EFFECT OF PARTIAL TIBIAL PERIOSTEAL RESECTION ON PROXIMAL TIBIA GROWTH PLATE IN GROWING RABBITS

By

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ABSTRAK

Pengenalan:

Graf periosteum telah digunakan untuk tujuan mempercepatkan proses penyembuhan tulang, kecederaan rawan sendi atau dijadikan sumber sel osteoblast bagi tujuan kejuruteraan tisu. Kesan pada plat pertumbuhan tibia sekiranya periosteum diambil secara keliling tulang penderma sudah sedia maklum namun kesan pada plat pertumbuhan tibia yang turut menyebabkan pemanjangan tulang apabila pengambilan periosteum dibuat secara separa masih belum diketahui. Kami menjalankan kajian ini bagi melihat kesannya pada plat pertumbuhan tibia sekiranya periosteum diambil secara separa.

Kaedah:

Kajian ini melibatkan lapan ekor arnab jantan New Zealand yang berumur lapan minggu. Kesemua arnab ini telah menjalani pembedahan membuang sebahagian lapisan periosteum sepanjang 2 cm pada bahagian depan kiri dan depan kanan tulang tibia kaki kiri bermula pada 2 cm daripada sendi tibia femoral. Kesemua arnab ini telah dimatikan pada minggu keempat selepas pembedahan dan kedua-dua tulang tibia menjalani proses penyediaan histologi bagi tujuan analisa dan diwarnakan dengan hematoxylin dan eosin. Ketebalan plat pertumbuhan tibia beserta zonnya diukur dengan aplikasi ImageJ versi 1.8.0_172. Seterusnya keputusan yang diperolehi daripada tibia penderma dan tibia kawalan dibandingkan dan dianalisa secara statistik.

Keputusan

Min ketebalan plat pertumbuhan adalah lebih tinggi pada tibia penderma (0.448mm) berbanding tibia kawalan (0.388mm) dengan nilai-p 0.004. Perbandingan diantara tibia penderma dan tibia kawalan bagi setiap zon plat pertumbuhan; zon simpanan (0.067mm lawan 0.065mm, nilai-p 0.708), zon percambahan (0.241mm lawan 0.221mm, nilai-p 0.222), zon hipertrofi (0.141mm lawan 0.102mm, nilai-p 0.036) dan bilangan salur darah yang memberi nutrisi kepada plat pertumbuhan (28.4 lawan 27.1, nilai-p 0.348). Walaubagaimanapun, hanya perbezaan min ketebalan plat pertumbuhan dan zon hipertrofi didapati secara statistiknya adalah ketara.

Kesimpulan

Pembuangan sebahagian daripada lapisan periosteum juga akan menyebabkan kesan positif pada perkembangan plat pertumbuhan tibia. Ini disebabkan oleh ketebalan secara menegak sel kondrosit pada zon hipertrofi. Ia juga menunjukkan tiada kesan morbiditi yang ketara pada tulang penderma, justeru prosedur ini selamat untuk tujuan graf.

ABSTRACT

Introduction

Periosteal graft has been used to enhance bone healing, cartilage defect, or source of osteoblast in tissue engineering. The effect on the growth plate of the circumferential periosteum resection is known, but the effect on the growth plate that leads to bone elongation in partial periosteum resection is not explored yet. We conducted this study to understand the effect of a partial periosteum resection on the growth plate of the donor tibia.

Methodology

This study was carried out on eight 8-week-old white New Zealand rabbits. All rabbits underwent partial periosteum resection of a 2 cm segment of anteromedial and anterolateral left tibia, starting at 2 cm distal to the tibia-femoral joint. All rabbits were euthanized 4 weeks after surgery. The growth plate of both tibias were prepared for histological analysis and stained using haematoxylin and eosin. The thickness of the growth plate and its zones were measured using ImageJ software version 1.8.0_172. The results of the donor and control tibia were compared and analyzed statistically.

Result

The mean thickness of the growth plate was more on the donor side (0.448mm) compared to the control side (0.388mm) with a p-value of 0.04. The comparison of donor versus control tibia on each zone of the growth plate was; the reserve zone (0.067mm versus 0.065mm, p-value 0.708), the proliferative zone (0.241mm versus 0.221mm, p-value 0.222), the hypertrophic zone (0.141mm versus 0.102mm, p-value 0.036), and the number of feeding

vessels (28.4 versus 27.1, p-value 0.348). However, only the growth plate and hypertrophic zone thickness mean differences were statistically significant.

Conclusion

Partial resection of the periosteum of the growing rabbit tibia has a positive effect on its growth plate activity. This was mainly contributed by the axial thickness of the hypertrophic chondrocyte in the hypertrophic zone. The result also proves no significant morbidity on the donor tibia, thus it is safe for grafting purposes.

Chapter 1

INTRODUCTION

1.1 INTRODUCTION

The periosteum is a specialized connective tissue that contributes to the circumferential growth of the bone. It is capable to induce osteogenic cell differentiation and regeneration for bone healing [1,2].

Ollier et al. [3] described the ability of the cambium layer of the harvested periosteum was capable to produce bone when used as a graft in treating fractured bone. This finding was later validated by Ito et al. [4] and has stimulated interest in a few other studies to use it as a graft [5–8].

Crilly et al. [8] showed that transverse periosteal resection has a growth-stimulating effect in a chicken model. Following this, many other animal studies have proven that the removal of a circumferential strip of periosteum has accelerated the elongation growth of the bone [9–13]. Limpaphayom et al. [14] found that circumferential periosteum stripping and transverse section can cause femur overlengthening in humans. With this knowledge, D'Souza et al. [15] and Wilde et al. [13] used the periosteal resection technique for the treatment of minor limb length discrepancy. Those authors believed that the mechanical tension and restraint released from the periosteal sleeve across the growth plate were responsible for bone elongation [9,13,16].

The effect of circumferential periosteum transection, resection, and stripping has been studied in depth, but partial periosteum resection was not. [18,23,24]

To our knowledge, there is no study on histological changes to the growth plate once the periosteum is partially resected. The information on the outcome of the donor's bone is crucial as partial resection of the periosteum is the real procedure used in clinical conditions involving the donor site of the periosteum. It is more feasible to be performed and less likely to cause iatrogenic major neurovascular injury during the surgery compared to circumferential resection. This knowledge is important to relate to some metabolic bone diseases like rickets or bone dysplasia like achondroplasia and epiphyseal dysplasia.

Chapter 2

OBJECTIVES OF THE STUDY

General Objective

To study the effect of partial periosteum resection on the growth plate of the donor tibia in growing rabbits.

Specific Objective

1. To compare the thickness of the growth plate in the left tibia (partially resected periosteum) to the right tibia (control tibia).

2. To compare the thickness of the reserve zone in the left tibia (partially resected periosteum) to the right tibia (control tibia).

3. To compare the thickness of the proliferative zone in the left tibia (partially resected periosteum) to the right tibia (control tibia).

4. To compare the thickness of the hypertrophic zone in the left tibia (partially resected periosteum) to the right tibia (control tibia).

5. To compare the number of the blood vessels that supplied the growth plate in the left tibia (partially resected periosteum) to the right tibia (control tibia).

Chapter 3

MANUSCRIPT

3.1 TITLE: A STUDY ON THE EFFECT OF PARTIAL TIBIAL PERIOSTEAL RESECTION ON PROXIMAL TIBIA GROWTH PLATE IN GROWING RABBITS

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3.2 ABSTRACT

Introduction

Periosteal graft has been used to enhance bone healing, cartilage defect, or source of osteoblast in tissue engineering. The effect on the growth plate of the circumferential periosteum resection is known, but the effect on the growth plate that leads to bone elongation in partial periosteum resection has not been explored. We conducted this study to understand the effect of a partial periosteum resection on the growth plate of the donor tibia.

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The mean thickness of the growth plate was more on the donor side (0.448mm) compared to the control side (0.388mm) with a p-value of 0.04. The comparison of donor versus control tibia on each zone of the growth plate was; the reserve zone (0.067mm versus 0.065mm, p-value 0.708), the proliferative zone (0.241mm versus 0.221mm, p-value 0.222), the hypertrophic zone (0.141mm versus 0.102mm, p-value 0.036), and the number of feeding

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Conclusion

Partial resection of the periosteum of the growing rabbit tibia has a positive effect on its growth plate activity. This was mainly contributed by the axial thickness of the hypertrophic chondrocyte in the hypertrophic zone. The result also proves no significant morbidity on the donor tibia, thus it is safe for grafting purposes.

Keywords; *periosteum, segmental resection, complete circumferential resection, growth plate, axial elongation.*

3.3 INTRODUCTION

The periosteum is a specialized connective tissue that contributes to the circumferential growth of the bone. It is capable to induce osteogenic cell differentiation and regeneration for bone healing [1,2]. Ollier et al. [3] described the ability of the cambium layer of the harvested periosteum was capable to produce bone when used as a graft in treating fractured bone. This finding was later validated by Ito et al. [4] and has stimulated interest in a few other studies to use it as a graft [5–8].

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The effect of circumferential periosteum transection, resection, and stripping has been studied in depth, but partial periosteum resection was not. [18,23,24]

To our knowledge, there is no study on histological changes to the growth plate once the periosteum is partially resected. The information on the outcome of the donor's bone is crucial as partial resection of the periosteum is the real procedure used in clinical conditions involving the donor site of the periosteum. It is more feasible to be performed and less likely to cause iatrogenic major neurovascular injury during the surgery compared to circumferential resection. This knowledge is important to relate to some metabolic bone diseases like rickets or bone dysplasia like achondroplasia and epiphyseal dysplasia.

3.4 MATERIAL AND METHOD

This study was carried out on eight male New Zealand white rabbits. The age of the rabbit in this study was 8 weeks of life, which corresponds to 8 years of human age[17]. They were kept in the Animal Research Unit and Service Centre (ARASC) USM Health Campus in optimum condition with food (Altromin) and water available.

The rabbits were chosen for this study as their tibiae are anatomically similar to the human tibiae, docile, and easy to handle compared to other animals, especially in the surgical procedure. The fractured bone also heals faster in comparison to the human subject [16].

Pre-operative Preparation

Pre-operative fasting is not necessary as vomiting during induction has not been reported in this species [16]. The limb of interest was marked prior to surgery.

Operative Procedure

The operation on the left tibia was done under anaesthesia using an intramuscular injection of xylazine 5mg/kg body weight, followed by an intramuscular injection of ketamine 35mg/kg body weight and prophylactic antibiotics (Enrofloxacin 5mg/kg for stat dose). The operative region was shaved using an electric hair clipper before the skin incision. The surgical site was cleaned with povidone-iodine and draped under aseptic techniques [16]. An anterolateral skin incision over the tibia was adopted in this study. The cut in the skin began 2 cm away from the tibiofemoral joint and went 2 cm farther away.

The muscle surrounding the bone was elevated until the periosteum was visualized. Two cm of periosteum segment was resected distally from 2 cm distal to the tibiofemoral joint. Only the anterolateral and anteromedial tibial periosteum were resected (Figure 1). The periosteum at the posterior tibia was left undisturbed. Following that, the wound was irrigated using normal saline, the muscles were allowed to fall back, and the skin was closed in layers. A dressing and bandage were applied over the surgical site.

Post-Operative Care

The rabbits were left free in the cage for four weeks, but they had to wear a cone around their necks to keep them from biting the operated back leg (Figure 2). The wound inspection was done on day 3 post-operative and no regular dressing was done.

Rabbit Euthanization

All rabbits were euthanized 4 weeks post-surgery. Overdose intravascular injection of Pentobarbitone 100mg/kg was used for this procedure [16]. Both the donor and control tibiae bones were disarticulated through the knee and ankle joints. The tibias were then put in a labelled container with a neutral buffered formalin solution in it (Figure 3). The tibias were then decalcified and prepared for the H&E staining process.

Histology Preparation

The harvested tibiae were immersed in the decalcifying agent 5% nitric acid solution for a duration of 4 days. The decalcified specimen was cut horizontally 2 cm from the tibia-femoral joint. The specimen was then further cut in a sagittal plane at the midline (Figure 4). At this stage, each tibia will have 2 specimens and be labelled based on coding [L/R (Left/Right)-G (Growth plate)-M/L (Medial/Lateral)]. The specimen was then placed on a labeled cassette for fixation, then paraffin wax impregnation. The trimming and sectioning processes were done using a rotary microtome. Tissue block sectioning was done at 3 micrometers thickness.

Sections numbers 6, 11, 16, 21, and 26 from the midline were then stained using haematoxylin and eosin.

Histology Analysis

The images of the histology slides were captured using an Olympus BX51 light microscope with F-cell software. The magnification was set to x4 on the microscope and the software. The scale was set to be embedded in the image. The images were then captured at the anterior one-third, middle one-third, and posterior one-third of the growth plate. Each image was analyzed using the ImageJ software version 1.8.0_172.

The thickness measurement was done at the reserve, proliferative and hypertrophic zone. Those three zones were identified based on their morphology and measured in the region of the middle third of the image, and the well-visualized point will be taken as a measurement point. The growth plate thickness will be based on the sum of the measurements of all 3 zones (Figure 5). The zone of provisional calcification was left out because it was hard to tell where it ended.

The vascularity of the growth plate was analyzed using a light microscope, and the measurements were taken at a random point over the anterior one-third, middle one-third, and posterior one-third of the growth plate. The vessels or their extension that are close to the reserve zone will be counted as feeding vessels of the growth plate.

Statistical Analysis

A total of 8 rabbits were operated on and included in the data analysis. The growth plate, reserve zone, proliferative zone thickness, and the number of feeding vessels in the reserve zone were measured and analyzed using a paired T-Test as the data was normally distributed. Meanwhile, the hypertrophic zone was analyzed using the Wilcoxon Signed Rank Test as the data was a non-normal distribution based on the normality test with the Kolmogorov-Smirnov test, which produced a p-value of 0.007, and the Shapiro-Wilk test, which produced a p-value of 0.035.

3.5 RESULTS

The growth of the proximal tibia physis was accelerated in the tibiae who underwent partial periosteal resection at a 4-week follow-up. These growth accelerations were seen in the individual thickness of the growth plate, reserve zone, hypertrophic zone, and the number of feeding vessels. The mean thickness of the growth plate on the affected side was greater than on the control side (0.448mm versus 0.388mm, p-value 0.004). These findings were also seen in the reserve zone (0.067mm versus 0.65mm, p-value 0.708), and hypertrophic zone (0.141mm versus 0.102mm, p-value 0.036) (Table 1). The mean number of feeding vessels on the donor side was 28.4 while 27.1 on the control side with a p-value of 0.348 (Table 2).

Even though data analysis based on individual rabbits showed some of the zones were higher on the control side compared to the affected side, such as in reserve zone (R1-R5), proliferative zone (R6-R8), and hypertrophic zone (R2 and R5), the mean growth plate thickness showed the affected side was still higher in the thickness. The same goes for the number of feeding vessels, where only R1 has more feeding vessels on the control side compared to the affected side, while R2-R7 showed more feeding vessels on the affected side.

3.6 DISCUSSION

Periosteum grafting is one of the options for treatment for non-union in growing bone. Its cambium layer has a rich number of osteoprogenitor cells, which has the capability to stimulate bone formation and assist in fracture healing. However, harvesting the periosteum from the normal bone is not without risk. Our previous radiological study on the same rabbit tibia showed that partial resection of the periosteum led to a lengthening of the tibia compared to the control side [25]. Thus, in this study, our primary goal was to assess changes that occur at the proximal tibia growth plate after partial periosteum resection. In this study, we only studied the proximal growth plate because the effect of periosteal resection appeared limited to the proximal growth plate rather than the distal tibia growth plate [18]. In this study, we chose to do partial resection instead of circumferential resection because it is more feasible to do on a human subject and less likely to injure the nerves or blood vessels during the harvesting process.

The growth plate is a crucial component of the endochondral bone that contributes to the elongation of the long bone. Any injuries or disorders that involve the growth plate will halt the lengthening process of the long bone [19]. The chondrocyte maturation process in the physis can be divided into five phases. In a step-by-step process, the chondrocyte goes through the resting, dividing, pre-hypertrophic, hypertrophic, and terminal phases.

In the resting phase, the cells are relatively inactive and express the *Col2a1* gene, which encodes type II collagen. In the proliferative phase, the chondrocyte rapidly divides and expresses *Col2a1* and *Acan*, which encode the proteoglycan aggrecan. These chondrocytes later enter the pre-hypertrophic phase and start to express *Col10a1* for type X collagen and IHH, which encodes *Indian Hedgehog*. Subsequently, the cell size increases and the cell enters the hypertrophic phase. Once the chondrocytes stop producing collagen and die, it is considered a terminal phase where the cartilage is replaced by blood vessels and organized bone tissue [20].

This linear process is coordinated by a wide variety of signaling cascades that interact with genes, receptors, and transcription factors [20]. The periosteal intervention piqued our interest. A few authors have reported accelerated longitudinal growth of the long bone after circumferential resection of the periosteum [9,10,21]. The authors attributed this phenomenon as being due to vascular and mechanical theories. The vascular theory explained the increase in vascularity at the growth plate, which stimulated activity over the growth plate, while the mechanical theory explained the release of the periosteum tension leads to the proliferative zone being able to grow without tension [14,22]. However, we couldn't find any published literature regarding the effect of partial periosteum resection on the long bone in human or animal subjects.

In our study, we found that partial periosteum resection has resulted in an increment in the mean thickness of the growth plate and its zones: the reserve zone, proliferative zone, and hypertrophic zone of the donor tibia. The statistical analysis showed statistically significant results in the growth plate thickness (p-value 0.004) and hypertrophic zone thickness (p-value 0.036). The mean number of feeding vessels to the reserve zone is also higher in the donor tibia than in the control tibia. Here we can conclude that even with partial periosteal resection, it has a significant effect on the growth plate where it increases the activity of the growth plate and leads to the lengthening of the tibia.

Our study findings were comparable in a certain area to the findings of Sansone et al. [18]. They did a study on the lambs' tibial growth plate measurement using implanted micro transducer technology near the growth plate and histomorphometry and stereological analysis after circumferential tibia periosteal resection. They discovered that periosteal resection has increased growth plate velocity on the donor tibia than on the control tibia (273 µm/day versus 201 µm/day) with a p-value less than 0.05. Their histomorphometry correlation to this phenomenon found that the cellular basis of this acceleration was most likely resulting from hypertrophic chondrocyte axial elongation rather than changes in chondrocyte proliferation, the magnitude of hypertrophic chondrocyte swelling, or increased matrix production. Even though the statistical analysis for hypertrophic chondrocyte axial elongation was not significant with a p-value of 0.08, its value approached statistical significance compared to the other three potential mechanisms. There were no changes found in the proliferative zone chondrocyte. In our study, an increase in the growth plate thickness which correlated with growth plate velocity after partial periosteum resection was statistically significant and we found that this phenomenon was also mainly contributed by the hypertrophic zone with a p-value of 0.036.

In correlation with some of the metabolic bone diseases such as rickets or bone dysplasia like achondroplasia and epiphyseal dysplasia, this procedure as one of the treatments to stimulate bone healing is questionable as the growth stimulus is diminutive even though it was statistically significant. We would recommend this procedure be employed as an adjunctive procedure such as distraction osteogenesis. Further studies are needed to explore the potential of periosteum resection as one of the treatments for those diseases.

3.7 CONCLUSION

Partial periosteal resection has a positive effect on the growth plate activity evidenced by axial thickening in the hypertrophic zone, outright increasing the overall growth plate thickness of the donor tibia at the end of the study. This positive growth activity was consistent across all eight rabbits. However, the thickness of the reserve zone, proliferative zone, and the number of vessels that supply the growth plate of the donor tibia were statistically insignificant despite the mean result showing an increment in the thickness and numbers. This result proves no significant morbidity on the donor side, thus it is safe to harvest the periosteum for grafting purposes in the case of fracture non-union.

The limitation of this study was the period of lockdown during the COVID-19 endemic. We were facing difficulties in getting supplies of rabbits and equipment; procedures couldn’t be done as our animal house and laboratory were closed. Due to a lack of supply, the price of rabbits and our equipment was raised, which led to an increase in costing of this project.

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3.9 TABLES AND FIGURES

Figure 1: The 2cm periosteum segment harvested 2cm distal to the tibia-femoral joint.

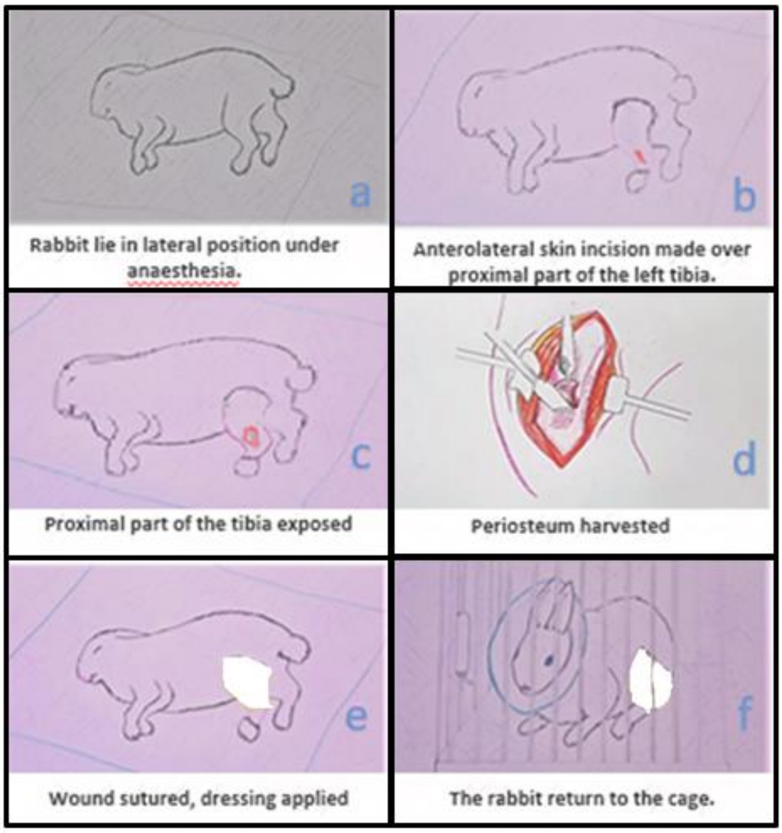

Figure 2: The operative process of the rabbit from induction until release back into the cage.

Figure 3: The specimens were kept in a container containing neutral buffered formalin solution.

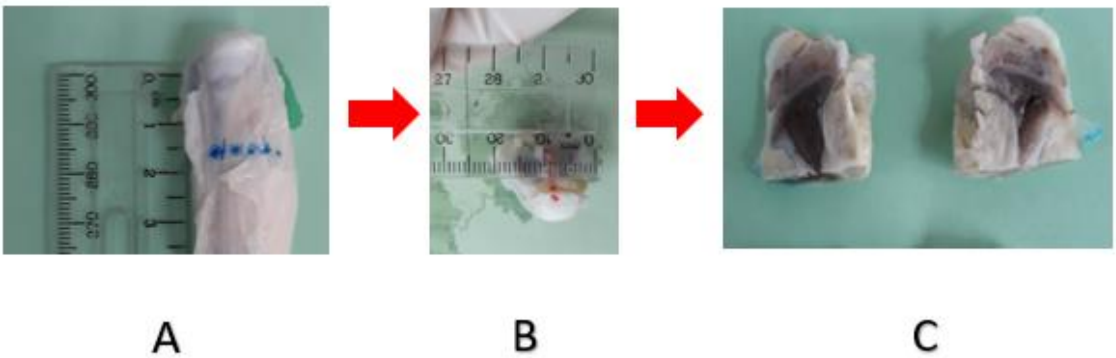

Figure 4: A. The proximal 2cm segment was cut horizontally from the tibia-femoral joint. B. The segment was cut in the sagittal plane at the midline. C. Two fragments of the proximal tibia.

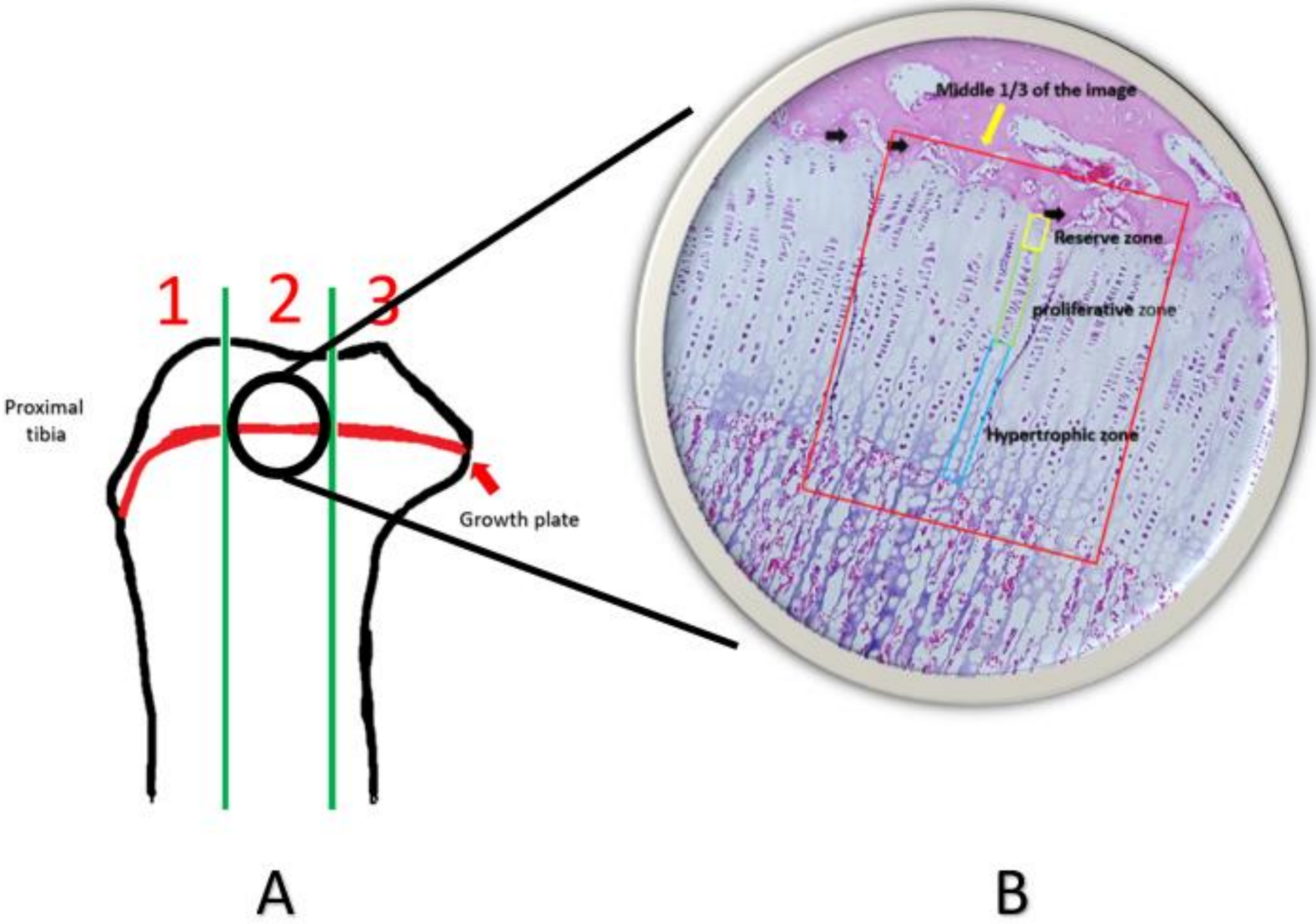

Figure 5: A. The image shows a sagittal cut of the proximal tibia and divided into 3 divisions. B. Division 2 was taken for measurement. The well-visualized area of the growth plate was then measured. An example of the feeding vessels to the growth plate is shown by the black arrow.

Table 1: Comparison of the growth plate and its zones thickness (mm) between donor and control tibia.

Rabbit	Donor (mm)	Control (mm)	Diff. (Donor-Control)	Donor (mm)	Control (mm)	Diff. (Donor-Control)	Donor (mm)	Control (mm)	Diff. (Donor-Control)	Donor (mm)	Control (mm)	Diff. (Donor-Control)
	Growth Plate			**Reserve Zone**			**Proliferative Zone**			**Hypertrophic Zone**		
R1	0.474	0.357	0.117	0.084	0.089	-0.005	0.210	0.162	0.048	0.179	0.106	0.073
R2	0.411	0.359	0.052	0.064	0.074	-0.010	0.247	0.181	0.066	0.100	0.103	-0.003
R3	0.295	0.261	0.034	0.058	0.061	-0.003	0.166	0.138	0.028	0.071	0.062	0.009
R4	0.411	0.328	0.083	0.064	0.072	-0.008	0.252	0.179	0.073	0.095	0.077	0.018
R5	0.372	0.372	0.000	0.041	0.045	-0.004	0.236	0.229	0.007	0.095	0.097	-0.002
R6	0.498	0.386	0.112	0.066	0.052	0.014	0.228	0.245	-0.017	0.204	0.089	0.115
R7	0.560	0.530	0.030	0.064	0.061	0.003	0.307	0.339	-0.032	0.188	0.130	0.058
R8	0.566	0.513	0.053	0.093	0.066	0.027	0.278	0.297	-0.019	0.195	0.150	0.045
Mean	**0.448**	**0.388**	**0.060**	**0.067**	**0.065**	**0.002**	**0.241**	**0.221**	**0.019**	**0.141**	**0.102**	**0.039**
			P= 0.004			**P= 0.708**			**P= 0.222**			**P= 0.036**

Table 2: Comparison of the number of feeding vessels on the growth plate of donor and control tibia.

Rabbit	Donor	Control	Diff. (Donor-Control)
R1	26.0	32.7	-6.7
R2	33.8	28.4	5.4
R3	28.0	24.9	3.1
R4	27.6	26.7	0.9
R5	24.8	24.0	0.8
R6	27.2	24.1	3.1
R7	30.4	29.9	0.5
R8	29.5	26.3	3.2
Mean	**28.413**	**27.125**	**1.289**
			P=0.348

3.10 GUIDELINES/INSTRUCTIONS TO AUTHORS OF SELECTED JOURNAL

- The selected journal is the Malaysia Journal of Medical Sciences

The *Malaysian Journal of Medical Sciences* (MJMS) welcomes manuscripts on all aspects of medicine/health/biomedical science from any part of the world, especially developing countries.

Please read and follow the Guidelines for Author carefully. The Editorial Office reserves the right to return manuscript for revision when the preparation is not in accordance with the Guidelines for Author or to reject manuscripts without peer-review when Turnitin plagiarism check shows similarity score of more than 30%.

Upon verification that a submitted manuscript has not been published elsewhere or has not been under consideration for publication elsewhere or is not simultaneously submitted to other journal and has not previously been posted on a non-profit preprint server, it is considered to undergo double-blinded peer-review process.

These guidelines are in accordance with the Uniform Requirement for Manuscripts Submitted to Biomedical Journals of the International Committee of Medical Journal Editors (ICMJE).

MJMS uses highly rigorous editorial, peer and statistical review processes to evaluate manuscripts for scientific accuracy, novelty and importance. Therefore, authors **must adhere** to our standards for reporting statistical results, as outlined in our Editorial (http://www.mjms.usm.my/MJMS23052016/01MJMS23052016_ED.pdf). To ensure the quality of the submitted manuscript, authors are advised to follow the relevant reporting