GTLabs

Research Article

Identification histological structure of femur and antebrachium *Oryctolagus cuniculus* as a biology learning

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ABSTRACT

Background: Preparations are used as learning resources in histology practicum, for this reason, it is necessary to seek various ways to improve the quality, one of which is the manufacture of femoral and antebrachium preparations of *Oryctolagus cuniculus*. The rubbing preparations were obtained through the microtechnical method by boiling and rubbing the bones as thinly as possible.

Objectives: The purpose of this study was to identify the histological structure of the femur and antebrachium tissue of *Oryctolagus cuniculus* which could be observed microscopically through bone rub preparations.

Methods: This research method is descriptive. The research sample is taken from the femur and antebrachium Oryctolagus cuniculus. The data collection method was by direct observation of the preparations using a microscope and documented using an HP Realme camera directly from the microscope. The data analysis technique was carried out in a qualitative descriptive manner. The research was conducted at the Biology Laboratory of the University of Muhammadiyah Malang

Results: Unstained femur and antebrachium preparations of *Oryctolagus cuniculus* show parts of the haversian system, namely Canalis havers, Osteocytes, Lacunae, Canaliculi, Lamella, and Canalis Volkmann.

Conclusion: The research results can be used as learning resources or histology practicum media.

Keywords: Bone, micro technique, *Oryctolagus cuniculus*, preparation, rubbing method

SDGs Relevance: One of the Sustainable Development Goals (SDG) is quality education. The purpose of this study was to identify the histological structure of the femur and antebrachium tissue of Oryctolagus cuniculus with bone smear media. The results of this study can be used as reference material in histology learning and histology practicums at junior high school to college levels.

Laboratory Affiliation: All research activities were carried out in the Biology Laboratory, University of Muhammadiyah Malang

INTRODUCTION

The preparation is used as a learning resource in histology practicum, so various ways need to be attempted to improve the quality of microtechnique preparations. One of them is through the creation of rubbing preparations. Microtechnique is one of the techniques for making plant or animal preparations that aims to facilitate the observation of plant or animal parts. This preparation must be small, thin, and transparent so that it can be penetrated by light (Harijati et al., 2017). To obtain the preparation, several methods or techniques are needed to make the preparation. One of them is the rubbing method. The purpose of this method is to obtain a preparation that is difficult to slice or difficult to obtain a preparation with an even thickness, in addition to making hard tissue (Wahyuni, 2015). The method is to boil the bone until soft and saw it. After getting the bone pieces, rub it in one direction using a chisel or sandpaper.

The use of preparations is closely related to the science of histology. Histology is a branch of biology that studies in general the tissues that make up the body, tissue chemistry, and cells which are studied using microscopic analytical methods (Amirazad et al., 2022). In histology, there is a great emphasis on discussing

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the cells that make up a tissue (Soesilawati, 2020). One of the studies of Histology studies connective tissue. Connective tissue is divided into 3, including: 1) ordinary connective tissue consisting of loose connective tissue (areolar), 2) dense connective tissue, namely connective tissue with special properties such as adipose tissue, reticular tissue, hematopoietic tissue, 3) special connective tissue or supporting tissue, namely bone and cartilage (Wangko & Karundeng, 2014).

Bones are the main organs that make up the body that can grow and develop. The main function is to form a skeleton and a means of movement for the body (Dewi et al., 2017). Bones include mineralized connective tissue and specialized connective tissue (Leeson et al., 1996). The composition of bone tissue consists of organic matrix and inorganic matrix (Antonio, 2017). Cells in the bone include osteoblasts, osteocytes, osteoclasts and osteoprogenitor cells. Osteoblasts are found in the bone tissue layer that mediates the mineralization of osteoid. Osteocytes are the main cell components that form the bone matrix. Osteoclasts are phagocytic cells that erode bone and repair bone with osteoblasts. Osteoprogenitor cells are cells that produce osteoblasts and osteocytes. Bones form a strong and rigid endoskeleton (Hartowiryono, 2018).

This research has something new compared to previous research, one of which was conducted by (Susetyarini et al., 2019) with the title "Histological Structure of Femur Bone and Subcutaneous Tissue of New Zealand Rabbits". The study focused on the histological structure of the femur bone and subcutaneous tissue in New Zealand rabbits. The results of the study obtained a havers system that was clearly visible in the femur bone with a microscope magnification of 100x, 400x, and 1000x and using a Scanning Electron Microscope (SEM), but in the study there has been no research on the antebrachium bone. Other innovations are bone cutting techniques using mechanical methods with saws, and in making preparations without using synthetic dyes or natural dyes so that in this study it is a novelty found in identifying the antebrachium and femur bones.

In this study, rabbits were chosen rather than mice or guinea pigs because rabbit femurs are larger in size and have the characteristic of faster bone growth, this is due to the rapid ossification process, but they are more fragile (Susetyarini et al., 2019). This study aims to identify the histological structure of the femur and antebrachium tissue of *Oryctolagus cuniculus* that can be observed microscopically through bone rubbing preparations. The results of this study are expected to provide opportunities to obtain quality bone rubbing preparations. Quality bone rubbing preparations will facilitate the study of microtechnical preparations of bone tissue parts and the Havers system. The results of this study are expected to contribute to one of the Sustainable Development Goals (SDG), namely quality education. In the implementation of educational activities, media or learning resources are needed, so that the results of the identification of the histological structure of the femur and antebrachium tissues of *Oryctolagus cuniculus* with media in the form of bone rubbing preparations can be used as media and learning resources for histology.

METHODS

The type of research chosen is descriptive, the research sample was taken from the femur and antebrachium of *Oryctolagus cuniculus*. The data collection method was by direct observation of the preparations using a microscope and documented using a Realme cellphone camera directly from the microscope. The data analysis technique was carried out qualitatively descriptively. The research was conducted at the Biology Laboratory of the University of Muhammadiyah Malang. The research began in November to December 2023. The tools and materials used included a binocular microscope, object glass, 1 set of surgical instruments, experimental animals, alcohol, xylol, and aquades. The procedure for observing bone preparations using the rubbing method according to (Wahyuni, 2015) and(Sari & Harlita, 2020), the steps include: 1) boiling the bones until all muscles, fat, ligaments are clean; 2) cutting the bones $\pm$ 1 cm; 3) rubbing the bones until thin evenly and in one direction while dripping water using a whetstone and rubbing paper; 4) putting in absolute alcohol for 15 minutes; 5) transferring to a glass object and pure xylol; 6) observing using a microscope.

RESULTS

This study used rabbit bones (*Oryctolagus cuniculus*). The results of histological observations of the femur and antebrachium bones in rabbits (*Oryctolagus cuniculus*) without staining and sliced transversely were then identified to obtain the results of observations of the preparations presented successively in Figures 1

to 6 using an Olympus type CX21LED light microscope and then documenting the observation images from the microscope using the rear camera of the Realme cellphone which has a camera pixel of 64 MP. The histological description of the femur and antebrachium rubbing preparations of *Oryctolagus cuniculus* with magnifications of 4×, 100×, and 400× are presented in Figures 1 and 2.

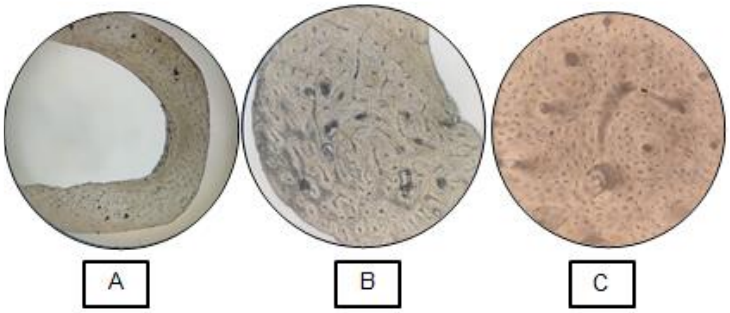

Figure 1. Femur Preparation *Oryctolagus cuniculus*
Description: A. Magnification 40×, B. Magnification 100×, C. Magnification 400×

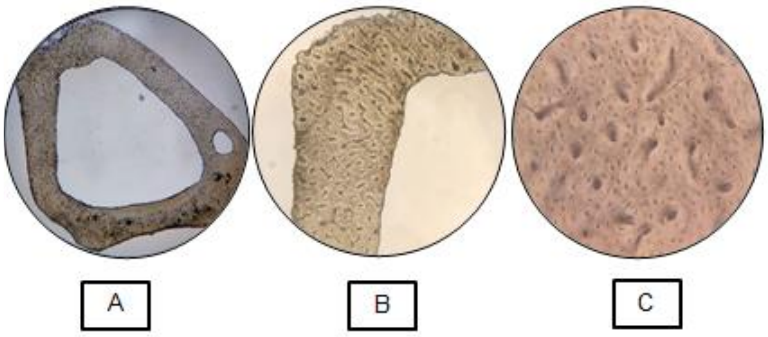

Figure 2. Antebrachium Preparation Oryctolagus cuniculus
Description: A. Magnification 40×, B. Magnification 100×, C. Magnification 400×

Based on the research results, the histological structure of the femur and antebrachium bones of Oryctolagus cuniculus with a magnification of 400× can be seen in figures 3, 4, 5 and 6.

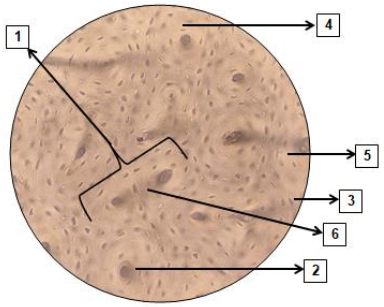

Figure 3. Femur Histology Preparation (personal document)
Description: 1. Haversian system, 2. Haversian canal, 3. Lacuna, 4. Canaliculi, 5. Lamella, 6. Volkmann canal

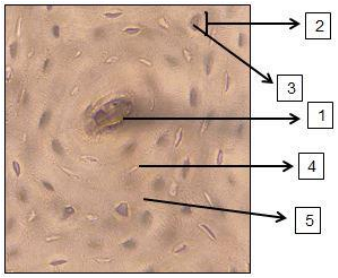

Figure 4. Femur Histology Preparation (personal document)
Description: 1. Canalis haversi. 2. Lacuna, 3. Osteocytes, 4. Canaliculi, 5. Lamella

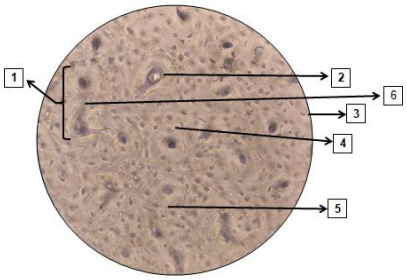

Figure 5. Histology Preparation of Antebrachium (personal document)
Description: 1. Haversian system, 2. Haversian canal, 3. Lacuna, 4. Canaliculi, 5. Lamella, 6. Volkmann canal

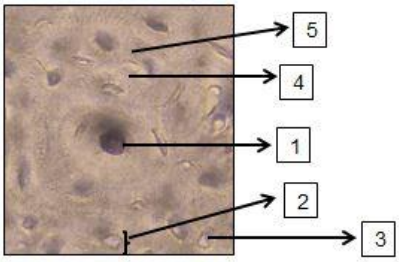

Figure 6. Histology Preparation of Antebrachium (personal document)
Description: 1. Canalis haversi. 2. Lacuna, 3. Osteocytes, 4. Canaliculi, 5. Lamella

DISCUSSION

Based on the documentation results above, it shows that the preparations with a magnification of 400x and without staining showed that the femur and antebrachium preparations had parts of the haversian system which included canalis havers, lacunae, canaliculi, lamellae, and canalis volkmann. This is in accordance with (Susetyarini et al., 2019) who stated that the haversian system consists of canalis centralis, osteocytes, lamellae, canaliculi, and canalis volkman.

The Haversian system is the entire system found in bone tissue (Achmad et al., 2018). Osteocytes are osteoblasts that are embedded in the bone matrix. Osteocytes are shaped like lacunae which are their place of residence. Osteocytes and their branches are not attached directly to the surrounding matrix but are separated from the lacunae and canaliculi walls by thin amorphous areas (Sihombing et al., 2013). Lamella are parts that are arranged concentrically (Wahyuni & Tosiyana, 2018), a circular lamella layer covers the osteons in the cortical bone and its structure is woven (Ardhiyanto, 2020). Volkman's canal or Volkman's bridge that looks like a connecting canal between one central canal and another central canal so that it forms a system called the harvest system. Canaliculi are fine channels that extend from one lacuna to another and extend to the surface of the bone, the canaliculi in each lacuna extend out from the lacuna, Canaliculi that are like fibers attached to each lacuna which are actually fine channels (Bella et al., 2021). Canaliculi are visible in each lacuna and serve as a connection between osteocytes in the lacunae of one lamella and those of the other lamella (Yuliana & Rosida, 2024).

In the process of making femur and antebrachium bone rubbing preparations, there are several limitations including the bone boiling process which takes a long time, around 2-4 hours, in cutting the bones still using a saw instead of a microtome so that the thickness is uneven which results in the rubbing results being thick on one side. In addition, bones that are too small are difficult to rub, so that there is concern if they break and disappear when dripped with distilled water, in the sanding process and the bone suddenly falls it is difficult to determine the direction of rubbing again, because if the direction of rubbing is different it will damage the bone structure, and rubbing on rough sandpaper and whetstones and for a long time can injure the skin of the fingers and erode fingernails (Wahyuni & Tosiyana, 2018).

According to Wahyuni (2015) stated that the factors that cause failure in this rubbing method include: a) improper boiling can result in the bone still being too hard so that it is difficult to slice or too soft so that the bone is crushed and cannot be sliced, b) uneven bone cutting can affect the process of sharpening and rubbing the bone which is also uneven so that the bone tissue is not very clear and the bone becomes prone

to fracture because it has uneven thickness, and c) lack of precision during the rubbing process results in damage to the bone structure.

The results of the femur and antebrachium bone smears of *Oryctolagus cuniculus* can be used as a source or media for histology practicum learning because they have met several requirements. According to Syah (2007) there are two criteria, namely general criteria and based on objectives. General criteria include economical, practical, easy, flexible, and in accordance with learning objectives. While the criteria based on objectives include learning resources that can be used as motivation, for learning, for research, for solving problems, and for presentations. Based on this, the bone preparations that have been produced have also gone through several stages of checking with several other learning resources such as the Atlas of Histology Practices by Dr. Johannes Halim, the Atlas of Histology in Fiore, and several other learning resources.

CONCLUSION

Unstained femur and antebrachium preparations of *Oryctolagus cuniculus* show parts of the haversian system, namely Canalis havers, Osteocytes, Lacunae, Canaliculi, Lamella, and Canalis Volkmann. The results of this study can be used in learning, namely as a media or source of learning histology practicums.

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Conflicts of Interest: The authors declare no conflicts of interest.

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