Effect of Castration on Bone Healing in Male Rabbit Model; Clinical, Histopathological and Radiological Study

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Abstract

Twenty male rabbits were used in the current study. The rabbits were classified into two groups (castrated and un-castrated). Injected by solution consists from Ketamine HCL (40 mg /Kg/B.W/IM) and xylazine HCl (20mg /Kg B.W/IM). The first group was uncastrated while the second group was castrated and waiting one month. For both groups, at the middle of lateral aspect of the femoral region, a 5mm in diameter bore in the femoral bone was made by a medical drill device. The clinical signs showed some manifested such as pain, edema and fever in the castrated group more than the un-castrated group. The last clinical signs disappeared during two to three days in the un-castrated group and (5-7) days in the control group. Examination of radiological of both groups revealed reaction of the periosteal was begin at 2nd week in the second group while in the castrated group which started at the ending of 3rd week in the first group. The fracture was disappeared at 4th week in the second group. The bony mass was redesigned into the lamellar bone in 4th week in the second group, that characterized by adhesion of the bone with the external callus. Our study showed that the fracture healing morebest quantity and quality in the second group than in the first group. The histopathological examination showed osteoclast, osteoblast and the periosteal reaction started at (15-30) days after operation more in the second group than the first group. Moreover, the osteocytes is a presence in more in the uncastrated group than the first group.

Keywords: *Bone healing, Castration, Hormonal effects and Rabbits*

Introduction

The mechanical characterization of bone has tension as same as cast iron, but it is more flexible and lighter. The material inside the bone is not homogenous like plastic, metal and iron that used in orthopedic implants [(1)]. Bone is a highly specialized form of connective tissue that offers support and protection to the internal organs. Mechanical functions include protection, shape formation, aid in movement, and sound transduction. Synthetic function occurs in the process of "Haematopoiesis" as the bone marrow contains hematopoietic stem cells. Bone tissue represents the main mineral storage of calcium and phosphate in the form of "Hydroxyapatite". Fatty marrow offers a fat-storage function as well. Acid-Base balance forms another metabolic function of bone as it is considered a buffering system to the blood against excessive pH changes by absorbing or releasing alkaline salts [(2)]. Bone cure is a complicated, unique procedure [(3,4)]. It could be finished without scar formation, some the fractures do not heal (5, 6, and 7). The angiogenesis has a significant role in the regeneration process [(8)]. The fracture heals through many reactions complex steps that included regeneration of the tissue, which results in healthy bone [(9)]. Bone regeneration leads form bone support functional requirements [(10)]. The fracture cure is remarkable repair processes to form bone same original form. The explanation of the bone healing process is complicated because it required cellular homeostasis [(11)]. Dysfunction of adults' sex organs is main cause of bone problems and its loss. Declines of estrogen lead to osteoporosis in females. The decline of testosterone leads to hypogonadism that is marked by low testosterone levels. The decline of estrogen in females and the decline

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of testosterone in males lead to loss of the elements from the bones and decrease mineral density and an increase in the probability of the fracture occurrence. Wherever hypogonadism in male has related to the bone problem [12].

Materials and Methods

Twenty male rabbits were chosen from Animal house in Veterinary Medicine college - Al-Qasim Green Uni. Weight Average of animals was (1.5-2.5) Kg, and Age Average of animals was (6- 11) months.

Surgical procedure:

The animals were not allowed to eat food for one day and water for 12 hours before the operation, Sterilization of the surgical site and side area of the femoral bone. Administration penicillin-streptomycin ten g/20 mg/kg B.W/IM before operation (60) minutes to reduce the side effect. Administration by ketamine HCL 40 Mg/ kg of body weight/IM (5)% and xylazine HCL (20) mg/kg B.W/IM (2)%.

A 5mm hole by a drill, up to my concern, will not show a major difference between the two groups in reference to healing.

Clinical examinations:

The animals were examined clinically and physically where temperature degree, pulse, respiration rate, urination and defecation during one week after the operation.

Radiological Examination:

Both groups were examined by radiographic every week by for estimation of fracture healing degree; the images were taken in lateral position.

Histopathological Examinations:

Biopsy of the bone was taken at (15) day and (30) day after the operation. A biopsy was kept in formalin (10) %, then treated by alcohol and put in paraffin wax after the block it at (5-6) mm and stained with Eosin and Hematoxyline then examined (13).

Results and Discussion

Clinical inspection:

According to our results, clinical signs were local edema, fever, pain, and Pus in the castrated group.

While the clinical signs in non-castrated group are less than the second group but it disappeared at the (4-5) days after operation, it is included redness, edema, fever and pain at the second day of the operation because the blood flow is increased and blood vessels become dilated leading to increase permeability, that results in transport of WBC and inflammatory cells outside of blood vessels associated with production of local edema, this result was in agreement with [14]. The testosterone is stimuli biomechanical stability of fractures in gonadal male. The testosterone has great role in bone maintenance and development. The receptor of androgen was founded for representing in osteoblasts, osteoclasts, osteocytes and pluripotent mesenchymal bone marrow stromal cells, and the androgens have a direct role in bone cells function and bone metabolism [15,16]. Some reports of androgen insensitivity syndrome showed partial or complete decreasing of testosterone receptor[17]. These studies support a direct role of testosterone in bone maintenance. The testosterone hormone has indirect e□ects on bone by aromatization [18], in the males who suffer from aromatase lack showed osteoporosis or osteopenia [19,20,21,22]. Decrease or loss of androgen receptor leads to decrease in bone mass and an increase in separation [23,24,25,26]. The pain was due to edema that formed around the fracture resulting in increased pressure on the nerves. The inflammation stimulates secretion of prostaglandin that leads to vasodilatation resulting in aggregation of inflammatory cells in the fracture area. All these processes ceased after (6^{th} -7^{th}) days after operation in castrated group, but in non- castrated group the clinical signs, the severity was less than castrated group and the process disappear at (3^{rd}-4^{th}) day due to thyroid hormones which have vascular effect and formation of phagocytes cells and caused increased inflammation severity and stimulated growth of blood vessels, that same with results of [27]. Furthermore, the decrease of the blood supply leads to the formation of WBC and plasma that causes increased acidity and oxygen, decreasing resulting swelling and inflammation.

Radiological examinations:

Examination of fracture radio-graphically of

both groups was done every week to determine the healing degree as: In the 1st week, in both groups, the radiological sign was clear fracture line, local swelling without periosteal reaction. At the secon[nd] week no clear fracture in the castrated group was observed, while the fracture was not clear with the beginning of the periosteal reaction in non- castrated group. In 3rd week the periosteal reaction occurs away of the fracture line in non- castrated group (Fig. 1). At 4th week the fracture is invisible with regeneration, for taking the normal bone shape in non- castrated group.

1- Castrated Group:

The 1st week: periosteal reaction was not apparent (Fig. 1).

The 2nd week: periosteal reaction was not apparent (Fig.2).

The 3rd week: line of the fracture was visible with little periosteal reaction.

The 4th week: line of fracture is an invisible clear periosteal reaction, forming of callus and formation bridge for filling area(Fig. 3).

2- Non-castrated Group:

The 1st week: apparent fracture line, without periosteal reaction (Fig.1).

The 2nd week: unapparent fracture, little periosteal reaction (Fig.2).

The 3rd week: apparent periosteal reaction; invisible fracture line, the callus production, and forming bridge were observed.

The 4th week: many try to uniting the extended and new bone production (Fig. 3).

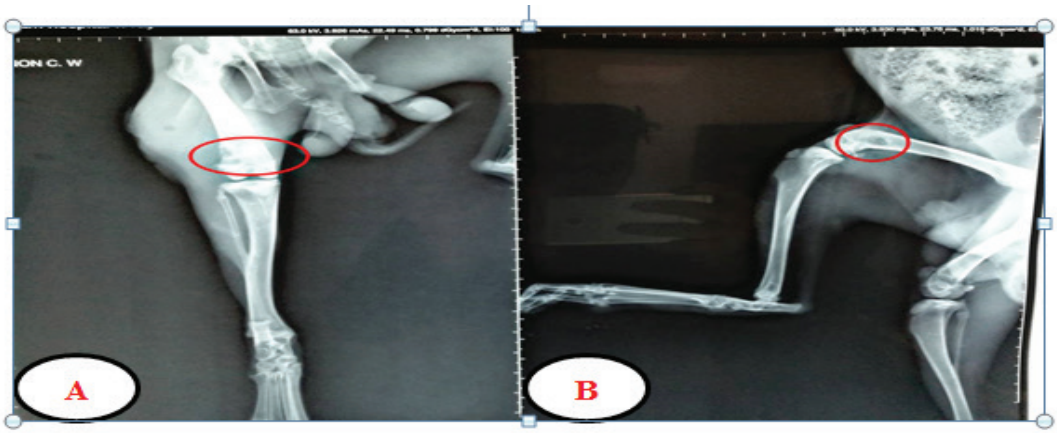

Fig. (1): Radiographic image at first week, **A**: Non-Castrated group: No periosteal reaction near the fracture, clear fracture with smooth edges of hole. **B:** Castrated group: No periosteal reaction surrounding the hole site, clear fracture.

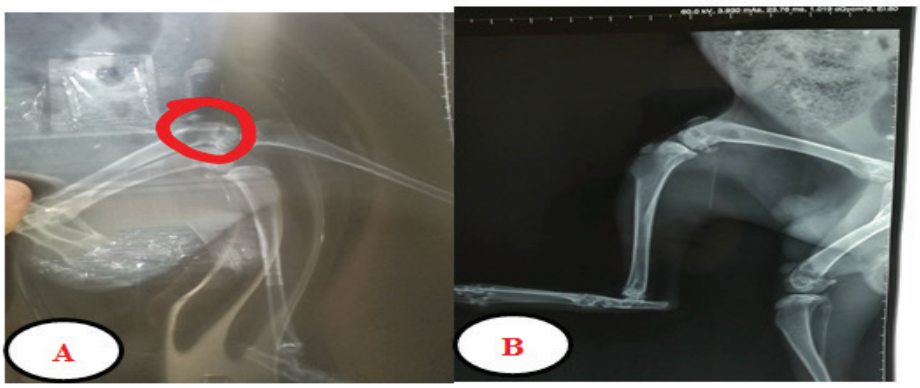

Fig. (2): Radiographic image at second week, **A**: Castrated group: No periosteal reaction around the fracture, still clear fracture. **B**: Non- castrated group: slight periosteal reaction begin projected in the hole site.

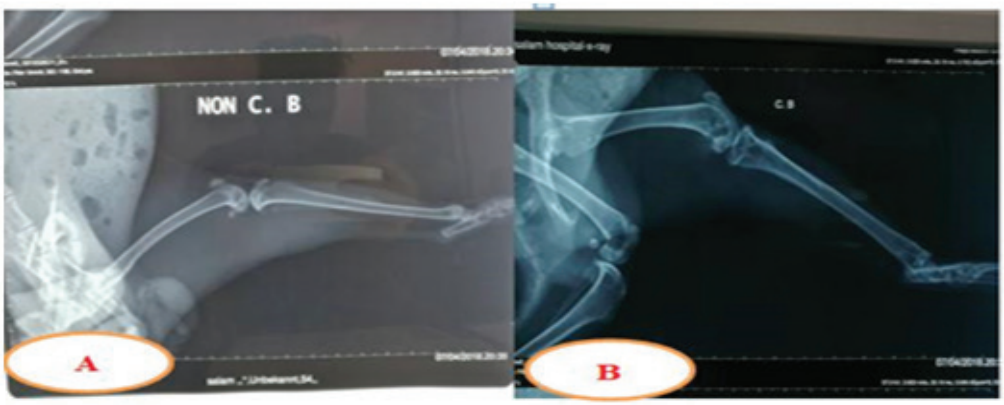

Fig. (3): Radiographic image at fourth week, **A**: Non- Castrated group: New bone formation with the contour of the bone itself, the bone may be taken normal shape. **B**: Castrated group: the callus cross the hole and try make bridge filled the hole, invisible fracture line .

The fracture cure could occur without noticeable callus in cancellous or cortical bone, that called primary bone healing, it does not include the formation of the callus if the periosteum was removed due to low blood supply (28).

Histopathological Results

1- Castrated groups:

Subgroup the fifteenth day after operation:

The histopathological of the castrated group at fifteenth day after operation demonstrated the formation of a large amount of granular tissue with thick gelatin fiber that pierd with early mineralized bone trabecula. (Fig. 4A)

Subgroup the thirtieth day after operation:

The histopathological of castrated group at thirtieth day after the operation revealed a thick bone trabecula that loss of normal lamellar pattern with a large number of osteoclast surrounding (Fig.5A)

2- Non- castrated groups:

Subgroup the fifteenth day after operation:

The histopathological of non- castrated group at fifteenth day after operation showed the formation of a large number of osteoprogenitor cell from the surrounding periosteum (to Fig. 4B)

Subgroup the thirtieth day after operation:

The histopathological is during this period that characterized the formation of normal bone lrabecuile structure with clear trabecula lamellae. (Fig.5B)

When we are showing the histopathological results, significant differences between castrated and uncastrated groups were observed. However, the results of the un-castrated group were better than castrated group and these results cause by the testosterone role in healing because the fracture healing including differentiation, proliferation, and mineralization of osteoblasts. The critical-size fractures do not cure without mechanical supporting [29]. The testosterone hormone helps to build the bone. The histological examination is making on cortical and trabecular bone revealed castrated and un-castrated treated fractures [30].

Testosterone has a direct and indirect effect on the bone [31] by transferring to estrogen by a process called aromatization directly. By indirect methods, the testosterone reacts with and activates AR, and transport it inside the nucleus to link with androgen response. Which lead to the transcription of some genes, for example, Bglap, Colla1, and AKP2 are activated [32,33]. The testosterone has non-genomic influenced by stimulation PI3K/Akt [34,35]. The testosterone reacts with c-Jun/c-Fos resulting in inhibition of the c-Jun N-terminal kinase. Since these actions are anti-apoptotic, that are useful for osteoblasts differentiation. The testosterone has significant effect on the bone by

increase regeneration by the production of IGFs and TGF-b and reduces the production of IL-6 receptors [36]. IGFs and IGF-binding proteins help to increase differentiation and osteoblast proliferation [31]. Moreover, the testosterone has an anti-resorptive effect by decreasing bone reabsorption [33]. Also, these pathways have main roles in many stages of bone cure. Our results found that osteoclastic activity was stimulated by testosterone hormone. Testosterone causes many different effects on several bones cells types, and increase bone cell proliferation and stimuli apoptosis in the bone [11].

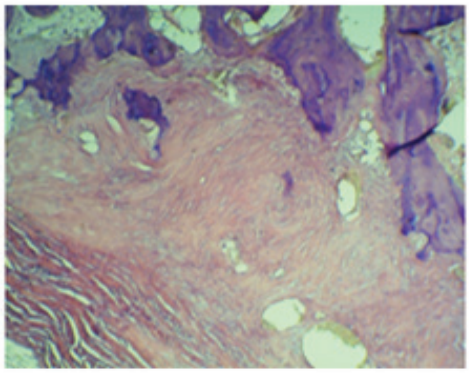

Fig. 4A: Castrated. 15 days Showing formation of large amount of granular tissue with thick glatein fiber that pierd with early mineralized bone trabecuile H, E (100x) Fig.

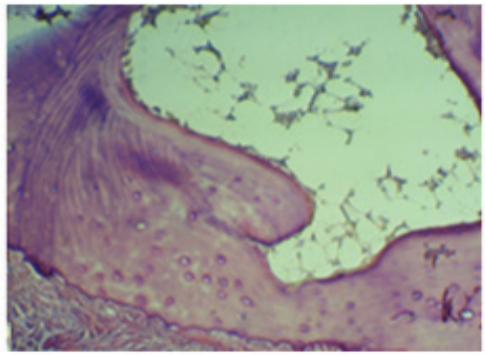

Fig.4B: Non- castrated 15 days Showing formation of the large number of ostue proginater cell from the srounding preiostum (how shape lacui also seen)

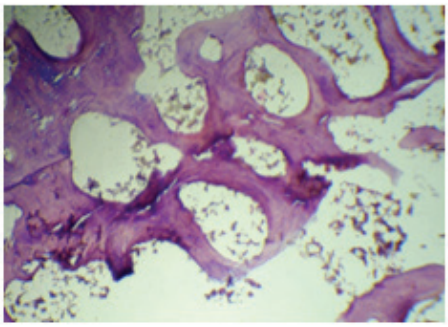

Fig.5A: Castrated 30 days Showing formation of thick bone trabecuile that loss of normale lammller pattern with large number of ostue clast srounding (how shape lacuni)

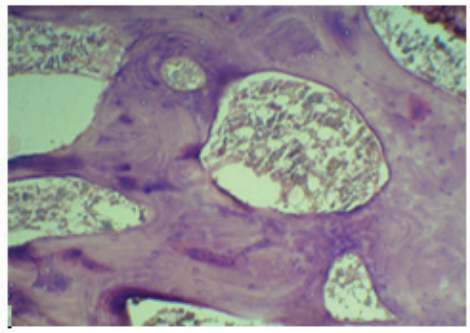

Fig. 5B: Non – castrated 30 days showing the formation of normale bone lrabecuile structure with clear trabecuile lamllai

Conclusion

In this study we were obtained that the androgens very important to healing the wound and regeneration the bon, so the un-castrated animal give best result when compare with castrated group

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

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