanced according to physiological need (Glowacki, 1998). The role of the vascular response in the healing of fractures has been well documented in the literature (Kurdy et al., 1997), so that the fracture of the tibia in the dog mostly suffering of delay and non-union due to poor in blood supply leading to slow healing (Leonard, 1971).

Vascular invasion of the fracture hematoma is a crucial step in the progress to union, as Chidgey et al. (1986) demonstrated that the return of mechanical integrity of the fractured bone is in direct relation to the vascular reorganization at the fracture site. The vascular response observed during fracture healing may be conveniently divided into two phases, an initial and generalized vasodilation response for whole injured limb (Gregg et al., 1983) and more simultaneous of localized vascular invasion of the hematoma and fracture gap, which characterized by new capillary and small vessel formation (Rhinelander et al., 1962). The maximum increase in blood flow at the fracture site has been observed during the first 14 days after injury (Taylor et al., 1991).

In this study, the callus was more prominent in the treatment group and also the vasculogenesis was significantly higher in treatment group. Since the only variable between the two groups was the presence of greater omentum transposition in treatment group, this difference can be attributed to it. In tibial fractures, the pattern and rate of fracture healing is ultimately dependent on the viability of the local circulation and its capacity to elicit a response (Trueta, 1963).

The greater omentum might play important role in the healing that might be a number of polypeptide growth factors possessed potent angiogenic properties have recently been identified. Zhang et al. (1997) analyzed the level of vascular endothelial growth factor (VEGF) protein in a number of rat tissues. The omentum demonstrated the highest VEGF secretion rate as well as the highest concentration of VEGF protein of the various rat tissues and organs examined. They suggested that VEGF is the major angiogenic factor produced by omentum and possibly underlies the mechanism omentum-induced angiogenesis. Also, it has been demonstrated that VEGF activity is essential for normal angiogenesis and appropriate callus architecture and mineralization in response to bone injury and the production of this growth factor is the major mechanism by which angiogenesis and osteogenes are tightly coupled during bone repair (Peng et al., 2002.; Carano and Filvaroff, 2003; Vadasz et al., 2004).

In the other study by Matoba et al. (1996), omental implantation was used for repair of perforated gastric ulcer in rat. Greater anti-inflammatory, angiogenic activity and accelerated collagen synthesis were seen in omental implantation group. Basic fibroblast growth factor (bFGF)-mediated angiogenesis was noted in this group, as well as transforming growth factor beta-1 (TGF-b1) activity within and around the omentum, resulting in abundant collagen production. Both bFGF (Liang et al., 1999) and TGF-b (Bostrom and Asnis 1998) are reported to have positive effects on bone repair. The angiogenic activity of omental fat is also documented (Silverman et al., 1988).

Activation of the omentum at the site of the injury created, led to the expansion of the mass of non-adipose part (milky spots), increased blood vessel density and as well as increased macrophages, B-lymphocyte, T-lymphocyte, mast cells, and stromal cells population which were important factor for bone regeneration. Macrophage release platelet-derived growth factor (PDGF) and transforming growth factor-beta 1, both of which stimulate bone production (Lieberman et al., 2002).

The fracture bone within hours, a transient extraosseous blood supply emerges from surrounding soft tissues, revascularizing the hypoxic fracture site (McLaughlin, 1991). Mononuclear phagocytes delivered by these new vessels assist in the removal of necrotic bone and aid in construction of the callus. Macrophages are also believed to orchestrate the orderly sequence of cutaneous wound healing and would play a similar role in fracture repair. They contain several growth factors, such as fibroblast growth factor (FGF), and initiate fibroplasia both in soft tissue as well as in bone repair (McLaughlin, 1991).

Low oxygen tension, poor vascularity, growth factors, and interfragmentary strain influence the elaboration of a cartilaginous callus. The periosteum surrounding the fracture site thickens prior to undergoing chondrogenic transformation; thereby producing an external callus entirely vascularized by extraosseous vessels (Remedios, 1999). An internal or medullary callus develops from the endosteal cell layer and is confined to the medullary canal and receives its blood supply derived from medullary arterioles (Dudley et al., 1997). The presence of a fibrocartilage layer within the medullary canal temporarily interrupts the medullary blood flow across the fracture gap. Both the external and the internal callus constitute the "bridging callus" (Binnington, 1990). Although the mechanical properties of this calcified fibrocartilaginous tissue have not been reported, these structures contribute greatly to the restoration of strength and stiffness within the fracture gap, thus allowing formation of compact bone. Mineralization of the soft callus proceeds from the fragment ends toward the center of the fracture site and forms a "hard callus" (Rahn, 2002).

The most extensively studied osteoinductive factors which are released from omentum such as transforming growth factor-b superfamily of morphogenetic proteins, comprising structurally and functionally related proteins, such as transforming growth factor-b and bone morphogenetic protein (BMP), which play significant roles in embryonic development and tissue repair as well as in bone development (Miyazono et al., 2004). Transforming growth factor-b, one of the major growth factors present in bone matrix, is a polypeptide synthesized and secreted in bone cultures. It is believed that the primary role of transforming growth factor-b in bone formation is to increase the pool of committed osteoblasts. BMPs are identified and cloned after the discovery that extracts from demineralized human bone matrix induce ectopic bone formation (Wozney et al., 1988). The major angiogenic factors include fibroblast growth factors, platelet-derived growth factor and vascular endothelial growth factor (VEGF). Fibroblast growth factors and VEGF stimulate endothelial cell production of proteases and plasminogen activators that degrade the vessel basement membrane and allow endothelial cell proliferation and migration (Cross and Claesson-Welsh 2001). Endothelial cells and endothelial progenitor cells then assemble to form new blood vessels and secrete key factors, such as platelet-derived growth factor and angiopoietin-1, to recruit smooth muscle cells and pericytes to stabilize and support the newly formed blood vessels (Lindahl et al., 1998). Growth factors, which stimulate angiogenesis, also drive bone repair and regeneration. In conclusion, it is appeared the omental pedicle enhancing healing of the distal third tibial fracture.

Acknowledgements:
Authors would like to thank Surgery Department/ College of Veterinary Medicine/ Baghdad University which was provided the facilities for accomplished the research.

Volume : 3 | Issue : 9 | Sept 2013 | ISSN - 2249-555X

REFERENCE

Agner, C, Yeomans, D and Dujovny, M (2001). The neurochemical basis for the application of the greater omentum in neurosurgery. Neurolog Resear-ch 23: 7-15. | Beelen, RH (1991). The greater omentum: physiology and immunological concepts. Neth J Surg., 43(5):145–149. | Binnington, AG (1990). Bone remodeling and transplantation. Wittick WG (ed), Canine Orthopedics. 2nd ed. Section III: Preparation, principles, and procedures for surgery. Philadelphia: Lea & Febiger, pp. 166–189. | Boone, EG, Johnson AL, Montavon P and Hohn RB 1986. Fractures of the tibial diaphysis in dogs and cats. J Am Vet Med Assoc, 188: 41-45. | Bostrom, MP and Asnis, P (1998). Transforming growth factor beta in fracture repair. Clin. Orthop. Suppl., 355: 124–131. | Carano, RA and Filvaroff, EH (2003). Angiogenesis and bone repair. Drug Discov Today., 8: 980–989. | Chidgey, L, Chakkalakal, D, Blotcky, A and Connolly, JF (1986). Vascular reorganization and return of rigidity in fracture healing. J. Orthop. Res., 4: 173-192. | Clark, DM (1986). Treatment of open comminuted intraarticular fractures of the proximal ulna in dogs. J Am Anim Hosp Assoc, 23:311-336. | Cross, MJ and Claesson-Welsh, L (2001). FGF and VEGF function in angiogenesis: signalling pathways, biological responses and therapeutic inhibition. Trends Pharmacol Sci., 22: 201–207. | Dudley, M, Johnson, AL, Olmstead, M, Smith, CW, Schaeffer, DJ and Abbuehl, U (1997). Open reduction and bone plate stabilization, compared with closed reduction and external fixation, for treatment of comminuted tibial fractures: 47 cases (1980–1995) in dogs. J Am Vet Med. Assoc., 211(8): 1008–1012. | Ferrara, N (2002). Role of vascular endothelial growth factor in physiologic and pathologic angiogenesis: therapeutic implications. Seminars in Oncology., 2910-14. | Glowacki, J. 1998. Angiogenesis in fracture repair. Clin. Orthop. Suppl., 355, S82–S89. | Glyde, M and Arnett R (2006). Tibial fractures in the dog and cat: options for management. Iris Vet J, 59: 290-295. | Gregg, PJ, Barsoum, MK and Clayton, CB (1983). Scintigraphic appearance of the tibia in the early stages following fracture. Clin. Orthop., 175: 139–146. | Kurdy, NM, Weiss, JB and Bate, A. (1996). Endothelial stimulating angiogenic factor in early fracture healing. Injury, 27: 143–145. | Leonard, E (1971). Orthopedic surgery of the dog and cat. Sauders. W. B. pp. 97-144. | Liang, H, Pun, S, Wronski, TJ (1999). Bone anabolic effects of basic fibroblast growth factor in ovariectomized rats. Endocrinology., 140: 5788–5789. | Lieberman, JR, Daluiski, A and Einhorn, TA (2002). The role of growth factors in the repair of bone. J Bone Joint Surg., 84(6):1032–1044. | Liebermann-Meffert, D (2000). The greater omentum. Anatomy, embryology and surgical applications. Surg Clin North Am., 80 (1):275–93. | Lindahl, P, Hellstrom, M, Kalen, M and Betsholtz, C (1998). Endothelial- perivascular cell signaling in vascular development: lessons from knockout mice. Curr Opin Lipidol., 9: 407–411. | Matoba, Y, Katayama, H, Ohami, H (1996). Evaluation of omental implantation for perforated gastric ulcer therapy: findings in a rat model. J. Gastroenterol. 31: 777–784. | McLaughlin RM (1991). The evolution of the understanding of bone healing. Vet Comp Orthop Traumatol., 4: 16–20. | Miyazono, K, Maeda, S and Imamura, T (2004). Coordinate regulation of cell growth and differentiation by TGF-beta superfamily and Runx proteins. Oncogene., 23: 4232–4237. | Oloumi, MM, Derakhshanfarb, A, Molaeia, MM and Tayyebi, M (2006). The angiogenic potential of autogenous free omental graft in experimental tibial defects in rabbit: Short-term preliminary histopathological study. J. Exper Ani Sci., 43: 179–187. | Peng, H, Wright, V, Usas, A, Gearhart, B, Shen, HC, Cummins, J and Huard, J (2002). Synergisitic enhancement of bone formation and healing by stem cell-expressed VEGF and bone morphogenetic protein-4. J. Clin. Invest. 110: 751–759. | Piermatti, D and Flo, G (1997). Small animals orthopedic and fracture repair.3rd Ed.Saundera Comp. pp 581-606. | Rahn, BA (2002). Bone healing: histologic and physiologic concepts. Fackelman GE (ed), Bone in Clinical Orthopaedics. Stuttgart New York: Thieme. pp. 287–326. | Remedios, A. (1999). Bone and bone healing. Vet Clin North Am Small Anim Pract., 29(5): 1029–1044. | Rhinelander, FW, Phillips, RS, Steel, WM and Beer, JC (1962). Microangiography in bone healing. II. Displaced closed fracture. J. Bone Jt. Surg., 44: 1273-98. | Seaman, JA and Simpson AM (2004). Tibial fractures. Clin Tech Small Anim Pract, , 19,151-167. | Silverman, KJ, Lund, DP, Zetter, BR, Lainey, LL, Shahood, JA, Freiman, DG, Folkman, J, Barger, AC (1988). Angiogenic activity of adipose tissue. Biochem. Biophys. Res. Commun., 153: 347–352. | Singh, AK, Patel, J, Litbarg, NO, Gudehithlu, KP, Sethupathi, P, Arruda, JA and Dunea, G (2008). Stromal cells cultured from omentum express pluripotent markers, produce high amounts of VEGF, and engraft to injured sites. Cell Tissue Res., 332 (1):81–88. | Taylor, CM, Thompson, JM and Weiss, JB (1991). Matrix integrity and the control of angiogenesis. Int. J. Radiat. Biol., 119: 61–64. | Trueta, J (1963). The role of the vessels in osteogenesis. J. Bone Jt. Surg. Ser. A 45: 402–418. | Unger, M, Montavon PM and Heim UFA (1990). Classification of fractures of the long bones in the dog and cat: Introduction and clinical application. Vet Comp Ortho Traumatol, 3: 41-50. | Vadasz, Z, Misselevich, I, Norman, D, Peled, E and Boss, JH (2004). Localization of vascular endothelial growth factor during the early reparative phase of the rats' vessels deprivation-induced osteonecrosis of the femoral heads. Exp. Mol. Pathol., 77: 145–148. | Vasseur, PB, Johnson AL, Buderberg SC, Linwln JB, Toombs JP, Whitebain JG and Lentz, EL (1995). Randomized, controlled trails of the efficacy of carprofen, a non-steroidal anti-inflammatory drug in the treatment of osteoarthritis in dogs. J Am Vet Med Assoc, 206: 807-811. | Wozney, JM, Rosen, V, Celeste, AJ, Mitsock, LM, Whitters, MJ, Kriz RW, Hewick, RM and Wang, EA (1988). Novel regulators of bone formation: molecular clones and activities. Science., 242: 1528–1534. | Zhang, QX, Magovern, CJ, Mack, CA, Budenbender, KT, Wilson, K and Rosenger, TK (1997). Vascular endothelial growth factor is the majorangiogenic factor in Omentum: mechanism of the Omentum-mediated angiogebesis. Journal Surgery Research 67: 147-154. |