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Using Xeno -Bovine Bony Implantation as Space Filler in Femoral Defect in Rabbits

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Abstract:

The aim of the present study was to employ the xeno- bovine bony implantation (XBBI) which prepared from ulna of calves as space filler in the induced femoral defect in rabbit s. Fifteen adult local breed rabbits were used. Under general anesthesia with highly aseptic technique, about 1cm of femoral bone diaphysis was removed and the empty space was replaced by the same size and diameter of XBBI and fixed internally by intramedulary pin (FGSR), the clinical observation revealed that the animals can use the limb normally in walking ,running and bear the weight at the end of the 4th week post operation (p. o.) with no complication or body rejection, while the radiological finding at the 4th months p. o. shows that the XBBI stable and well fixed with profuse external callus formation , with marked increase density of the sclerotic area at the junction of the end fragment and the bony implantation .The conclusion the xeno- bovine bony implantation (XBBI) which prepared from the ulna of calves can be used successfully as space filler ,support body weight and promote bone healing of the induced femoral bones defect in rabbits .

Key words: Xeno- bovine bony implantation (XBBI), intramedullary pinning, food grate stainless steel rods (FGSR), space filler.

Introduction:

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Bone grafting/implantation is widespread used in veterinary medicine, which can be used for the treatment of fractures healing complication like delay and nonunion fractures healing , and as a framework to provide stability , promote healing ,and filling osseous defect (Myerson and Uribe 2003).Many authors refer that bone graft/implantation substitution can be used successfully as bones scaffolds, for bone bridge defect, space filler, this bone graft / implantation can be cortical , cancellous ,or corticocancellous source (Ken et al., 1995;). Myerson and Uribe 2003; Nazht et al.,2018).

Treatment of bone fractures which characterized by loss part of bone as in pathological fractures (bone osteosarcoma) or in delay or noun union fractures healing ,need special strategies for stimulating healing processing of bone defect which may include bone implantation with different types and origin (Lohmann et al ., 2001). Bones grafting/ implantation may be either natural in source, like auto, allo or xeno, or may be biochemical synthetic substances (Nazht,2000; Schell et al.,2008; Ben Ali,2018).

The advantages of using xeno-bony grafts are easily prepared from animal sources; with wide range of sizes, shapes, strength, and may be cortical or cancellous ratio. Fresh xeno-bony graft have been shown to be unacceptable due to the antigenicity of the foreign tissue, they are unable to generate satisfactory bony repair (Ken et al .,1995).

Using Bone graft stimulates the differentiation of mesenchymal or pleuropotential cells in the surrounding tissue into osteoblast cells (Brinke et al.,1991), thus bringing about healing through the same sequence of events as in the healing of a fresh fracture.

The body reaction toward the grafting/implantation can be processed through fifth stages. **The primary stage** is “creeping substitution” (Upton et al .,1984; Stevenson,1985; Stevenson,1990; Weigel and Joseph, 1993).**The second and third phases** are characterized by revascularization and osteoinduction phases which determined the rate of repair or incorporation of bone graft. The factors that affect vascularization are the type of graft; the nature of recipient bed and the stability of the graft (Stevenson,1985; Stevenson,1990; Weigel and Joseph,1993; Schrueder et al 1997). The **fourth phase** is osteoconduction in which capillary ingrowths into the graft occur from the recipient bed, This may take place on a framework or a scaffold of the dead graft itself (Holmes et al .,1986).The **final phase** of bone graft is mechanical, during this phase of incorporation, on graft material which remains and functions as a mechanical weight bearing, or stress-transmitting fashion (Stevenson,1985; Stevenson,1990).

The aim of the present study is to evaluate the use of xeno –bovine bony implantation (XBBI) which prepared from the ulna of calves as space filler and for promote fractures healing in the induced defect of the femoral bone of rabbits.

Materials and methods:

Experimental animals and design:

Fifteen adult local breed rabbits of both sexes were used to remove 1cm of bone from femoral diaphysis under general anesthesia and aseptic technique, the empty space replaced by same diameter and length of XBBI and fixed with the femoral bone by FGSR (Nazht,2019).

Prepare the xeno-bovine bony implantation (XBBI):

The bony devices were prepared by cutting 1 cm from the ulna of calves from the slaughtering hose, they well cleaned, boiled then sterilized by waves and kept in sterile tube.

Prepare the site of operation:

The lateral aspect of the thigh region well prepared by clipping, shaving the hair , wash with tape water and soap then disinfect the area with 70% ethyl alcohol.

Anesthetics protocol:

Intramuscular injection of 2% xylazine hydrochloride at dosage17mg/kg. B. W. and 10% ketamine hydrochloride at the dosage 35mg/kg.B.W. respectively, and for more anesthetic confidence local anesthetic injection of 2% lidocaine hydrochloride at the proximal aspect of the femoral bone and at the site of operation.

Surgical operation:

The skin and subcutaneous tissues sharply incised , 4cm length at the lateral aspect of the thigh region ,then fascia lata was incised the underlying muscles were bluntly dissected and the femur bone exposed ,the periostium fine separated and 1cm of diaphyseal femoral bone removed by electrical saw with dropping isotonic normal saline to prevent thermal necrosis ,the empty femoral space filled with the same size and diameter of the XBBI and fixed by FGSR ,the surgical area irrigate by normal saline and applied local antibiotic powder ,the soft tissues reposition by simple continuous pattern using 2/0 absorbable suture materials and the skin closed by using 2/0 of non absorbable suture materials by interrupted suture pattern .The operated animals followed for 4^{th} month's p. o.

Post operative examination and care:

1. Daily clinical observation the site of operation and animal's gait besides any complication or body rejection ,until the end of experimental periods
2. Systemic antibiotics for 3 days p. o. by i/m injection of penicillin streptomycin at 10000 iu /Kg. B.W. and 5mg / Kg. B. W. p. o. respectively.
3. Radiographic examination at the period immediately after operation and at the end of 1^{st} , 2^{nd} ,4^{th},8^{th} week and at the 16^{th}, week p. o.

Result:

1. **The clinical observation:**
   A. Immediately after the surgical operation, the animals suffering from body depression, loss appetite which remain for two to three days ,and hold the affected limb.
   B. One week p. o. the skin incision healed satisfactory and the swelling of the surgical site subsided, the animals can support body weight on the affected limb when standing but still hold the limb during walking.
   C. Two week p. o. the animal gait normally and can support the body weight in walking with normal appetite, but hold the limb when running.
   D. Third week p. o. the animals still hold the affected limb during running but normally bear the weight when walking.
   E. Fourth to Sixth week p. o. the animals can use the affected limb normally in walking and running

2. **The radiographic examination**

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A. immediately after operation:

The XBBI well fixed between the two femoral fragments by the FGSR, visible fractures line between the end of the two fractures fragments and the XBBI with sharp end (Fig.1).

B. At the end of 1st week p. o.:

The XBBI stable, well fixed, no periosteal reaction, the fractures line visible (Fig. 2).

C. At the end of 2nd week p. o. :

Clear periosteal reaction which achieved by new bone formation from the proximal and distal cortex of bone fragments, sclerotic area with increase bone density at the junction of the two femoral fragments and the XBBI (Fig 3). .

D. At the end 4th week p. o. :

Increase volume and bone density of the new bone formation from the adjacent cortex toward the XBBI, high density of sclerotic area at the junction of the end of two femoral segments, increase in the bone cortex due to periosteal reaction (Fig. 4).

E. At the end 8th week p. o. :

Revealed new bony formation that created from the adjacent bone cortex and cover the XBBI, but still not completed the bony bridge formation, the fractures line disappear between the bony device and the recipient femoral bone (Fig 5).

F. At the 16th week p. o. :

Revealed the XBBI stable and well fixed between the two femoral fragments by profuse amount of the external callus formation, with, increase in cortex density and size around the implantation (Fig. 6).

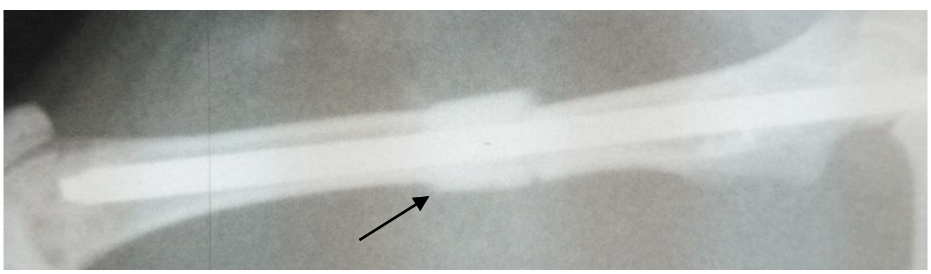

Figure 1 Immediately after operation, the XBBI well fixed and well alignment in the femoral diaphysis black arrow, clear fracture line between the two femoral fragment and the bony implantation, no periosteal reaction .

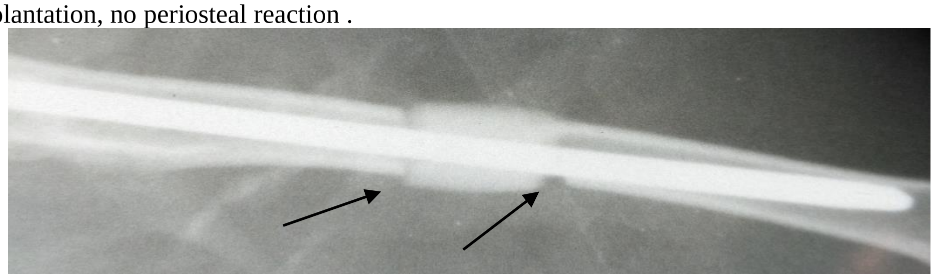

Figure 2 end of one week p. o. no periosteal reaction appear, the XBBI stable well fixed ,clear fractures line between the bony devices and the recipient femoral bone black arrow ,no periosteal reaction can be recognized.

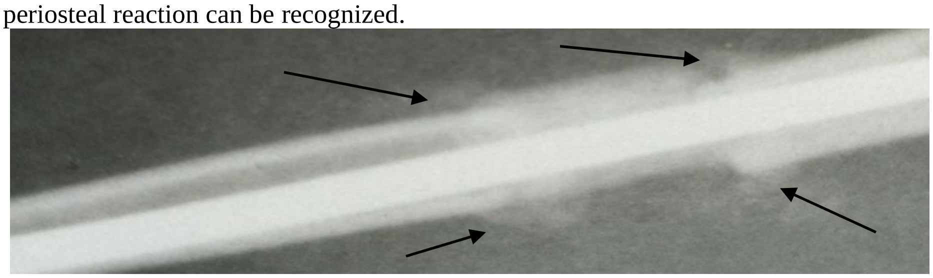

Figure 3 end of two week p. o. revealed by periosteal reaction from the femoral cortex toward the bony device black arrow characterized by elevation of the periostium and new soft callus bone formation

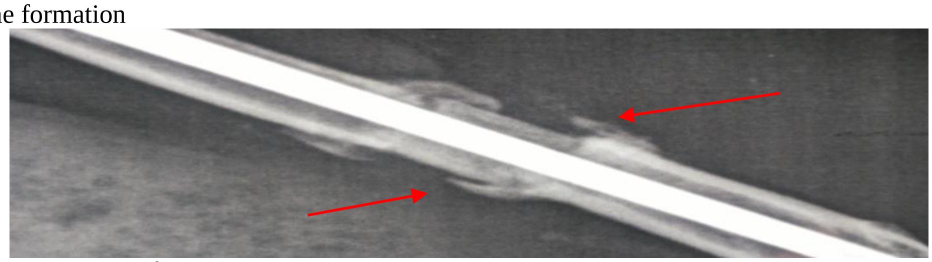

Figure 4 end of 4th week p. o. increase in density and volume of the external callus formation red arrow from the proximal and distal femoral fragments toward and cross the XBBI

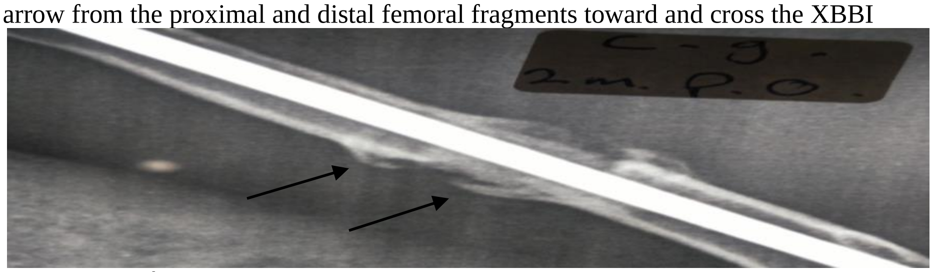

Figure 5 end of 8th week p. o. increase in density and volume of the newly formed bony callus formation from the recipient femoral bone black arrow to cross the junction line, but still not formed the bony bridge

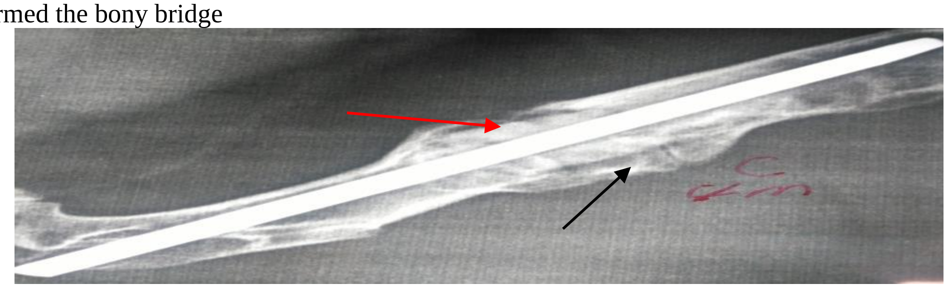

Figure 6 16th weeks p. o. revealed the external callus stopped formation, with increase bone density most of them pass from one side to the other to form bony bridge formation black arrow , especially the caudal aspect of the femoral bone ,increase density at the junction of the two femoral fragments with the bony device red arrow..

Discussion:

The first 3 days p. o., at the inflammatory phase which achieved by swelling the site of operation and reduced appetite, which gradually disappeared within 3-5 days p. o. and these signs were agree with (Shukla,1989; Singh et al., 2000; LaStayoet al.,2003).

The operated rabbits hold the affected limb during movement and in standing position for 2 days p. o. then only used it for supporting the body weight for 7-10 day p. o. these information agree with (Stevenson,1985; Singh,2000) they refer that Lameness or difficulty in movement disappears gradually as the fracture stabilizes.

The incision healed satisfactory, without complications as described by (Stevenson,1985; Abo-Elsaad et al., 2008) they found that the surgical wounds in dogs and rabbits, heal without complication or serious inflammation.

The animals that used XBBI as bone substitution can be normally use the limb normally in walking and running 4th to 6th week p. o. which achieved by well radiological finding ,in which the bony device stable well fixed by external callus formation and increase the size and density of mature bone formation these agree with (Shukla ,1989; LaStayo et al.,2003) that the purpose of grafting an empty bone space is for therapeutic purposes by support weight and provide a source for calcium, and facilitate healing ,and these finding also agree with (LaStayo et al.,2003; Abo-Elsaad et al.,2008; Schindeler et al.,2008) in which they mentioned that the radiographic finding at the end of the 1st week, showed the implant remained well positioned in the femoral diaphysis with no periosteal reaction.

The radiological finding are due to the osteoblast cells activation and stimulation for new bone formation, these agree with(Brinker et al.,1991), that bone graft stimulates the proliferation and differentiation of mesenchymal or pleuropotential cells in the surrounding tissue to osteoblast cells, and healing through the same sequence of events as in the healing of fresh fracture.

The periosteal reaction was visible radiographically at the 2nd week, which evident by high bone density at the junction of the implant with the ends of bone fragments. Many authors mentioned that periosteal reaction can be visible radiographically from 10 to 14 days p.o.(Breitbart et al.,1995;Ken et al.,1995;Toth and An 2000). .

At the 4th week p. o. , which achieved by an increase in volume and density of new bone from at the proximal and distal fragments of the femoral bone with no sign of any bridge formation, This is usually due to osteoblasts activation and osteoid formation with excessive bone mineralization (Stevenson,1985;Marsell and Einhorn 2011). .
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The increased density of the external callus formation at the 8th and 16th week p. o. is due to increased mineralization and osteoid formation by osteoblasts cells activation (Brinker,1991; Marsell and Einhorn 2011) they refer that bone graft can stimulates and differentiate the mesenchymal or pleuropotential cells in the surrounding tissue into osteoblast, they do the same sequence of events as in the healing of a fresh fracture.

At the end of the experimental period the animal which use the XBBI as bone substitution in the induced femoral defect in rabbits clinically use the limb normally and can strongly support the body weight, and this agree with (Valiati et al.,2012) that the advantages of bone graft/implant are functional mechanical and weight bearing or stress transmitting fusion .

Conclusion:

From the results above, xeno- bovine bony implantation (XBBI) which prepared from the ulna of calves can be used in filling the empty induced femoral bones defect , support body weight ,and promote fractures healing in rabbits.

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