Tikrit Journal for Dental Sciences 13(1) (2025) 189-199
https://doi.org/10.25130/tjds.13.1.20

Tikrit Journal
for
Dental Sciences
https://tjds.tu.edu.iq
ISSN:2073-1213

Star Volume Measurement of Marrow Space After Application of Hydroxyprogesterone Caproate on Surgically Created Defect

Huda A. Salim[(1)] *
Ziad Hazim Deleme[(2)]
Mohanad AlFahad[(3)]

[(1,2)] Department of Oral and Maxillofacial Surgery College of Dentistry, University of Mosul, Mosul, Iraq.
[(3)] Department of Pharmaceutics, College of Pharmacy, University of Mosu, Mosul, Iraq.

Keywords:
HPC, star volume, marrow space, trabeculae.

Article Info.:

Article History:
Received: 4/7/2024
Received in revised form: 27/7/2024.
Accepted: 30/7/2024
Final Proofreading: 30/7/2024
Available Online: 1/6/2025

Citation: **Salim HA, Deleme ZH, AlFahad M. Star Volume Measurement of Marrow Space After Application of Hydroxyprogesterone Caproate on Surgically Created Defect. Tikrit Journal for Dental Sciences. 2025; 13(1): 189-199.**
https://doi.org/10.25130/tjds.13.1.20

***Corresponding Author:**
Email:
hudaakram@uomosul.edu.iq

MSc. Department of Oral and Maxillofacial surgery, Dentistry College, University of Mosul, Mosul, Iraq.

Abstract

Introduction: For description of structural changes of trabecular bone, a new stereologic method can be used. Progesterone One of naturally occurring steroid hormone that is crucial for procreative purpose, progesterone has the Chemical Formula $C_{21}H_{30}O_3$.

Materials and Methods: 60 domestic adult male rabbits had been selected, randomly divided into three groups: control negative groups, control positive group, and study group. Each group sacrificed at day 3, 7, 21, and 28.

Results: star test measurement reveals bone trabeculae formation faster in study group than other two groups. Marrow space less in the study group.

Conclusion: HPC enhanced bone formation by recruitment of osteoblast for bone formation at the defect size.

Introduction:

The star volume is a stereologic new parameter that is defined as the average volume of an object's constituent parts, these parts in all directions should be un obscured from a certain location inside the object. This test demonstrates structural changes of bone. Use when Baddeley's vertical or isotropic sections only when it is impartial in this parameter [1, 2].

Two measurements can be used for definition of trabecular bone structure: the (V*tr) trabecular star volume and marrow space star volume (V*m. space). The average of trabecula volume along all directions to the margin of the trabecula as designated from internal point is V*tr. While V*m. space represents the average volume of marrow cavity measured from a particular point that is unobstructed by the trabecula in every direction. The V*tr becomes larger and the V*m. space becomes smaller, with increasing trabecular number and continuity. Conversely with decreasing trabecular number and continuity, the V*tr becomes smaller and the V*m. space become larger [3-5]. Progesterone is one of naturally secreting steroid hormone that is crucial for procreative purpose [6-8]. Progesterone has the Chemical Formula $C_{21}H_{30}O_3$ [9]. Progestogens are either natural or synthetic. Natural compounds are those similar to those produced by living organisms. In contrast, synthetic progestogens are made in the laboratory and their structures have been modified and are not like natural progestogen [10].

Hydroxyprogesterone Caproate is an oil solution available for intramuscular (IM) injection, naturally presenting progestational hormone composed of sterilized long-acting form of the caproate ester [11]. Progestins "an efficient pharmaceutical family of HPC" [12].

Hydroxyprogesterone caproate is known by the chemical name pregn-4-ene-3,20-dione, 17[(1-oxohexyl) oxy]. Its molecular weight of 428.60 and its empirical formula of C27H40O4. The crystalline powder known as Hydroxyprogesterone caproate is white to creamy white in color [11, 12].

In general, the actions of Progesterone and progestins result from their union with progesterone receptors (PRs), a member of a large family of ligand-activated nuclear transcription regulators, which are characterized by organization into specific functional domains and are conserved, to differing degrees, between species and family members [13-15].

Materials and Methods

The protocols of the study were approved by the research ethics committee of University of Mosul / Collage of Dentistry, REC reference number: UoM. Dent. 25/ 23. This study was held at College of Dentistry / University of Mosul / Iraq from May 2023 to September 2023.

Sixty domestic adult male rabbits had been selected, the average weight of these rabbits was 1500 ± 50 gm, and age between 8-10 months. Rabbits were randomly divided into three groups: control negative groups that lift without anything and sacrificed at day 3, 7, 21, and 28. Control positive group that received treatment by putting only gelfoam in holes prepared for receiving this treatment. Study group that includes adding hydroxyprogesterone caproate hormone into gelfoam. The HPC dose was calculated according to [16-18] to be 4mg/kg per bone defect for local delivery of HPC. This dose is still less than the lethal dose of progesterone as reported in rabbits, the LD_{50} is 26.5 mg/kg of body weight [7]. The surgical procedure involves anesthetized the rabbit by given 40 mg/kg ketamine injection [19,20] intramuscularly in the thigh muscle of the rabbit, mixed with xylazine 4 mg/kg of rabbit weight [19,21], then animal hair was removed and the area for surgery was cleaned with povidone iodine solution, after incision and exposure of the femoral bone, two holes were made by low-speed handpiece with round bur 3 mm with continuous irrigation of normal saline (0.9 %), the space between holes was measured to be about 1 cm. the application of materials according to the selected group.

All the samples stained by Hematoxylin & Eosin, read by digital light microscope,

attached to digital histomorphometric analysis software of OPTIKA.
Descriptive statistics of all variables are presented as mean ranks. Comparison
Between different time periods were evaluated by performing analysis by Kruskal -walls test. Statistical analysis was made by using Spss 25 computer software program.

Results:

Three days period: After surgery at this period, there were many cells within the defected area. The cells were mesenchymal stem cells (which derived locally from periosteum), which could be distinguished from cells morphology and would differentiate into osteoblasts with time. The activated mesenchymal osteoblasts proliferated and secreted high amount of collagen to form the osteoid later. In this period there is no bone formation either in control negative or control positive and study group just inflammatory cells infiltrate, except in study group, as seen in figure 1. So, the star volume test is zero.
Figures (5-8) represents the diagrams of vales of measures of marrow space by the histomorphometric software by micrometer unit (μ_m), in figure 5 there is no bone formation at three days period in all groups, while in figure 6 of seven days period only study group show bone formation. Figure 7 and 8 show bone formation in all groups but the study group show further bone formation, this is explained by that when decreased in star volume test measured by the software mean that increased bone formation, this is in inverse relationship.

Seven days period: At day 7 after surgery, the amount of the woven bone increased at the defected area with the matrix still randomly oriented. The formation of pre-existing trabeculae to form lamellar bone later. This can be seen in study group but in control negative and control positive there is just granulation tissue formation. As seen in figure 2.

21 days period: in this period there is bone formation in all groups, but the study group (the group that contain HPC material) show more bone trabeculae and less marrow space, as seen in figure 3.

28 days period: in this period bone formation approximately complete but the study group shows well organized bone formation, as seen in figure 4.

Discussion:

Progesterone is a steroid hormone that acts on the human body through various mechanisms. In addition to the traditional activity, it is linked to the female body and gestation, and it is crucial for male fertility as well as the neurophysiological condition and emotional state of both sexes [(6).] Neither Progesterone or synthetic progestins have no or only minor effects on bone. Osteoblasts and osteoclasts can be identified by Progestin receptors, and its derivatives have a limited effect on the density of bone. [(22).]
Star volume a stereological parameter that provides a three-dimensional assessment of the size of the trabeculae and marrow space, is used to quantify new bone structure and is a reasonably accurate way to assess the development of new bone from bone substitutes [(3).]
Inflammation is a key response in bone healing. Healing worsened by excessive inflammation, and impairment for inflammation lead to impede healing and rise rates of delayed osseus healing [(23).]
During the first 3 days of defect healing there is inflammatory cells infiltrate with no bone formation, an excess level of an inflammatory response can impair fracture healing.
During the period of day 5 to 11, vascular endothelial growth factor (VEGF) is released, causing angiogenesis at the defect site and the development of fibrin-rich granulation tissue inside the hematoma. Bringing in addition mesenchymal stem cells to initiate their differentiation to fibroblasts, chondroblasts, and osteoblasts. Chondrogenesis, as a result starts to happen, fibrocartilaginous collagen-rich network spanning started laying down at the fracture ends, with a surrounding hyaline cartilage sleeve. A layer of woven

bone laid down by osteoprogenitor cells, at the same time, adjacent to the periosteal layers [24]. Through this period after inflammation resolved, HPC thought to activate the cells present in the area around defect created, and accelerate the recruitment of cells to differentiate it to most important cells for bone healing. This explains the presence of bone trabeculae in study group at this period of healing.

During week 3 after surgery, finished cell proliferation was occurred. Scarcely any new woven bone could be seen in the wound. Formation of typical lamellar structure, by mineralization of newly formed trabecular bone [25]. At this period during our study almost all the trabecula of bone mineralized, the marrow space decreased with time.

At week four after surgery, BMU (Basic Multi-cellular Unit) appeared on the surface of almost all the newly formed trabecular bone at the fracture site and reached a peak at 6 weeks [25]. In our study at this time all the bone reached the remodeling to mineralize all the bone formed.

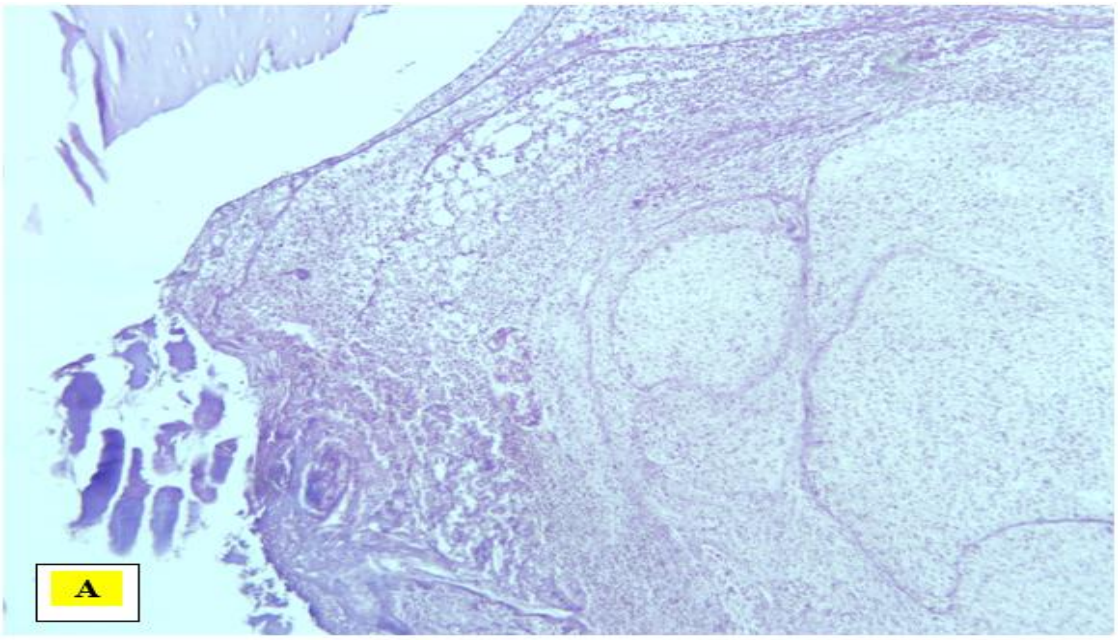
Conclusion:

Bone formation occurs in HPC group earlier than other group; this may be due to acceleration in the differentiation of cells that present defect site.

Funding:

This manuscript is self-funded.

Conflict of interest: No.



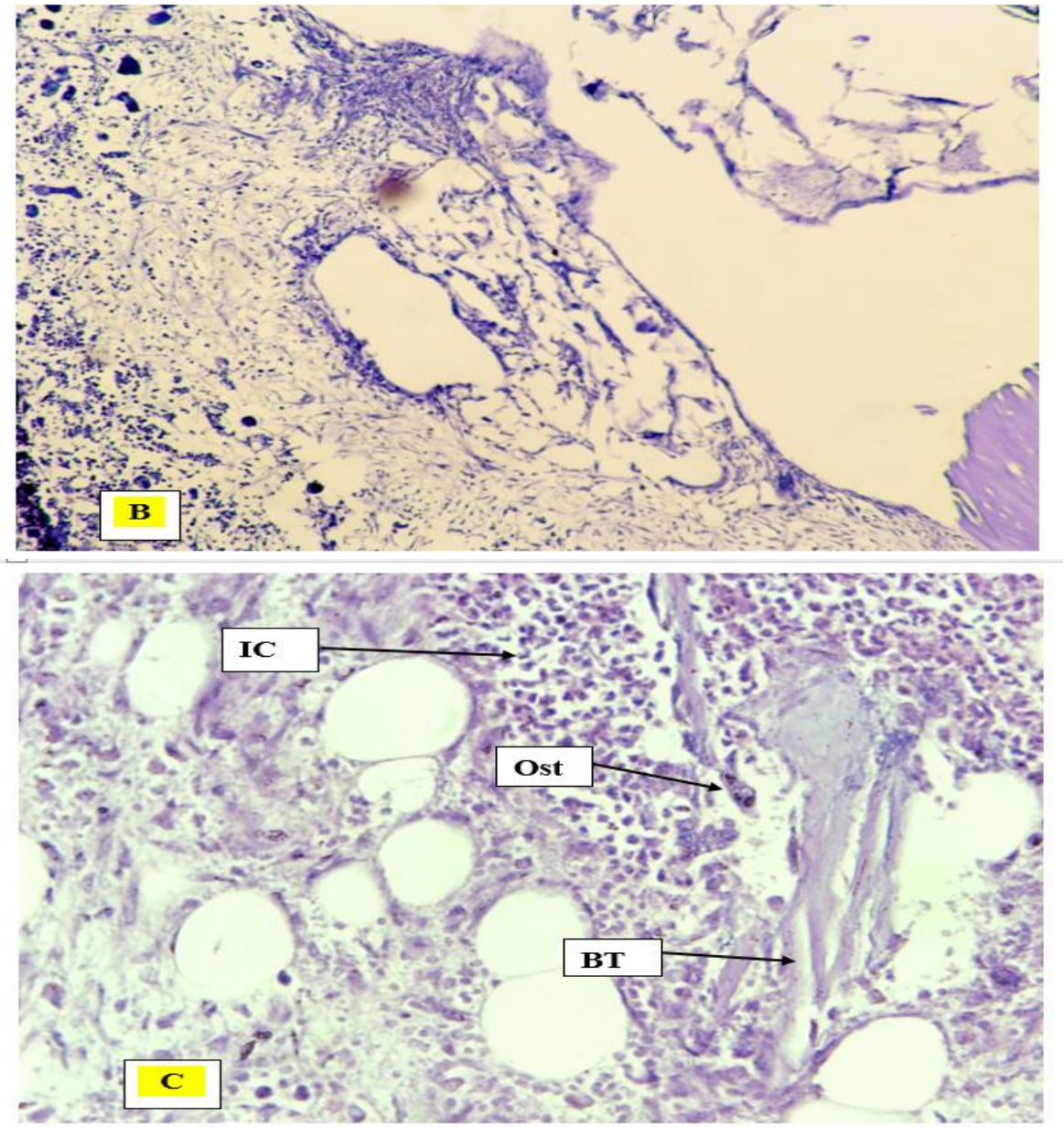


Figure 1: Three days period. Section in rabbit's femoral bone with H&E stain. A: control negative group,10x. B: control positive group, 10x. C: study group, the pointer reveals the osteoblasts aggregation (Ost). (BT) Bone trabeculae formation, Inflammatory Cells (IC),40X.

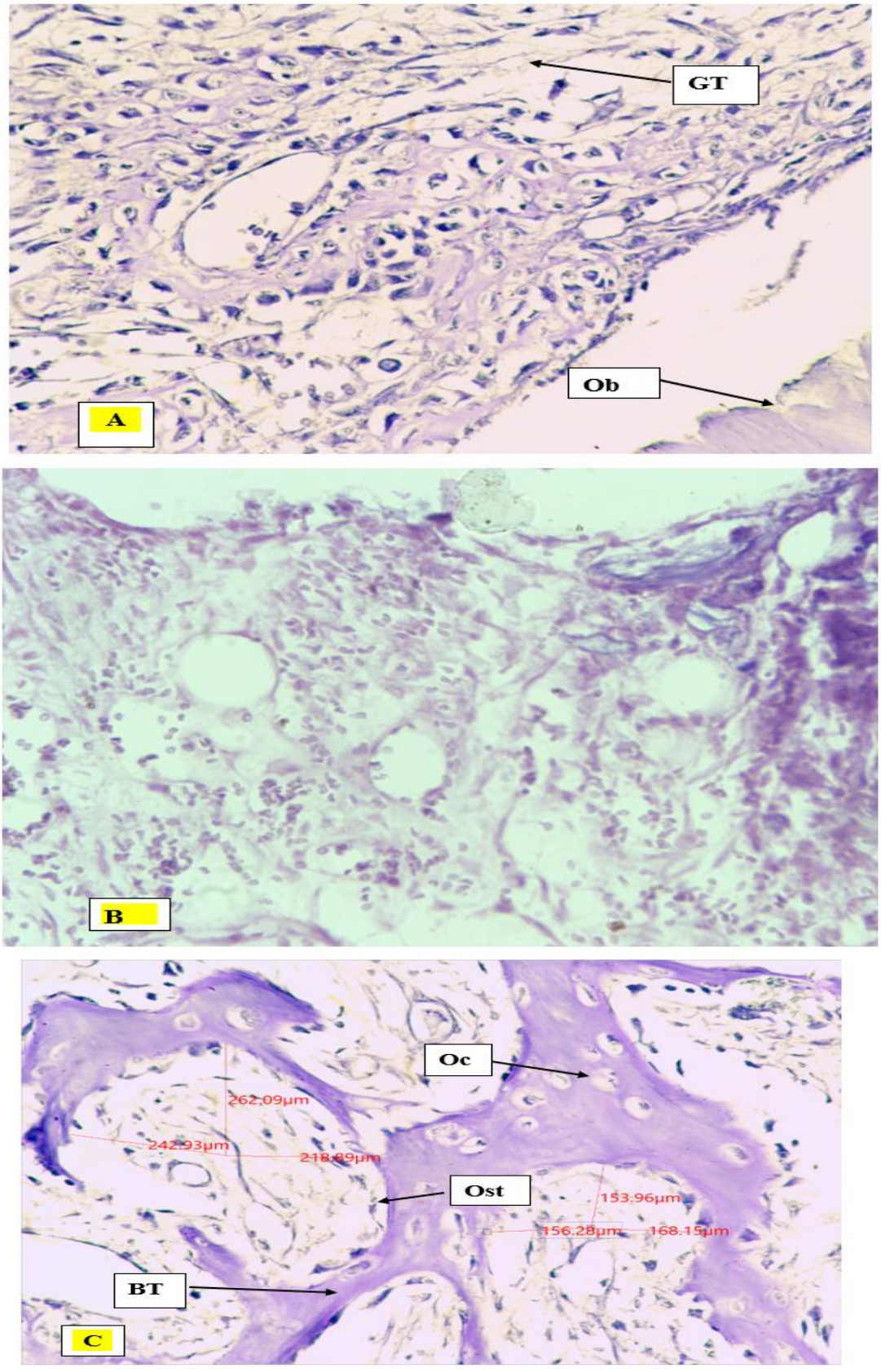


Figure 2: Seven days period. Section in rabbit's femoral bone with H&E stain. A: control negative group, show granulation tissue formation (GT), Old bone (Ob), 40x. B: control positive group, 40x. C: study group, appearing osteoblasts (Ost), Osteocytes (Oc), marrow space. 40X.

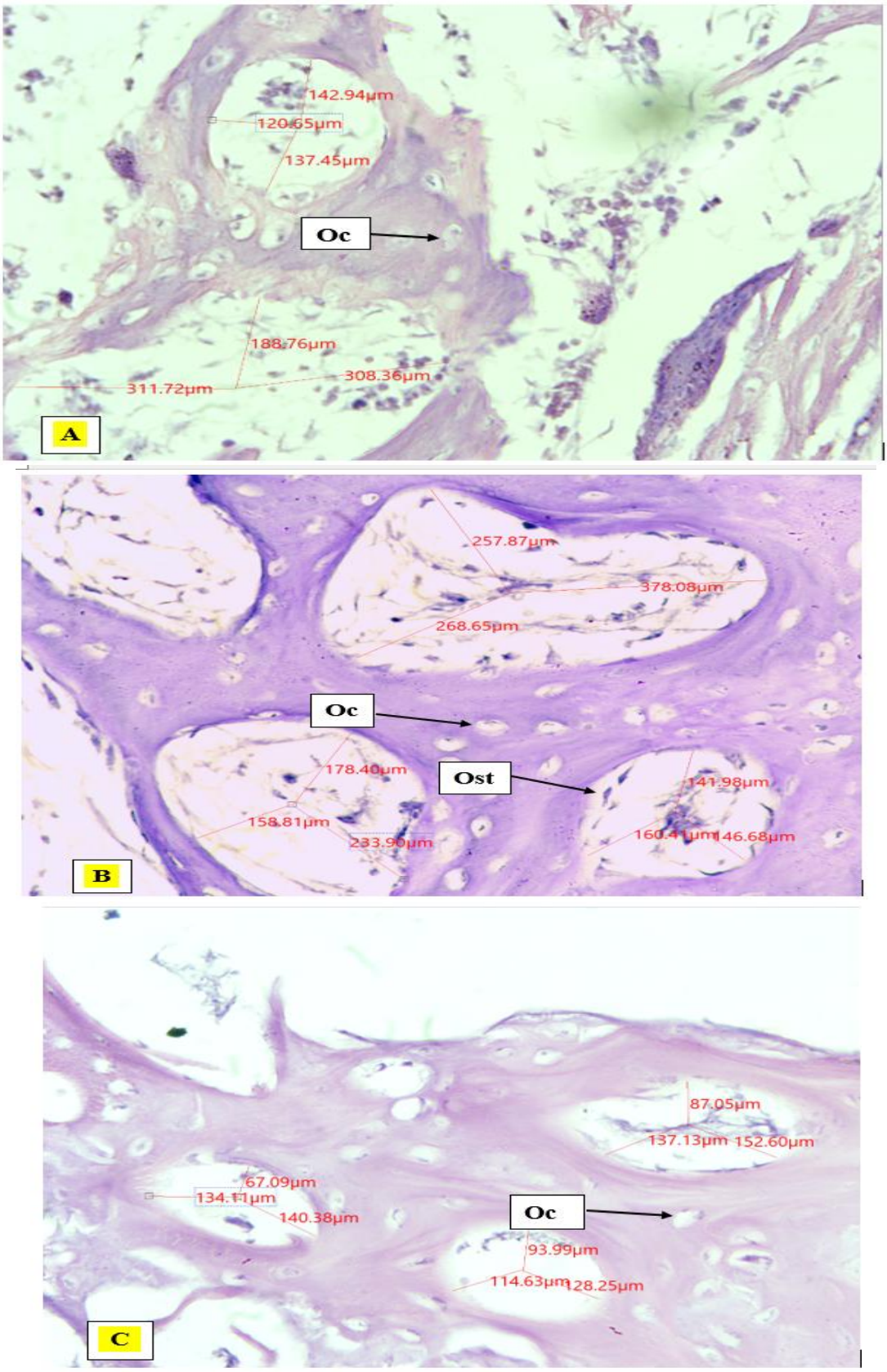


Figure 3: Twenty-one days period. Section in rabbit's femoral bone, H& E stain. A: Control negative group, 40x. B: Control positive group, 40x. C: Study group, 40x. Osteocyte (Oc), Osteoblast (Ost).

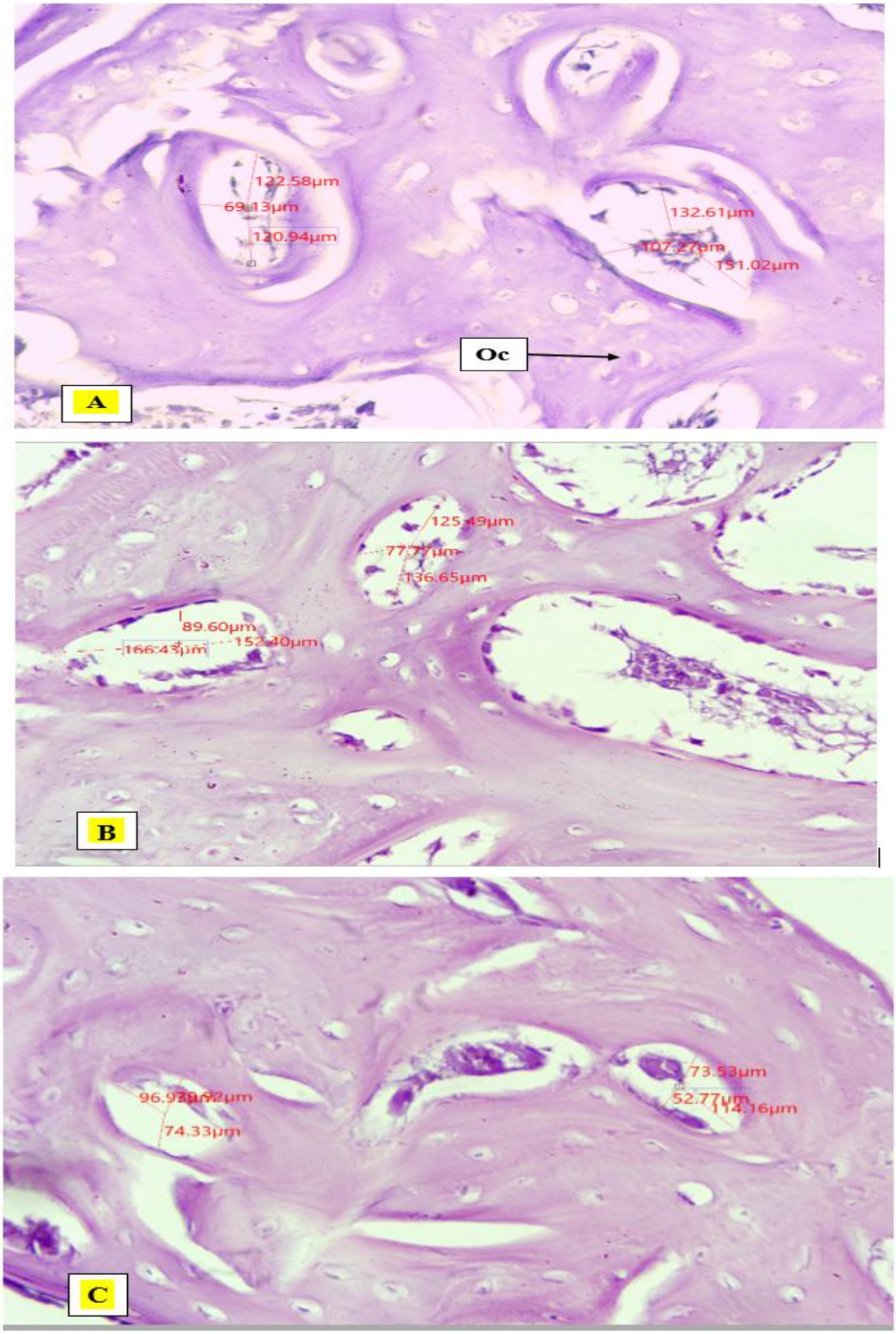


Figure 4: Twenty-eight days period. Section of rabbit's femoral bone with H&E stain. A: Control negative group, 40x. B: Control positive group, 40x. C: Study group, 40x.

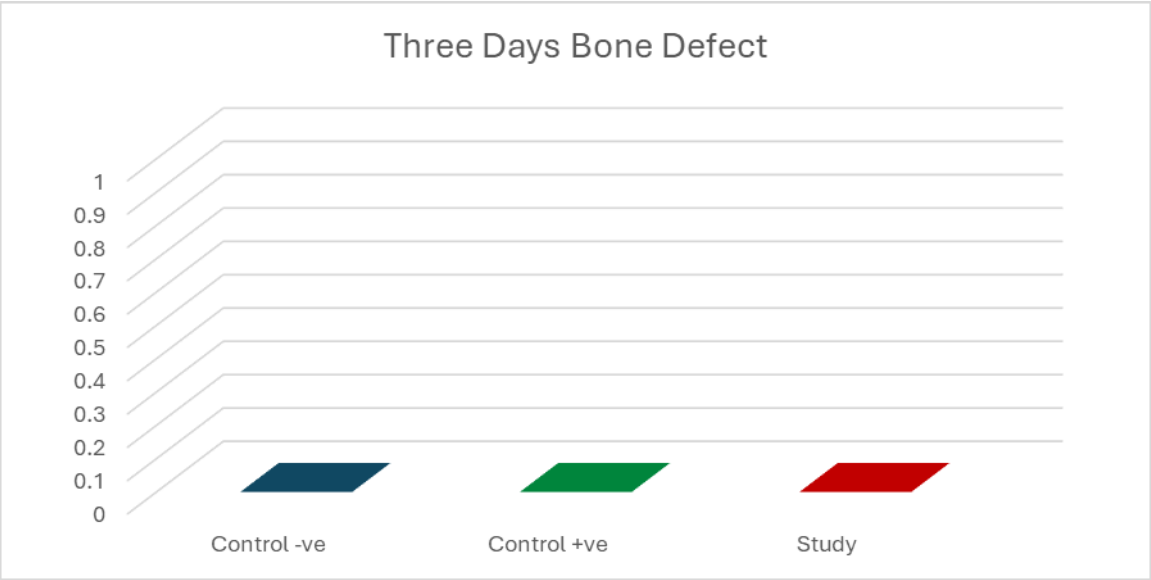


Figure 5: Diagram of Three days bone defect

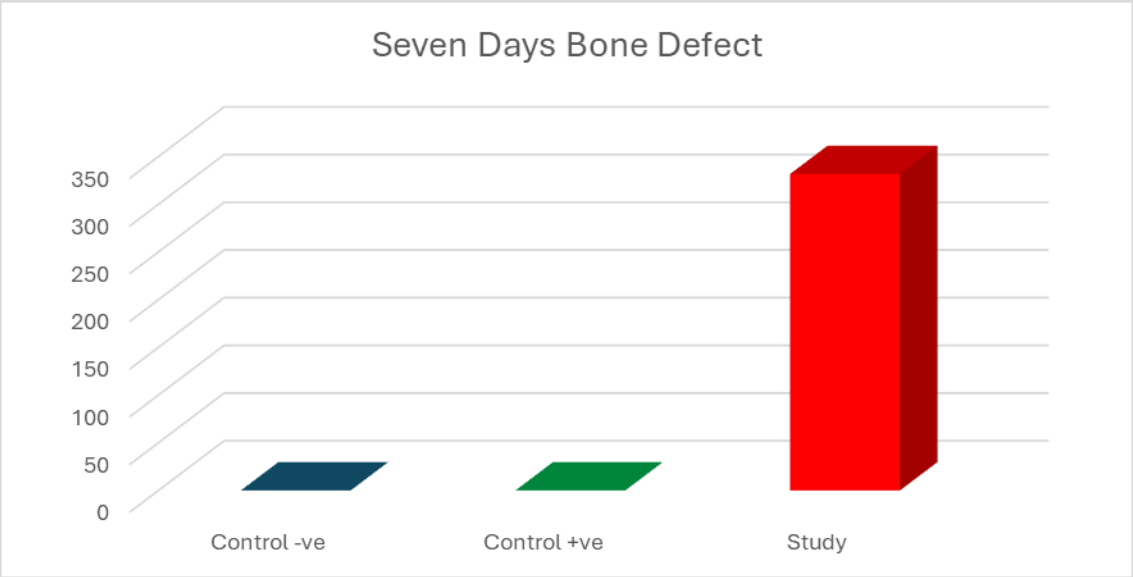


Figure 6: Diagram of Seven days bone defect.

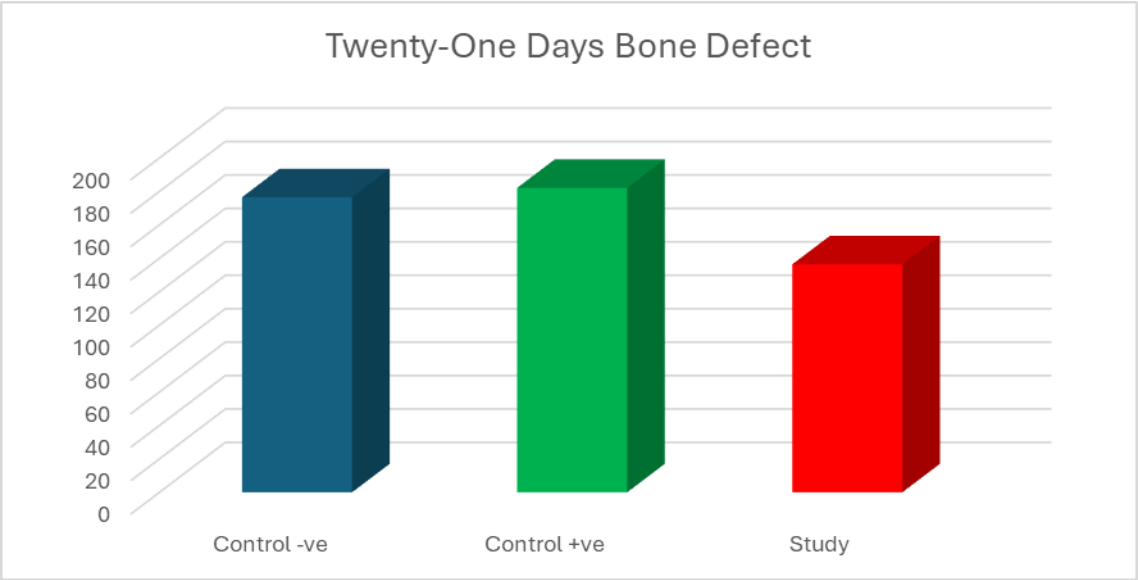


Figure 7: Diagram of twenty-one days bone defect.

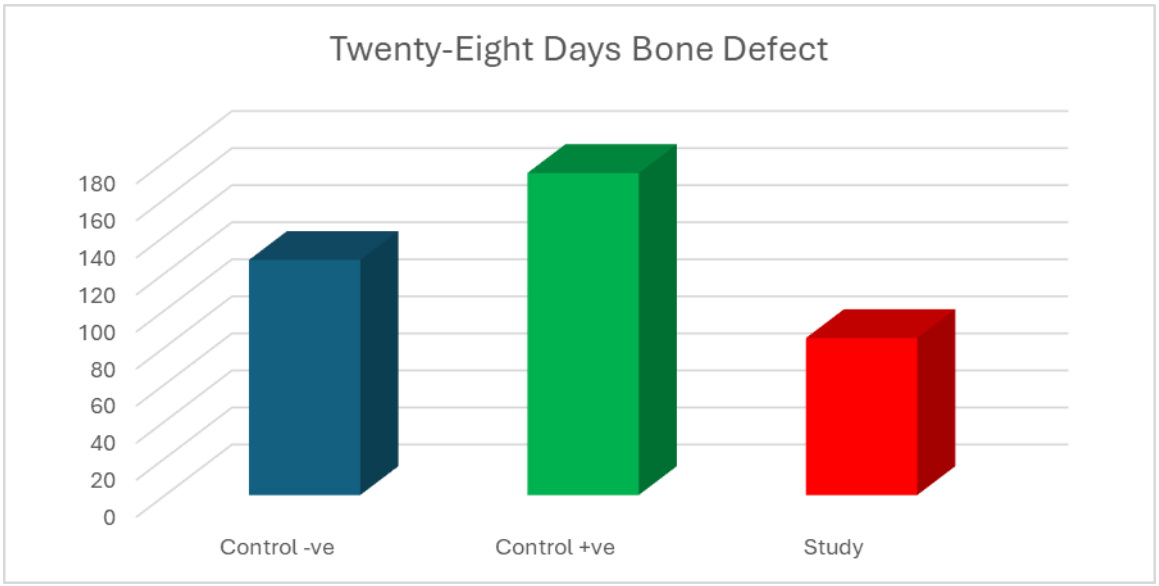


Figure 8: Diagram of twenty-eight days bone defect.

Table 1: Kruskal -Wallis Test among four different groups.

Groups	Day 3	Day 7	Day 21	Day 28
Con -ve	8.00	5.50	9.20	9.40
Con +ve	8.00	5.50	8.80	11.60
Study	8.00	13.00	6.00	3.00
P-valu	1.00	0.001	0.468	0.007

Note: table include mean rank with interquartile range (IQR).
P value ≤ 0.05 significance.
P value ≤ 0.01 highly significance.
There is highly significance at day 7 of defect healing, the P value is 0.001 which reveal bone healing at the study group at this period. Table 1 reveal the mean values and p-values of the three different groups at the different time intervals of the procedures.

References

1-Vesterby A. Star Volume of Marrow Space and Trabeculae in Iliac Crest: Sampling Procedure and Correlation to Star Volume of First Lumbar Vertebra. Bone. 1990; 11, 149-155.

2-Croucher P, Garrahan N, and Compston J. Assessment of Cancellous Bone Structure: Comparison of Strut Analysis, Trabecular Bone Pattern Factor, and Marrow Space Star Volume. Journal of bone and mineral research. 1996; Volume 11, Number 7, Blackwell Science, Inc.

3-Cantín M, Olate S & Del Sol M. Mandibular Trabecular Structures in Alveolar Ridge Preservation Using Different Grafting Materials After Tooth Extraction. Int. J. Morphol. 2018, 36(3): 1143-1148.

4-Thomsen J, Ebbesen E, and Mosekilde L. a New Method of Comprehensive Static Histomorphometry Applied on Human Lumbar Vertebral Cancellous Bone. Bone. 2000; Vol. 27, No. 1:129–138.

5-Vesterby A. Star Volume in Bone Research. The anatomical record 1993; 235:325-334.

6-Kolatorova L, Vitku J, Suchopar J, Hill M, and Parizek A. Progesterone: A Steroid with Wide Range of Effects in Physiology as Well as Human Medicine. Int J Mol Sci. 2022; 23(14): 7989.

7-Sitruk-Ware R. non-clinical studies of progesterone. Climacteric. 2018; 21(4): 315–320. doi:10.1080/13697137.2018.1463982.

8-Ibraheem H. Synthesis and Microbiological Investigation of Progesterone Derivatives. Eng. & Tech. Journal. 2013; Vol.31, Part (B), No. 6.

9-Enna S and Bylund D. Hydroxyprogesterone. 2007; Elsevier Inc. All rights reserved.

10-Shaamash A, Ali M, Attyia K. Use of hydroxyprogesterone caproate injection for prevention of preterm labor in women with different risk factors: a mini review. Curr Med Res Pract. 2020; 5:237–240.

11-Santa Ana. McGuff Pharmaceuticals, Inc. CA 92704 Printed in USA. 2015; MD381-0051.

12-Shirley. American regent. 2018; INC., NY 11967.

13-García-Sáenz M, Ibarra-Salce R, Pozos-Varela F, Mena-Ureta T, Flores-Villagómez S, Santana-Mata M, Santos-Aguilar R, Uribe-Cortés D and Ferreira-Hermosillo A. Understanding Progestins: From Basics to Clinical Applicability. J. Clin. Med. 2023; 12, 3388.

14--DeMayo F and Lydon J. New insights into progesterone receptor signaling in the endometrium required for embryo implantation. J Mol Endocrinol. 2020; 65(1): T1–T14. doi:10.1530/JME-19-0212.

15--Scarpin K, Graham J, Mote P and Clarke C. Progesterone action in human tissues: regulation by progesterone receptor (PR) isoform expression, nuclear positioning and coregulator expression. NRS. 2009; Vol. 7, Page 1 of 13.

16-Larson L, Spilman C, Dunn H, Foote R. Reproductive efficiency in aged female rabbits given supplemental progesterone and oestradiol. Faculty Papers and Publications in Animal Science. 1973; 734.

17-Pickworth S and Lamming G. Observations on pregnancy in intact rabbits and in ovariectomized rabbits treated with 17 a- Hydroxyprogesterone caproate. J. Reprod. Fert. 1967; 13, 457-468.

18-Huiming D and Allen W. Maintenance of Pregnancy in Castrated Rabbits by 17-alpha-Hydroxy-Progesterone Caproate and by Progesterone. Fertility & Sterility. 1959; Vol. 10, No.5.

19-Mahmood M. A comparison between ketamine-xylazine and ketamine-midazolam or all of them to induce balance anesthesia in rabbits. Iraqi Journal of Veterinary Sciences. 2022; Vol. 36, No. 2, (499-506).

20- Paknejad M, Rokn AR, Eslami B, Afzalifar R, Safiri A. Evaluation of Three Bone Substitute Materials in the Treatment of Experimentally Induced Defects in Rabbit Calvaria. Journal of Dentistry. 2007; 4(4): 171-175.

21-Kilic N. A Comparison between Medetomidine-Ketamine and Xylazine- Ketamine Anesthesia in Rabbits. Turk J Vet Anim Sci. 2004; 28: 921-926.

22-Taraborrelli S. Physiology, production and action of progesterone. Acta Obstetricia et Gynecologica Scandinavica. 2015; 94: 8–16.

23-El-Hawary H, Baradaran A, Abi-Rafeh J, Vorstenbosch J, Xu L, Efanov J. Bone Healing and Inflammation: Principles of Fracture and Repair. Semin Plast Surg. 2021; 35:198–203.

24-Sheen J and Garla V. Fracture Healing Overview. Stat Pearls - NCBI Bookshelf. 2019; 23:17.

25-Zhao Y, Yang S, Wang G. Study of trabecular bone fracture healing in a rabbit model. Int J Clin Exp Med. 2018; 11(8):7651-7665.