Iraqi Journal of Veterinary Sciences, Vol. 20, No. 2, 2006 (163-172)

THE EFFECT OF BONE MARROW AUTOGRAFT ON FRACTURE HEALING WITH DESTRUCTION OF PERIOSTEUM AND ENDOSTEUM IN RABBITS

***Eesa M J, Thanoon M G, Ibrahim S M**

*Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Diyala, Baa'kuba, Iraq

Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

(Received September 29, 2005; Accepted March 5, 2006)

ABSTRACT

Ten mature rabbits was used in this study. The animals were divided into two equal groups. Femoral mid–shaft fractures were induced, and destruction of both periosteum and endosteum for about one centimeter around the fractured ends was performed. The fractured bone was immobilized by intramedullary pinning. Group 1 considered as control, while group 2 was implanted by sufficient amount of auto–bone marrow at the fracture site. The radiographic results revealed that, the destruction of both periosteum and endosteum leads to delayunion and some percentage of nonunion. While the bone marrow in group two promoted and enhanced the fracture healing.

تأثير الزرع الذاتي لنخاع العظم على التئام الكسور بعد تدمير السمحاق الخارجي والداخلي في الأرانب

***محمد جواد عيسى، ميسر غانم ذنون، سحر محمد إبراهيم**

* فرع الجراحة والتوليد، كلية الطب البيطري، جامعة ديالى، بعقوبة، العراق

فرع الجراحة والتوليد، كلية الطب البيطري، جامعة الموصل، موصل، العراق

الخلاصة

أُستخدم في هذه الدراسة عشرة أرانب ناضجة ومن كلا الجنسين، قسمت إلى مجموعتين متساويتين. تم إحداث كسر في منتصف عمد عظم الفخذ مع تدمير السمحاق الداخلي والخارجي لمسافة واحد سنتمتر حول منطقة الكسر، ثم ثبت الكسر بوساطة سفود داخل النخاع. اعتبرت المجموعة الأولى مجموعة السيطرة أما في المجموعة الثانية فتم حقن نخاع العظم المأخوذ من نفس الحيوان في منطقة الكسر. أظهرت الدراسة السريرية والشعاعية بان تدمير السمحاق الداخلي والخارجي أدى إلى تأخر الالتئام فضلا عن نسبة من عدم الالتئام، في حين أن الزرع الذاتي لنخاع العظم في المجموعة الثانية أدى إلى تحفيز عملية الالتئام.

INTRODUCTION

Periosteum, endosteum and bone marrow next to the fracture focus provide cells that proliferate and differentiate in fibrous tissue, fibrocartilaginous and hyaline cartilage, all of them part of new bone formation (1, 2).

Quantitatively, the periosteum contributes more than bone marrow in healing in destruction (3, 4). The interaction between periosteum and bone marrow is significant (5, 6). The endosteum is a layer in the reticular connective tissue that lines the medullary cavity. Its pleuripotent cells sub serve both haematopoisis and osteogenesis (7). The periosteum together with endosteum acts as a limiting membrane of bone, controlling the ingress and egress of ions (8). Destruction of both periosteum and bone marrow result in absence of bone formation around the destructed area (9). Osteogenic precursor cells are stem cells derived from mesenchyme and have the potential to undergo mitosis and differentiation into mature cells (10). These cells are present in deep periosteal layer and in the endosteal layer, which lines the internal medullary surface (11).

Bone marrow contains many cellular elements that may contribute to fracture repair (6, 12). Bone marrow has been show to contain a population of rare mesenchymal stem cells that are capable of forming, bone, cartilage, and other connective tissue (13, 14). Bone marrow when placed in a fresh femoral defect and given in sufficient amounts, produced a rate of union comparable with that of autologus bone graft (15, 16). The object of this study was to investigate the ability of bone marrow for enhancing of fracture healing with destruction of both periosteum and endosteum.

MATERIALS AND METHODS

Ten mature rabbits from local breed weighting between 1.5–1.75 kg were used in this study. The animals were kept under the same conditions of housing and feeding during the experiments. Animals were divided into two main equal groups.

Group 1 (control group): The femoral bone was exposed and transverse mid–shaft fracture was induced. About one centimeter of periosteum and endosteum were destructed from each side of fractured ends.

Group 2 (experimental group): Similar procedure that described in group one, but in addition to that sufficient amount of bone marrow was injected directly into the fracture site.

Surgical operations were carried out under general anesthesia using atropine sulphate 1mg/kg B.W., intramuscularly, ten minutes later a mixture of xylazine hydrochloride and ketamine hydrochloride was administered intravenously at a dose 10, 40 mg/kg B.W., respectively. After inducing a transverse fracture in mid–shaft of femur, one centimeter of periosteum was stripped from each side of fractured ends by using surgical blade. In addition, one centimeter of endosteum was destructed from each side of fractured ends by using curved surgical blade. The fractured bone was immobilized by using intramedullary pinning (2.4ϕ x 120 mm Steinmann stainless steel). In group 1, closure of the muscles and subcutaneous tissue was performed by two rows of simple continuous technique using catgut No.3-0 and the skin closed by simple interrupted technique using silk No.1-0. While, in group two, following closure of muscles (as in group one), sufficient amount of bone marrow which was aspirated from the proximal part of femur of the other side from the same animal, was injected directly at the fractured site, then the surgical operation was completed similarly as in group one. After operation, the animals were injected intramuscularly penicillin–streptomycin at dose of 10.000 i.u, 10 mg/kg B.W., respectively for four days. The pins were removed at the end of ninth week in group 1, while at the end of

seventh week in group 2. The animals were observed daily for recording any information that related with movement of animals, condition of the fractured leg, and appearance of prominent callus formation. Radiographical studies of fracture healing were done weekly with anterioposterior and mediolateral views.

Results

Clinical observations revealed that, the inflammation at the site of operation appeared in both groups on 2nd days following operation. This inflammation then subsided on fourth and fifth days after operation. The callus formation was detected clinically by palpation at the site of fractured bone, which revealed that, the callus palpated at the end of 3rd week in both groups. However, the callus in group 2 was larger than group one. During palpation of fractured site, the movement of fractured ends disappeared at the 6th week in group 2, while in group 1 disappeared at 8th week in three animals, but this movement in remaining two animals was not completely absent until the end of experiment. Therefore depending on these results, the pins were removed at ninth and seventh week in group 1 and 2, respectively.

The results of radiographical studies of both groups are summarized in Table 1.

Table 1: Radiographic findings of the control and treated groups

Week	Group 1 (Control)	Group 2 (Treated)
1	There is slight periosteal proliferation at the distance from the fracture site. Clear fracture line.	There is slight periosteal proliferation at the distance from the fracture site. Clear fracture line.
2	The periosteum had become more active than in first week, and form external callus at the distance of the fracture site then begun to migrate into the fracture site. Clear fracture line (Fig. 1).	Increased external callus at the distance of the fracture site, which start to migrate to the fracture site. Clear fracture line.
3	The external callus became more prominent, but still not bridged the fracture site. Clear fracture line (Fig. 2).	The external callus was active and partially bridged the fracture site. Clear fracture line (Fig. 3).
5	Weak external callus bridged the fracture site. Clear fracture line (Fig. 4).	Thick external callus that completely bridged the fracture site especially from the medial side. Fracture line still clear.
6	External callus appeared thin in spite of bridging the fracture site. Still clear fracture line.	External callus similar to fifth week, while the fracture line mostly disappeared (Fig. 5).
7	Similar to sixth week.	The external callus was started to absorbed. The bone surface has begun to take the normal shape (Fig 6).

9	The external callus still appeared around the fracture site. Fracture line almost not clear (Fig. 7).	External callus mostly absorbed and the bone contour taken about its normal shape (Fig. 8).
12	Similar to that observed in ninth week. The fracture line not clear.	Absorption of external callus, and the bone taken nearly its normal shape (Fig. 9).
16	Absorption of external callus and the bone started to take its normal shape.	The bone was taken its normal shape.
20	The bone was taken nearly its normal shape.	

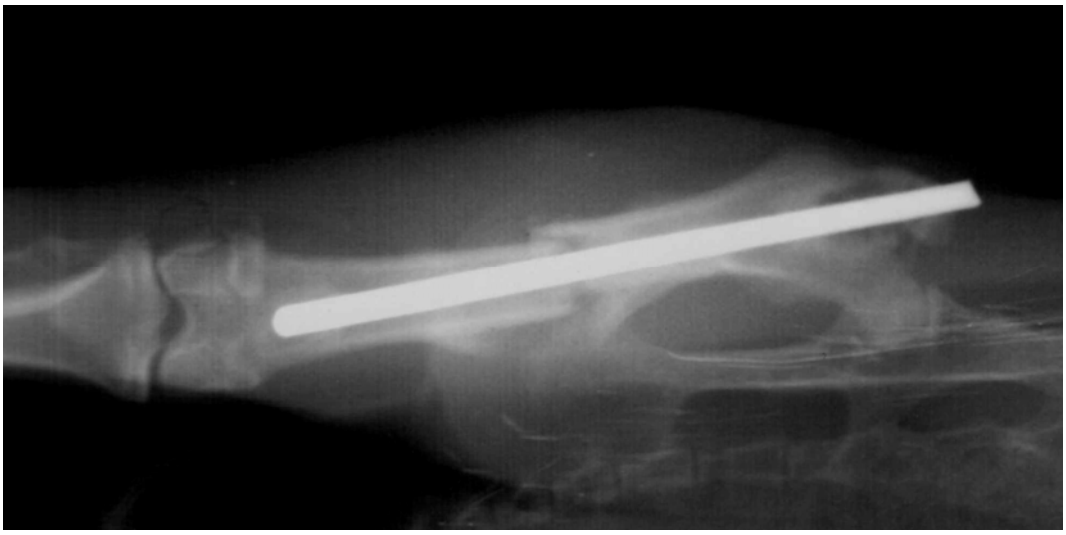

Fig. (1): Radiograph of the femoral bone of rabbit in group 1, two weeks after operation. Notice the external callus at a distance of the fracture site then begun to migrate into the fracture site. Clear fracture line.

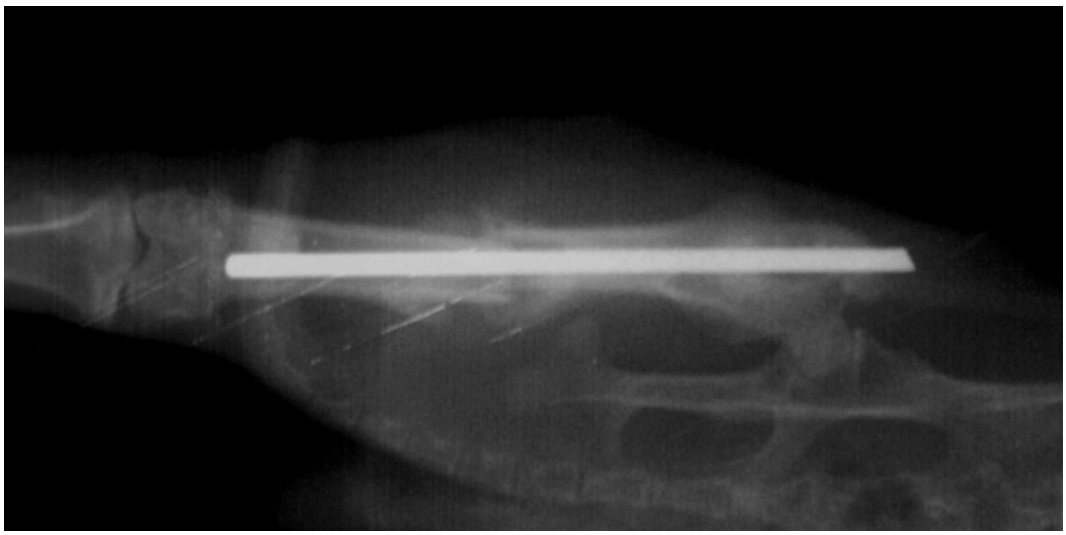

Fig. (2): Radiograph of the femoral bone of rabbit in group 1, three weeks after operation. Notice the external callus, which became more prominent, but still not bridging the fracture site. Clear fracture line.

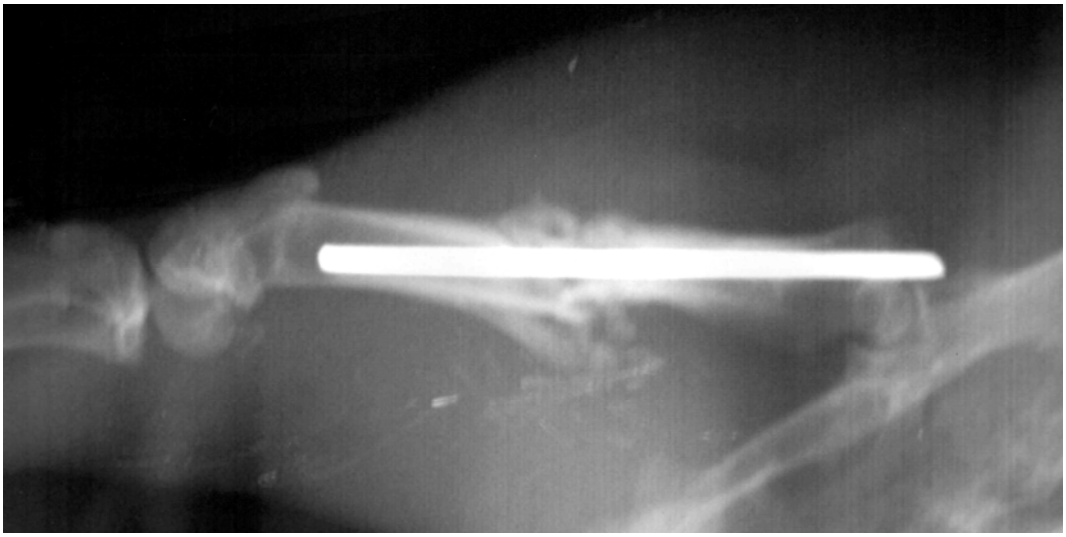

Fig. (3): Radiograph of the femoral bone of rabbit in group 2, three weeks after operation. Notice the external callus became more active and partially bridging the fracture site. Clear fracture line.

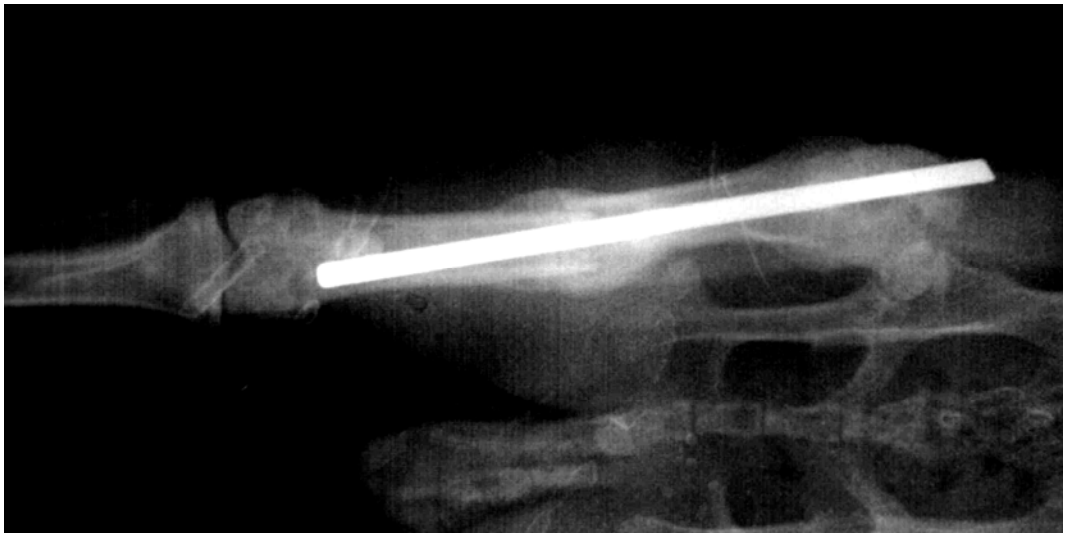

Fig. (4): Radiograph of the femoral bone of rabbit in group 1, five weeks after operation. Notice the weak external callus bridging the fracture site. Clear fracture line.

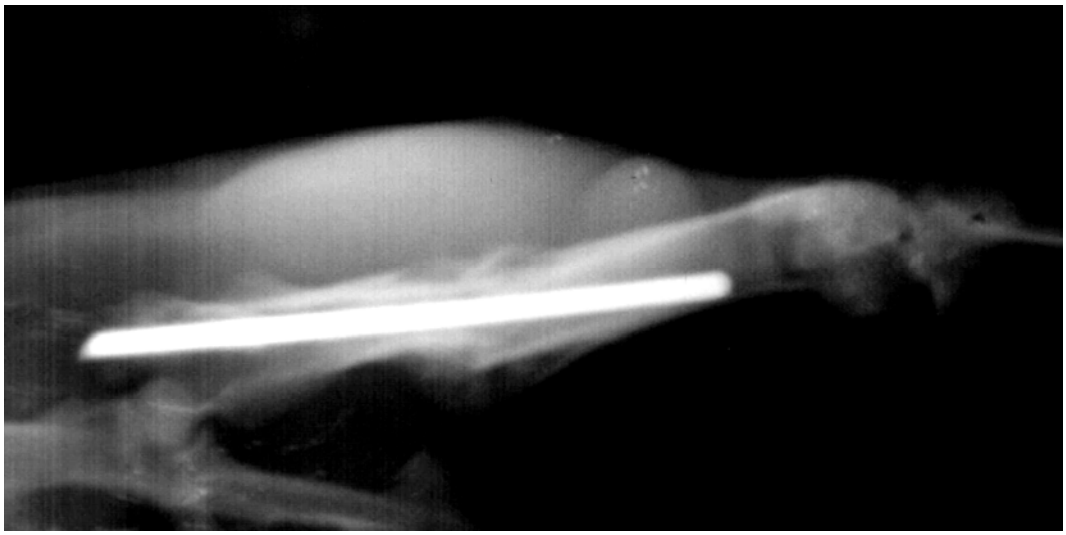

Fig. (5): Radiograph of the femoral bone of rabbit in group 2, six weeks after operation. Notice the thick external callus that completely bridged the fracture site especially from the medial side. The fracture line almost disappeared.

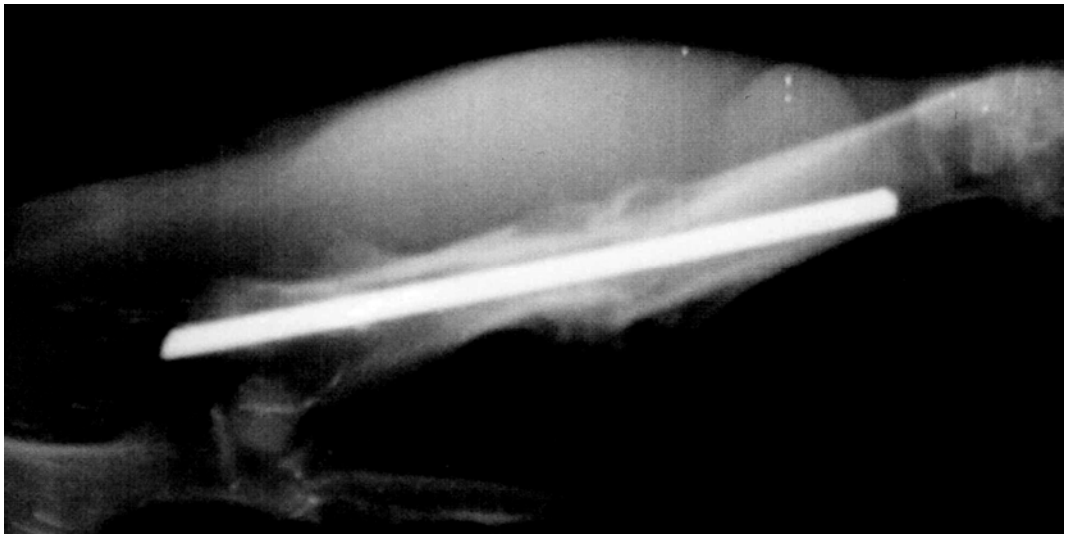

Fig. (6): Radiograph of the femoral bone of rabbit in group 2, seven weeks after operation. Notice the external callus that started to absorbed. The bone surface has begun to take the normal shape.

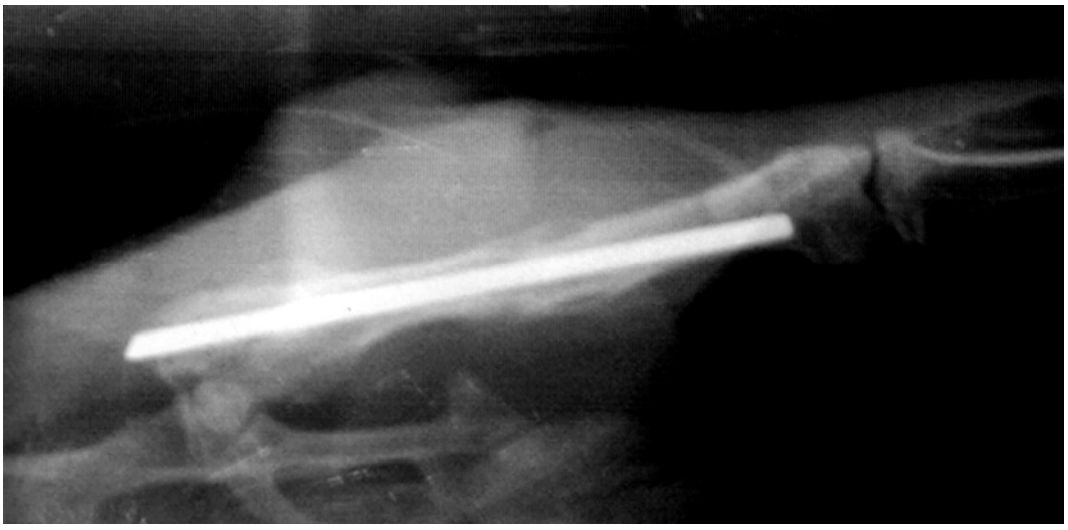

Fig. (7): Radiograph of the femoral bone of rabbit in group 1, nine weeks after operation. Notice that the external callus still appeared around the fracture site. Fracture line almost not clear.

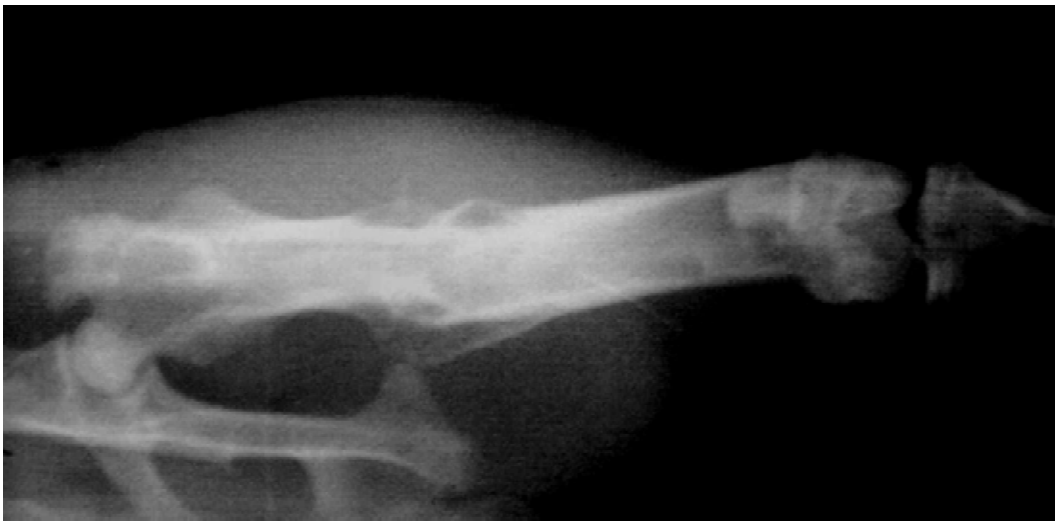

Fig. (8): Radiograph of the femoral bone of rabbit in group 2, nine weeks after operation. Notice the external callus almost absorbed and the bone contour taken about its normal shape.

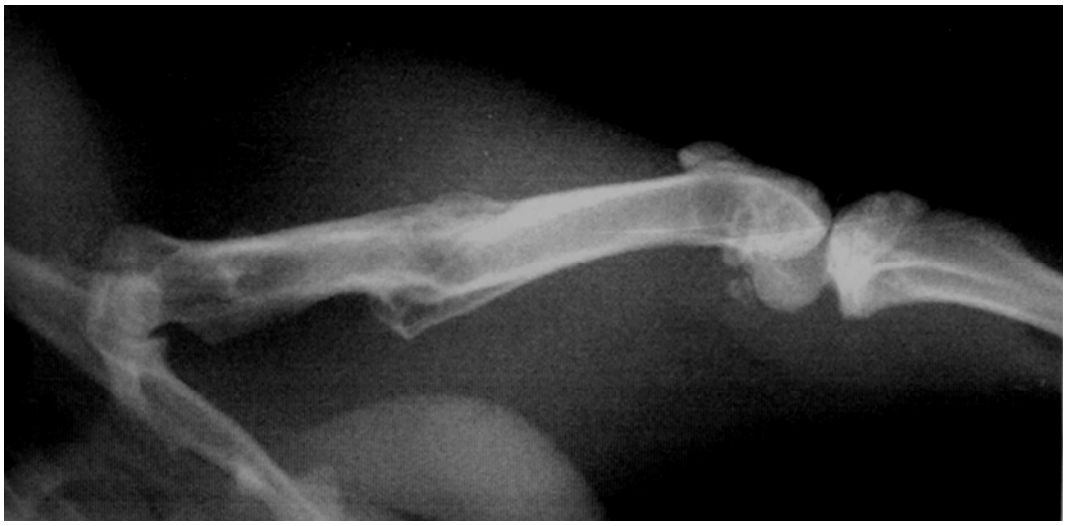

Fig. (9): Radiograph of the femoral bone of rabbit in group 2, twelve weeks after operation. Notice absorption of external callus, and the bone taken nearly its normal shape.

DISCUSSION

The periosteum, endosteum and bone marrow plays an important role in the fracture healing. Destruction of periosteum and endosteum lead to absence of bone formation around the destructed area (9). These observations was confirmed in our study whereby, the destruction of periosteum and endosteum, ended by nonunion in two animals of group 1, while the others (three animals) revealed delayunion. This variation of healing in the same group might be due to the activity and thickness of external callus produced from the periosteum and

migrated into the fracture site to connect the fractured ends together. The evidence of external callus at a distance from the fracture site at the 2nd week was observed in both groups. However, this callus appeared thicker and more active in group 2 than group one. These observations may be attributed to the role of bone marrow as a source of osteogenic cells, which agree with other workers (12, 17, 18, 9), whom said that, the bone marrow contains many cellular elements which may contributes to fracture repair.

The radiographical findings indicated that healing at the fracture site was observed in the seventh week in group 2. While, three animals of group one reached to the same stage of healing as in group two, at about the ninth week. These phenomena indicated that, there is a relationship between osteogenesis, periosteum, endosteum and bone marrow. These results coincides with other authors findings (1, 2). In group 2, the bone was taken about its normal shape at the twelfth week, while the bone in three animals of group one was taken at sixteenth week. This might be due to injection of fresh bone marrow at the fracture site which enhanced the steps of healing process and increased the rate of remodeling, and that was confirm by others (11, 19, 20), whom said that, bone marrow when placed in a fresh bone defects produces a rate of union.

In conclusion, the study indicated that, the periosteum and endosteum plays an important role in fracture healing. Destruction of both resulted in delayunion and nonunion in some cases, which depended on early, thick external callus formation to bridge the fractured ends. Implantation of sufficient amounts of bone marrow at the fracture site after destruction of periosteum and endosteum may be helped in the enhancement of fracture healing.

REFERENCES

1. Kirker–Head CA, Gerhart TN, and Armstrong R. Healing bone using recombinant human bone morphogentic protein 2 and copolymer. Clin Orthop 1998; 349: 205–217.
2. Street J, Winter D, Wang JH, and *et.al*. Is human fracture hematoma inherently angiogenic? Clin Orthop 2000; 378: 224–237.
3. Kierdorf V, Stoffels E, Stoffels D, Kierdorf H, Szuwart T, and Clemen G. Histological studies of bone formation during pedicle restoration and early antler regeneration in roe dear and fallow deer. Anat Rec A Discov Mol Cell E Vol Biol 2003; 273 (2): 741–751.
4. Song HR, Puri A, Lee JH, Park HB, Ra DK, Kim GS, and Yeon SC. Spontaneous bone regeneration in surgically induced bone defects in young rabbits. J Pediatr Orthop B 2002; 11 (4): 343–349.
5. Guichet JM, Braillon P, Bodenreider O, and Lascombes P. Periosteum and bone marrow in bone lengthening: a DEXA quantitative evaluation in rabbits. Acta Orthop Scan 1998; 69 (5): 527–531.
6. Zhou H, Choong PF, Henderson S, Chou ST, Aspenberg P, Martin TJ and Ng Kw. Marrow development and it's relationship to bone formation in vivo: a histological study using an implantable titanium device in rabbits. Bone J 1995; 17 (4): 407–415.
7. Roth GI, Calmes R. Oral biology. St Louis: CV Mobsy 1981; 153 – 156.
8. Coen PD. Spontaneous bone regeneration after mandible resection in a case of Ameloblastoma–A case report. Ann Acad Med Singapore 2004; 33 (suppl): 59–62.

9. Fawcett DW, Bloom and Fawcett. A textbook of histology. New York. Champmon and Hall 1994; 194–233.
10. Kalfes IH. Principles of bone healing. Neuro Surg Focus. 2001; 10(4): 1–4.
11. Devine MJ, Mierisch CM, Jang E, Anderson PC and Balian G. Transplanted bone marrow cells localize to fracture callus in a mouse model. J Othop Res. 2002; 20 (6): 1232–1239.
12. Bruder SP, Kraus K, Goldberg V and Kadiyala S. The effect of implants loaded with autologus mesenchymal stem cells on the healing of canine segmental bone defects. J Bone Joint Surg Incorporated 1998; 80A: 985–996.
13. Gregory CA, Ylostalo J and Prockop DJ. Adult bone marrow stem/ progenator cells (MSCs) are preconditioned by microenvironmental "niches" in culture: a two–stage hypothesis for regulation of MSC fate. Sci STKE 2005; 2005 (294): 37.
14. Werntz JR, Lane JM, Burstein AH, Justin R, Klein R and Tomin E. Qualitative and quantitative analysis of orthotopic bone regeneration by marrow. J of Orthopedic Res 1994; 14: 85–93.
15. Djapic T, Kusec V, Jelic M, Vukicevic S and Pecina M. Compressed homologous cancellous bone and bone morphogenetic protein (BMP)–7 or bone marrow accelerate healing of long–bone critical defects. Int Orthop 2003; 27 (6): 326–330.
16. Khanal GP, Garg M and Singh GK. A prospective randomized trial of percutaneous marrow injections in a series of closed fresh tibial fractures. Int. Orthop 2004; 28 (3): 167–170.
17. Siwach RC, Sangwan SS, Singh R and Goel A. Role of percutaneous bone marrow grafting in delayed unions, non unions and poor regenerates. Indian J Med Sci 2001; 55 (6): 326–336.
18. Matsuda Y, Sakayama K, Okumura H, Kawatani Y, Mashima N and Shibata T. Percutaneous autologous bone marrow transplantation for nonunion of the femur. Nippon Geka Hokan 1998; 67 (1): 10–17.
19. Ma HL, Chen TH and Hung Sc. Development of a new method in promoting fracture healing: multiple cryopreserved bone marrow injections using a rabbit model. Arch Orthop Trauma Surg 2004; 124 (7): 448–454.
20. Goel A, Sangwan SS, Siwach RC and Ali AM. Percutaneous bone marrow grafting for the treatment of tibial non union. Inj J 2005; 36 (1): 203–206.