



Haematological Profile of Fracture Rabbit with Horse Bone Graft Implant

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Abstract

Fractures are commonly found in the world of veterinary practitioners. Fractures do not only occur as a result of accidents, but can also occur due to bone diseases or due to an imbalance in nutrition in animals. One method that can be used to increase the healing process is through bone implantation. Bone graft is a material to speed up the bone healing process because it can provide mechanical support and signals to the body's molecules to maximize tissue growth. Bone implants that are often used and commonly found are bovine bone implants. Another implant material that we can use is horse bone. Bone grafts from horse bones have not been widely studied. Horse bone has the potential to be used as bone graft material because it has a more compact and harder structure. The aim of this study was to assess the haematological profile of fracture rabbit with horse bone graft. The experimental animal used was a rabbit. A total of 12 rabbits were divided into three groups, namely the control group, the group with horse bone graft, and the group with commercial bone graft. Horse bone graft is obtained from horse bone waste through sintering and hydroxyapatite synthesis processes. Blood collection was performed pre-surgery, week 2 and week 6 postoperatively to see the response and description of blood cells against bone graft. The results of hemoglobin and erythrocyte examinations showed no significant difference between the three groups ($P < 0.05$). Hemoglobin level in rabbits implanted with horse bone grafts was still at normal levels, except for the erythrocyte levels which were slightly below normal after surgery.

Keywords: Fracture, Bone graft, Rabbit

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Introduction

Bone is a special connective tissue used to support the muscular structure, protect vital organs which are soft and easily damaged, gives shape to the animal body and contains bone marrow as a place for blood cells to be formed. Bones also function as a reserve of calcium, phosphate and other ions which can be released or stored in a controlled manner to maintain the concentration of these ions in body fluids (Nurhidayat et al., 2018). Bone

consists of two main components, 30% in the form of organic matrix and 60% in the form of inorganic salts and 10% water, while the cellular components of bone consist of osteoprogenitors, osteoblasts, osteoclasts and osteocytes. Osteoprogenitor cells are undifferentiated flattened cells present in the cellular lining of the periosteum, in the endosteum and lining the Haversian canal. Osteoprogenitor cells proliferate and differentiate during bone remodeling. (Gartner and Hiatt, 2014). The existence of disorders such as fracture conditions and diseases of the bones can disrupt the skeletal system which results in an individual's activities.

Fracture is a break in the continuity of the bone either due to trauma, pressure or pathological abnormalities. Fractures or broken bones are damage of bone tissue which results in bones losing continuity. Fractures are generally divided into two, closed fractures and open fractures. Closed fractures are fractures without any wound complications and no bleeding. However, if treatment is slow, there will be damage to the tissues, blood vessels and the nervous system around the fracture. While open fractures show surface fractures and can cause infection. Fractures based on the direction of the fracture are divided into transverse, oblique, spiral, comminuted, impact, and fissure fractures (Fossum et al., 2013). Implantation is a surgical technique to replace lost bone using bone material or what is often called a bone graft.

Bone-grafting is an effort to replace damaged bone tissue using certain materials that come from the patient's own body, from synthetic/chemical materials, or from natural materials. Bone-grafting materials must have ideal characteristics for recipients, like having good biocompatibility, not causing reactions (effects), non-toxic, non-infectious, adaptable, and able to stimulate new attachments (Maulidah et al., 2018). Currently, there have been many studies developing bioceramic materials as an alternative to bone grafts derived from bovine, porcine and horse bones, because they have osteoconductive properties and can act as mediators for the development of osteogenic cells (Adiloglu et al., 2019).

According to statistical data from the Directorate General of Livestock and Animal Health (2020), horse slaughter data from 2016 to 2020 reached 11,400 tons of meat. Horse meat is used by the community as a consumption material or as an export commodity, but the bones are only organic waste that is not utilized. Horse bone waste has the potential to be used as bone graft material because it has several advantages. Horse bones have a more compact and tough structure. The mineral content of horse bones is very high, especially calcium and phosphorus. The availability of horse bone waste in Indonesia is quite a lot and the mineral content, especially calcium and phosphorus which can be synthesized into hydroxyapatite has the potential to make horse bone as an environmentally friendly bone graft material. Therefore, research on the effect of bone graft implantation from horse bone waste on the treatment of femoral os fractures in rabbits is very useful for adding innovation to fracture treatment.

Materials and Methods

Horse Bone Hydroxyapatite Material Synthesis

Bone preparation. The demineralization process includes cleaning horse bone waste from the rest of the muscle using a scalpel. The bones are dried in the sun for 1-5 days to remove fat. Furthermore, the bones were cleaned and cut with a size of 0.5 x 0.5 cm (Putra et al., 2020).

Deproteinization. The bones are then immersed in a solution of H₂O₂. Goal done This soaking is to remove the remaining fat and protein found in the bones. Bones that have been treated with proteinase will turn white. this process done repeatedly until the color

of the bone changes to pure white by replacing H₂O₂ solution. H₂O₂ solution also acts as an oxidizing agent to oxidize impurities on the surface layer of bones while killing bacteria attached to them. bones (Afifah and Sari, 2020).

Sintering. Deproteinized horse bone is dried at high temperature for 1-2 hours. Sintering aims to remove organic materials such as collagen from horse bones so that what is left is inorganic materials such as calcium and phosphorus for the manufacture of calcite material. After heating is complete, the material is allowed to stand until the temperature drops to the room temperature. Next, the bones were crushed using a pestle and mortar. The bones that are still rough will be blended and sifted, the result is bone powder the size of grained beach sand. Powder calcite or CaCO₃ is then mixed with phosphate and stirred using a magnetic stirrer for 3 hours. The calcite powder is then mixed with distilled water and heated inside the furnace at 30-40°C for 1-2 hours. The heated CaCO₃ is allowed to stand to form a precipitate and heated again for 2 hours. The powder is then added and stored in tubes until applied (Wathi et al., 2014; Jang et al., 2014).

Bone Graft Implant in Rabbit

Twelve rabbits were divided into three groups, namely the control group (CG), the implant group with horse bone graft (HBG) and the implant group with commercial bone graft (CBG). The part of the bone used is the femur. The operation is performed by making an incision in the skin in the lateral area of the femur, then the vastus lateralis and biceps femoris muscles are exposed so that the femur bone is visible. The diaphyseal part of the femur was perforated with a bone drill (bone drill) with a diameter of 0.35 mm until it penetrated the medullary cavity and was closed again.

Twenty-four hours before the operation, all rabbits in groups I, II and III had their blood collected via the auricular vein. Blood samples were collected in sterile tubes containing anticoagulants to be examined for hemoglobin (Hb) and total erythrocytes. Blood sampling was collected again on the 2nd and 6th week on two rabbits in each group.

Data Analysis

The hematological data, such as hemoglobin and erythrocyte was analyzed by Analysis of Variance (ANOVA) using SPSS

Results and Discussion

Blood is one of the parameters of animal health status, because blood is a component that has an important function in the physiological regulation of the body. The results of analysis on data with a 95% confidence level from the results of Horse Bone Graft on the haematological profile of Rabbits with Femur Os Fractures showed no significant difference or no effect of implants on hematological status, such as the number of erythrocytes and the hemoglobin level. Data analysis results are presented in Table 1.

Table 1. The average of hemoglobin levels (gr/dl) in the control and the treatment group

Week	Group		
	CG	CBG	HBG
0	8.65 ± 1.63	10.62 ± 2.35	11.37 ± 1.65
2nd	11.95 ± 1.40	11.27 ± 1.52	10.8 ± 1.65
6th	11.97 ± 1.45	11.47 ± 1.57	11.35 ± 2.13

*There is no significant difference (P>0.05).

The measurements of hemoglobin levels did not show significant differences between the various groups each week. Hemoglobin levels tended to increase in all groups, except for the Implant group with horse bones which tended to fluctuate but were still within normal level. The normal level for hemoglobin in rabbits is 10.0-17.4 gr/dL (Marshal, 2008). Measurement of Hb concentration is a part that is commonly done as part of a blood test. Low hemoglobin levels indicate anemia. Hemoglobin is an oxygen-carrying compound as well as a protein contained in erythrocytes to the tissues. Oxygen must be bound effectively with hemoglobin in the capillaries for gas exchange to occur (Forgue et al., 1989). A normal hemoglobin level indicates no bleeding problems and the implants in the rabbit's bones are working well. This shows that bone graft implants have no effect on blood hemoglobin levels.

Table 2. The average of erythrocyte levels ($\times 10^6/\mu\text{L}$) in the control and the treatment group

Week	Group		
	CG	CBG	HBG
0	2.46 \pm 0.08	2.35 \pm 0.26	2.22 \pm 0.15
2nd	4.29 \pm 0.67	3.99 \pm 0.63	3.14 \pm 0.59
6th	4.48 \pm 0.65	4.08 \pm 0.61	3.35 \pm 0.59

*There is no a significant difference ($P>0.05$).

Hemoglobin is closely related to erythrocytes, because hemoglobin synthesis occurs early in the formation of erythrocytes. Data on erythrocyte levels can be seen in Table 2. From the data above it can be seen that there was an increase in the number of red blood cells in the three groups. This increase is the body's response to low erythrocytes by producing more erythrocytes to return to normal conditions (Guyton and Hall, 2006). Under physiological conditions, an increase in the total number of erythrocytes correlates with an increase in the hemoglobin value. The normal level of red blood cells in rabbits is 3.7–7.5 $\times 10^6/\mu\text{L}$ (Hewit et al, 1989). Even though it tended to increase from the first week, the erythrocyte levels in the treatment group with bone graft implants were still lower than the values in the control group. The stress condition due to the installation of implants is thought to be the cause of the decrease in the value of the rabbit's erythrocytes. The function of red blood cells dynamically regulates the process of balancing oxygen demand and distribution of nutrients in the body and removing metabolic waste in the form of CO₂ (Arosa et al., 2004).

Conclusion

Blood hematological profile in rabbits after graft implantation from horse bone material did not have a significant difference with the control group and the implant group with commercial materials. The data is in the form of hemoglobin and erythrocyte. This shows that bone grafts made from horse bone can be used as fracture treatment.

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