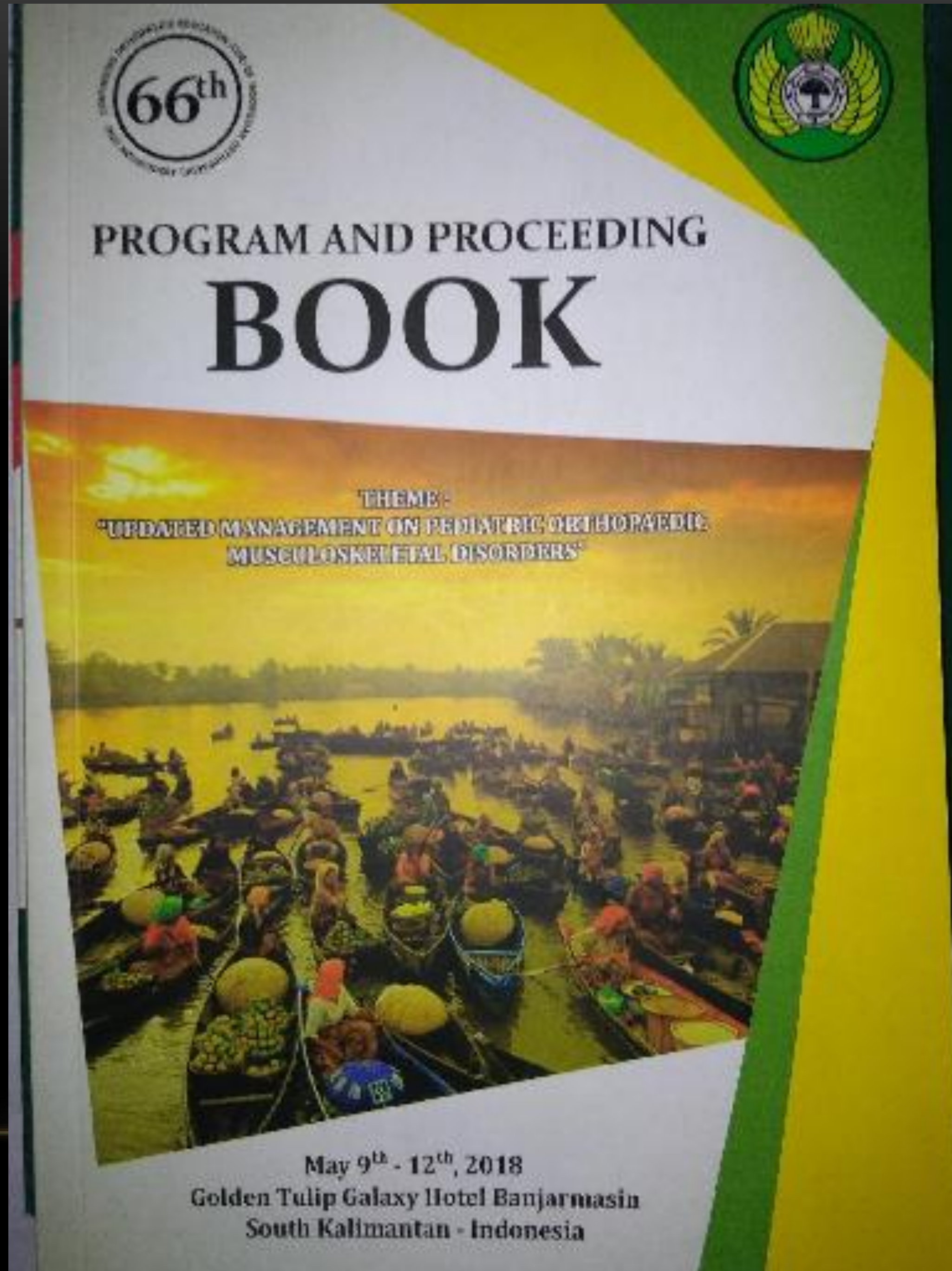
PROCEEDING


66th
PROGRAM AND PROCEEDING
BOOK
THEME :
"UPDATED MANAGEMENT ON PEDIATRIC ORTHOPAEDIC
MUSCULOSKELETAL DISORDERS"
May 9th - 12th, 2018
Golden Tulip Galaxy Hotel Banjarmasin
South Kalimantan - Indonesia

DAFTAR FINAL PAPER - COE KE 66

HOTEL GOLDEN TULIP GALAXY, BANJARMASIN

9 – 12 MEI 2018

Wednesday, May 9, 2018

FINAL PAPER FOR CANDIDAT – VENUS 1 – 2nd Floor

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FPA 08

EFFECT OF ELECTROMAGNETIC STIMULATED CHITOSAN BONE GRAFT ON CALLUS FORMATION TO FEMUR FRACTURE WITH BONE LOSS AT RATS

Hefrel Irammandar

There was [illegible] cases of fracture in [illegible] at 2014-2016 and 84 cases of it was nonunion fracture with bone defect. If it is significant, the bone can't regenerate normally. Widely used methods for management fracture with bone defect is bone stabilization and used of bone graft. The use of Pulsed Electromagnetic Fields (PEMF) in stimulate bone healing has been known its contribution to accelerate the fracture healing.

In both groups was performed: made a fracture and bone defect on the right middle shaft of femur, fixation with mini plate and screw, and chitosan bone graft administration. Stimulation was given at group I using PEMF, 4 hours per day for 30 days. Radiographic examination was taken at 1st week and at 6th week.

At 6th week radiological examination, callus formation occurred at 62.5% of group I samples and 37.5% of group II samples. If compared between 1st week and 6th week at each group, there was significant callus formation in group I (p of 5 points radiology score: 0.015, p of callus width: 0.018), while there was no significant difference in group II (p of 5 points radiology score: 0.102, p of callus width: 0.109). When compared the amount of callus formation between the two groups at 6th week, the amount of callus in group I was significantly more than group II (p of 5 points radiology score: 0.039; p of callus width: 0.046).

Chitosan bonegraft and PEMF on fresh femur fracture with bone defect will accelerate the callus formation.

Key word: Bone loss, Pulsed electromagnetic fields, chitosan bone graft.

FOTO


Effect of Electromagnetic Stimulated Chitosan Bonegraft on Callus Formation To Femur Fracture with Bone Loss

SERIFIKAT

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66th Continuing Orthopaedic Education (COE) of Indonesian Orthopaedic Association (IOA)

Theme :

"UPDATED MANAGEMENT ON PEDIATRIC ORTHOPAEDIC MUSCULOSKELETAL DISORDERS"

Golden Tulip Galaxy Hotel, Banjarmasin, May 9th - 12th, 2018

Chairman of Organizing Committee

Izaak Zoelkarnain Akbar, MD, PhD

Chairman of The Indonesian College of Orthopaedic & Traumatology

Ifran Saleh, MD

PRESENTASI

EFFECT OF ELECTROMAGNETIC STIMULATED CHITOSAN BONE GRAFT ON CALLUS FORMATION TO FEMUR FRACTURE WITH BONE LOSS AT RATS

Helmi Ismunandar

DEPARTMENT OF ORTHOPAEDIC AND TRAUMATOLOGY
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HASAN SADIKIN HOSPITAL
BANDUNG
2017

CHAPTER I
INTRODUCTION

INTRODUCTION

- Fractures incidence rate → 11,13/1000/year[1]
- In traumatic fracture with bone loss → influence the healing process[2,3]

Requirement of Alternative treatment with bone graft and adjuvant therapy[2,3]

1. Bulcholz et all. Rockwood and Green's Fractures in Adult. 7th Edition. Philadelphia: Lippincott Williams and Wilkins; 2010. Page. 53-84
2. Keating et all. The Management of Fracture With Bone Loss. The Journal of Bone and Joint Surgery. 2005; 87B (2): 143-150
3. Lan S. Chitosan-based Scaffolds for Bone Tissue Engineering. J Mater Chem B Mater Biol Med. 2014; 2: 3161-3184

- Autograft from the iliac crest and other local sources is used as the gold standard to replace the bone loss

Source limitation → 30% of the donor.

Required additional surgical procedure → increase morbidity and postoperative complications

Reducing the donor bone strenght[4,5,6]

4. Nandi et all. Orthopaedic Aplication of Bone Graft and Graft Subtitutes. Indian Journal of Medicine. 2010; 132: 15-30
5. Salvator JM. Understanding Bone Grafts and Bone Grafts Subtitutes [document on the internet]. USA: Biosurgery Baxter; 2012 [Download at March 2014]. URL: www.naot.org
6. Boden S et all. Bone Graft Subsitution: Facts, Fictions, and Aplication. 70th Annual Meeting of American Academy of Orthopaedic Surgeon; 2003 Februari 5-9; New Orleans, USA. Illinois; AAOS; 2003

- Last two decades → Chitosan plays an important role in bone tissue engineering[8]

Suitable for the cells growth, osteoconduction, biocompatibility, and biodegrabilitas

Natural intrinsic antibacterial

Low Toxicity[8]

8. Venkatesan J et all. Chitosan Composit for Bone Tissue Engineering. Journal of Marine Drug. 2010; 8: 2252-2266

- According Ezoddini (2012), Chitosan significantly accelerated the bone regeneration process in rat tibias.[10]

10. Ezoddini F et all. Histologic Evaluation of Chitosan as an Accelerator of Bone Regeneration in Microdrilled Rat Tibias. Dental Research Journal. 2012; 9: 694-699

According to Assiotis (2012), pulsed electromagnetic field (PEMF) stimulation effective as noninvasive theraphy for nonunion fracture at tibia.[11]

11. Assiotis A. Pulsed Electromagnetic Fields for The Treatment of Tibial Delayed Unions and Nonunions. A Prospective Clinical Study and Review of The Literature. Journal of Orthopaedic Surgery and Research. 2012; 7: 1-6

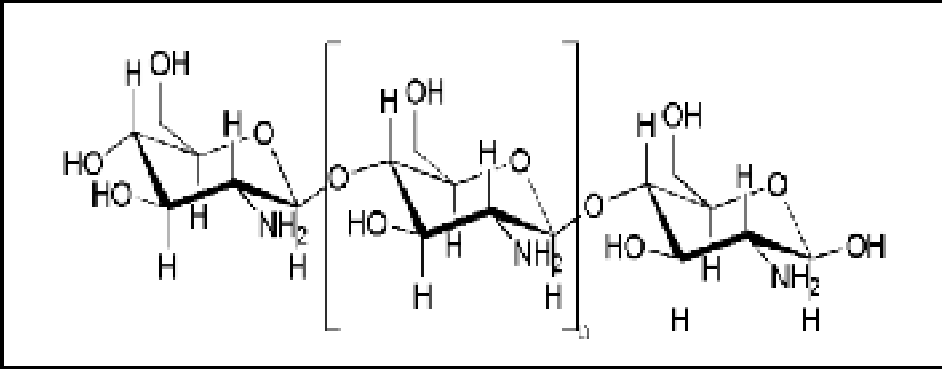
THE PROBLEMS IN THIS STUDY

Whether there is a positive effect of electromagnetic stimulated citosan bonegraft on callus formation to femur fracture with bone loss at rats?

CHAPTER II
LITERATURE REVIEW, FRAME OF MIND, PREMISES, AND HYPOTHESIS

CITOSAN AS BONE GRAFT

Chitosan is a natural polymer derived from chitin which is the main component of the exoskeletons of crustacean.[3]



Chemical Structure of Chitin[8]

3. Lan S. Chitosan-based Scaffolds for Bone Tissue Engineering. J Mater Chem B Mater Biol Med. 2014; 2: 3161-3184
8. Venkatesan J et all. Chitosan Composit for Bone Tissue Engineering. Journal of Marine Drug. 2010; 8: 2252-2266

- The nature cations effect of chitosan is important in bone tissue engineering applications.
- Chitosan cation effect → form a complex of polyelectrolytes with anionic biological macromolecules.
- These molecules include anionic glycosaminoglycans such as heparin and heparin sulfate → modulate the activity of cytokines and growth factors.[3]

3. Lan S. Chitosan-based Scaffolds for Bone Tissue Engineering. J Mater Chem B Mater Biol Med. 2014; 2: 3161-3184

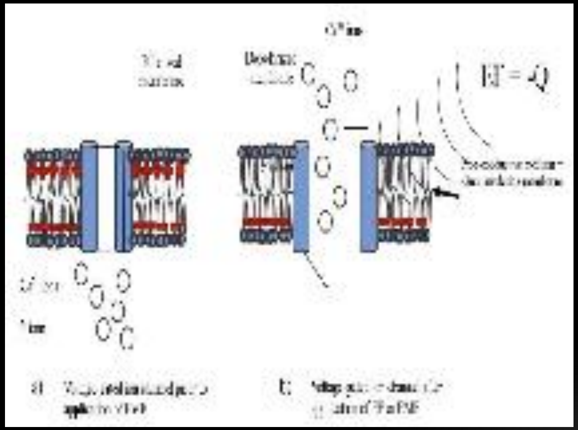
Inductive coupling (IC) → Noninvasive methode to create electric current at bone → PEMF[13]

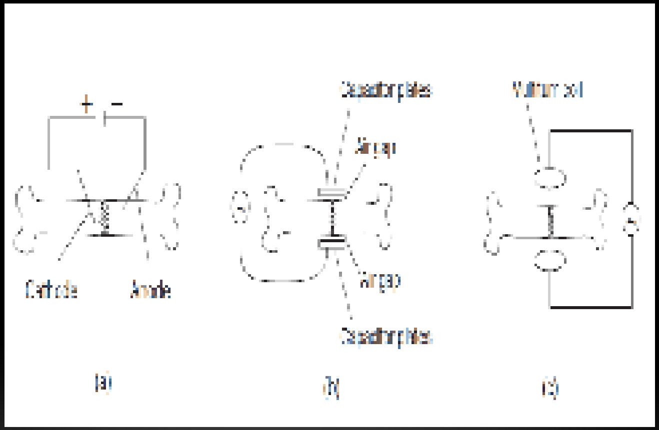
PEFM

Increase Intracelluler calcium ion

Canal calcium opening

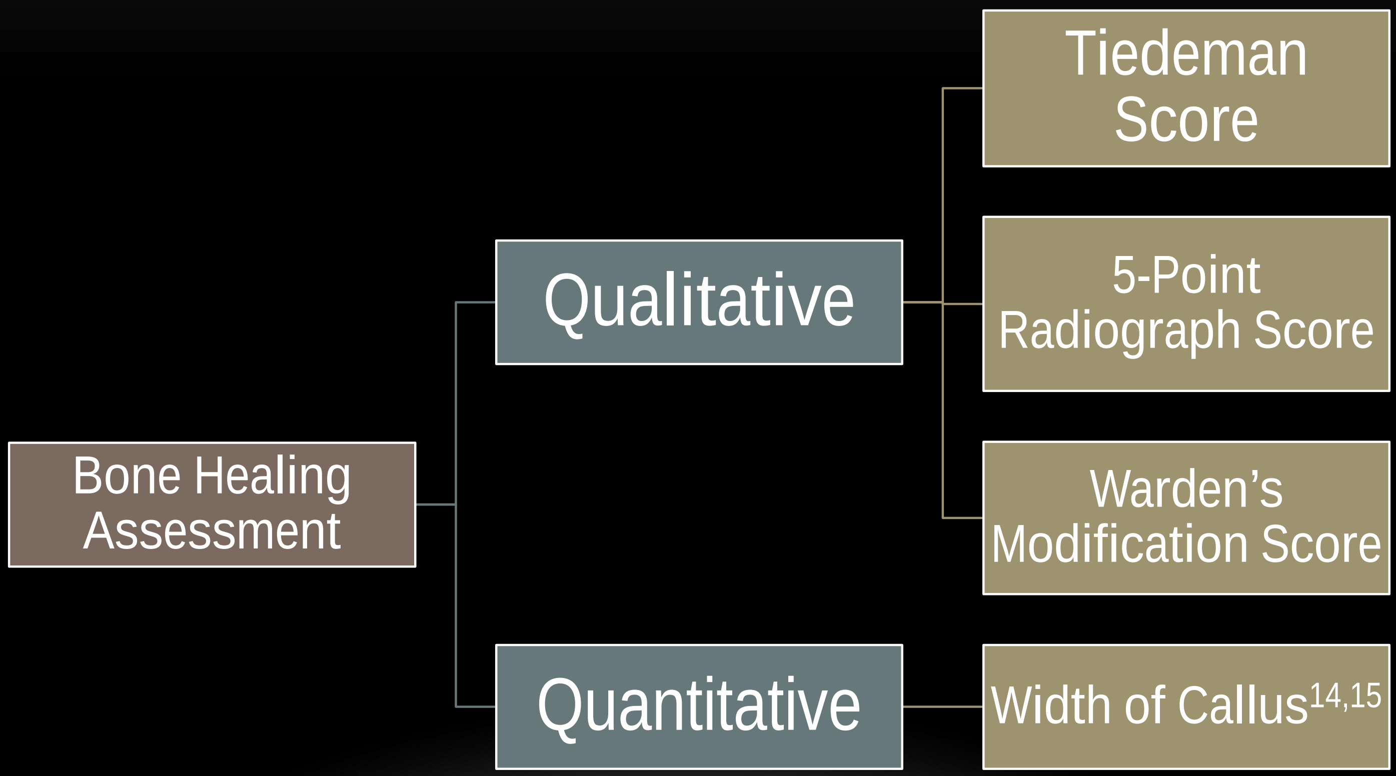
Release calcium ion from endoplasmic retinaculum[13]





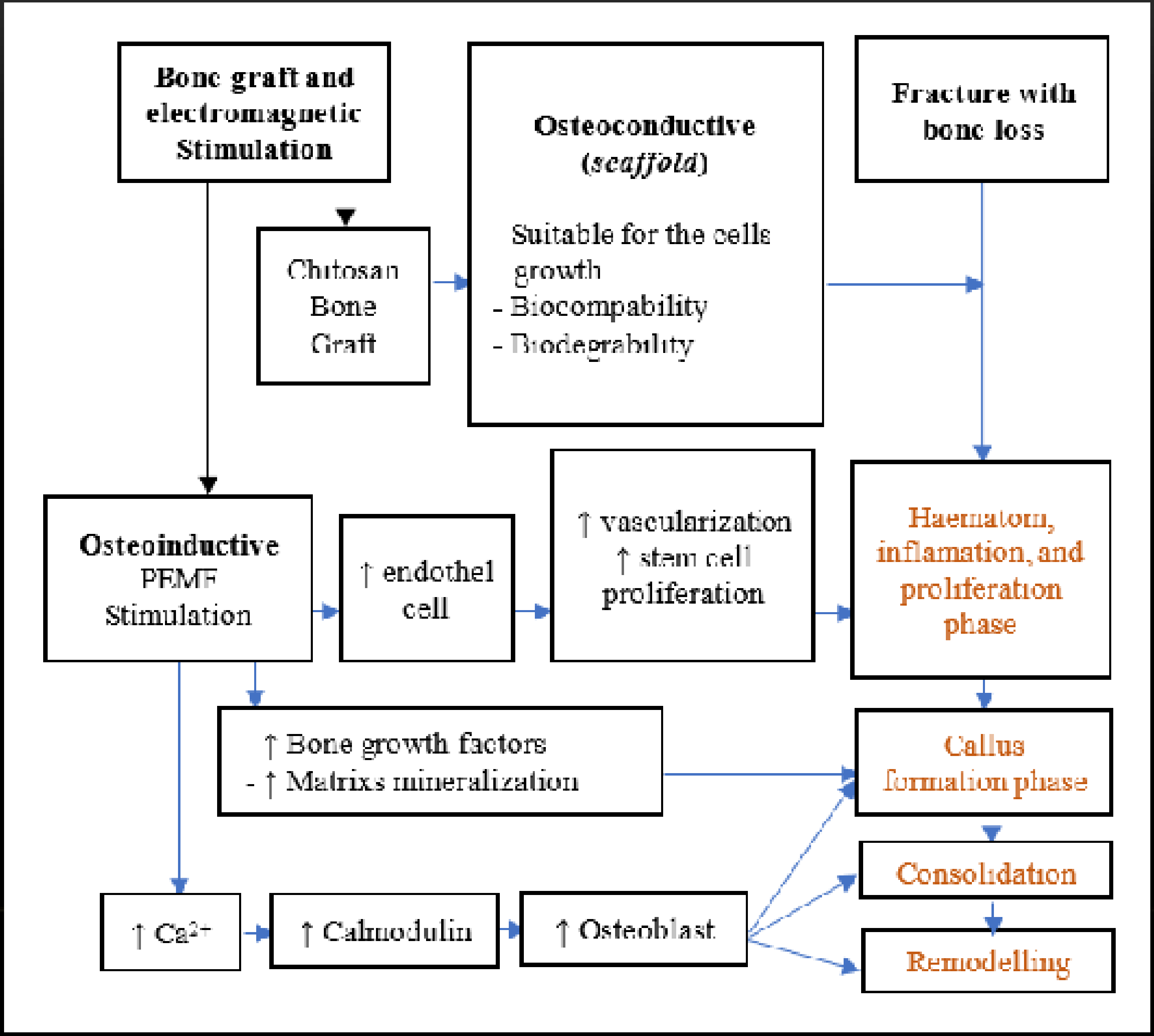
13. Netter F. The Netter Collection of Medical Illustrations: Physical Factor in Bone and Electric Stimulation of Bone Growth. Edisi 2. Volume 6. Philadelphia: Elsevier; 2013. Halaman. 62-64, 324, 325.

Assessment of Bone Healing and Callus Formation Radiologically



14. Anwar R. Stimulasi Pulsed Electromagnetic Field Pada Fraktur Terutup Tibia Tikus Terhadap Pembentukan Kalus Berdasarkan Pengukuran Radiografi. Tesis. Bandung: Universitas Padjadjaran; 2013
15. Estai M et all. *Piper sarmentosum* Enhance Fracture Healing in Ovariectomized Osteoporotic Rats: a Radiological Study. Clinics. 2011; 66(5): 865-872

CONCEPTUAL FRAMEWORK



PREMISES

PREMISE 1

In a fracture with significant bone loss, bone unable to regenerate normally.[3]

PREMISE 2

Chitosan scaffold suitable for cell growth, osteoconduction, biocompability, and biodegrability.[8]

3. Lan S. Chitosan-based Scaffolds for Bone Tissue Engineering. J Mater Chem B Mater Biol Med. 2014; 2: 3161-3184
8. Venkatesan J et all. Chitosan Composit for Bone Tissue Engineering. Journal of Marine Drug. 2010; 8: 2252-226

PREMISE 3

Due to chitosan cation property, It is able to form complex of polyelectrolytes with biological macromolecules including glycosaminoglycans. The molecules modulate the activity of cytokines and growth factors.[3]

3. Lan S. Chitosan-based Scaffolds for Bone Tissue Engineering. J Mater Chem B Mater Biol Med. 2014; 2: 3161-3184

PREMISE 4

Electromagnetic stimulation has a positive effect on different levels of bone healing by promoting the angiogenesis, chondrogenesis, osteogenesis, and regulating of normal bone healing factors.[16]

16. Einhorn T et all. Orthopaedic Basic Science Foundation of Clinical Practice. Edisi 3. USA: AAOS; 2007. Halaman. 65-87, 331-349

PREMISE 5

Pulsed electromagnetic fields regulate the release of calcium and calmodulin ions which results in an osteoblast stimulation.[17]

PREMISE 6

In the proliferation phase, electromagnetic stimulation will increase blood vessel formation resulting in increased of stem cells proliferation and differentiation.[18,19]

17. Selvamurugan N et all. Effects of BMP-2 and Pulsed Electromagnetic (PEMF) an Rat Primary Osteoblastic Cell Proliferation and Gene Expression. Journal of Orthopaedic Research. 2007; 25(9): 1213-1220
18. Barnes FS. Biological and Medical Aspect of Electromagnetic Fields: Mechanisms and Therapeutic Applications of Time-Varying and Static Magnetic Fields. Edisi 3. Boca Raton: CRC Press; 2007. Halaman. 352-386
19. Ameia GP et all. Endothelial Response to Pulsed Electromagnetic Fields: Stimulation of Growth Rate and Angiogenesis in Vivo. Journal of Cellular Physiology. 1988; 134(1): 37-46

HYPOTHESIS

From the premise can be drawn hypothesis that there is positif effect of electromagnetic stimulated citosan bonegraft on callus formation to femur fracture with bone loss at rats.

CHAPTER III
RESEARCH METHODOLOGY

Research Object

SAMPLES

$$N_1 = N_2 = 2\left(\frac{(Z\alpha - Z\beta)S}{X_1 - X_2}\right)^2$$

$$N_1 = N_2 = 2\left(\frac{(1.64 - 1.28)2.03}{0.5}\right)^2$$

$$N_1 = N_2 = 4.3 = 5$$

N	: Amount of Samples each group
Zα	: Alpha standart deviation
Zβ	: Beta standart deviation
S	: Combined of standart deviation
X_1-X_2	: Minimum mean different that considered to still meningful[20]

20. Sopiyudin M. Besar Sampel dan Cara Pengambilan Sampel dalam Penelitian Kedokteran dan Kesehatan. Edisi 2. Jakarta: Salemba Medika; 2009. Halaman 65-70.

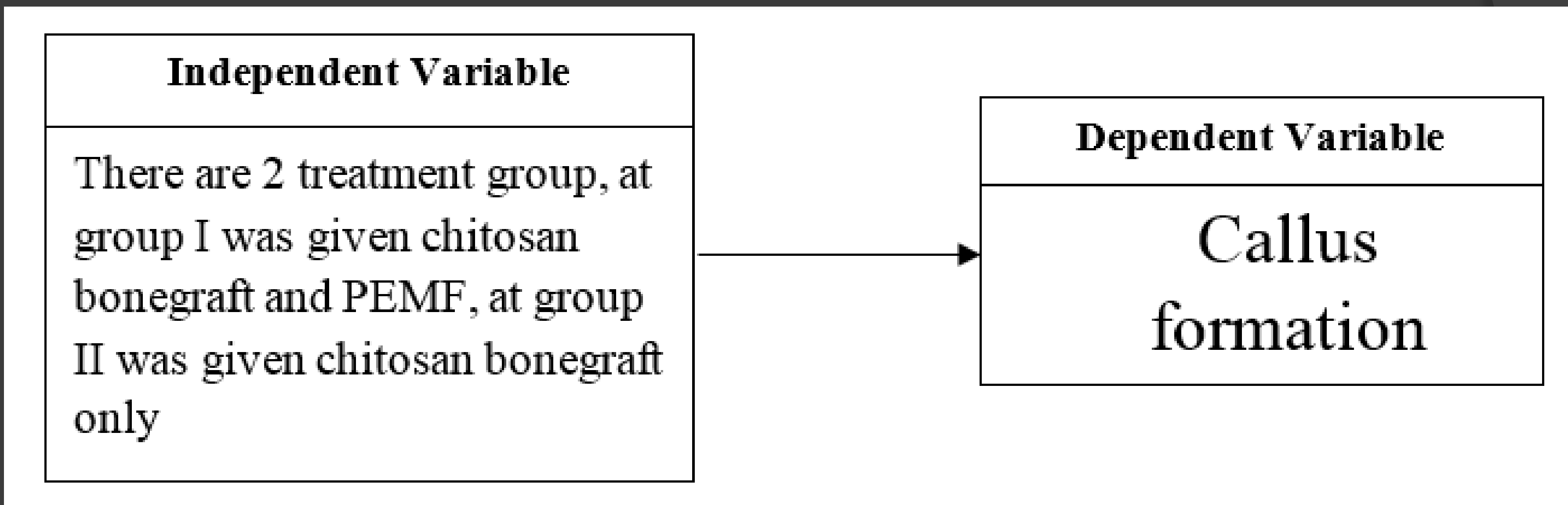
INCLUSION CRITERIA:

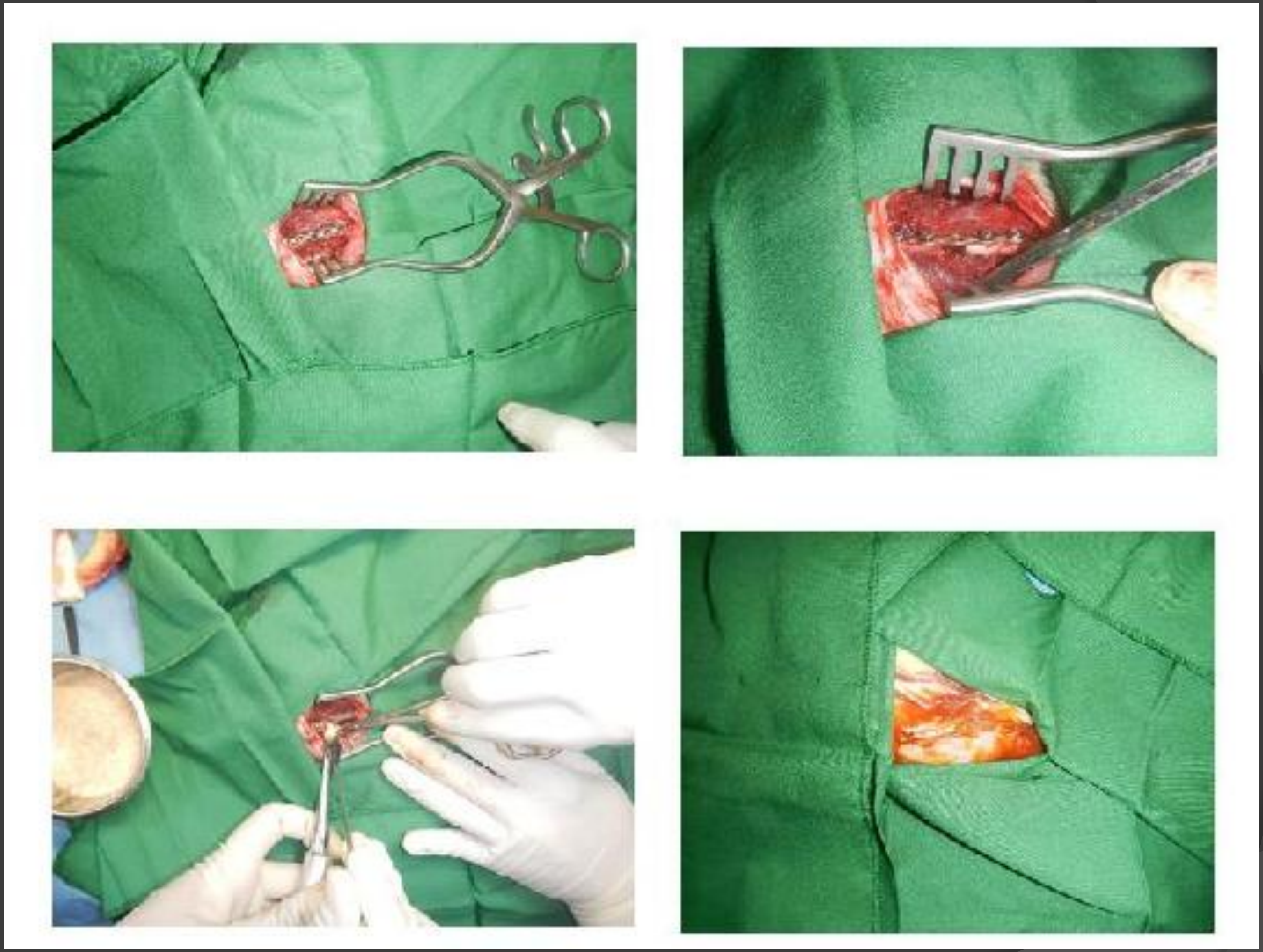
Healthy mature Wistar strain rats (male, age 2-3 months) with weight 350-400 gram.

Research Design

This is a comparative laboratory experimental study with two treatment groups.

Research Variable

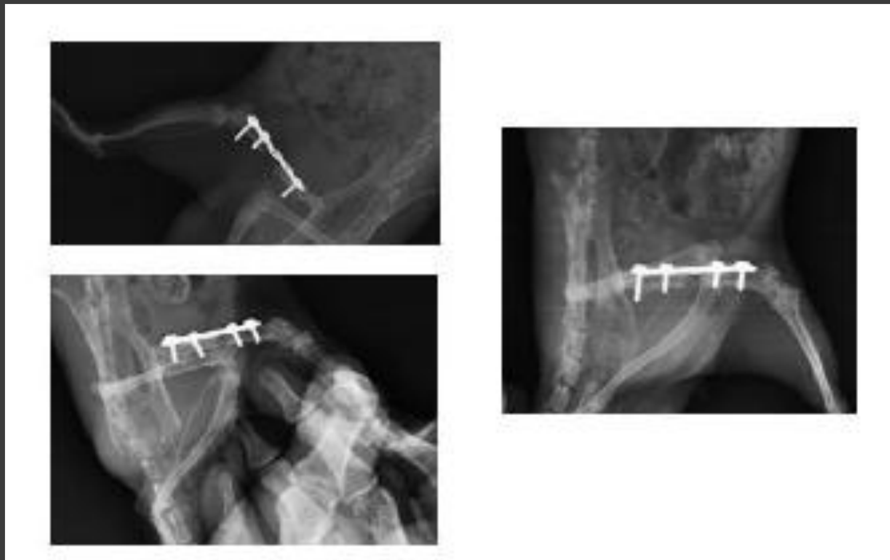




The Surgical Procedure

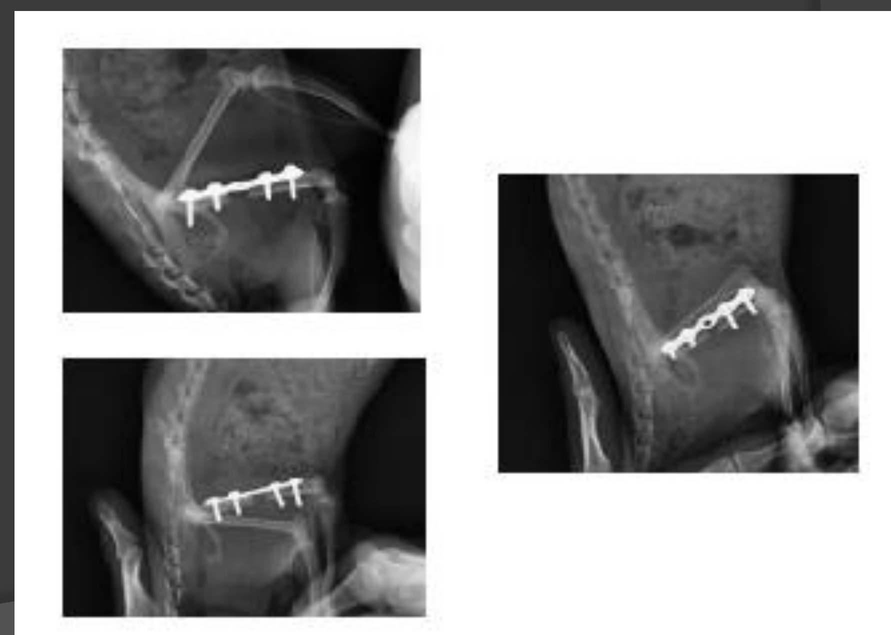
PEMF Device Preparation

Treatment at Group I

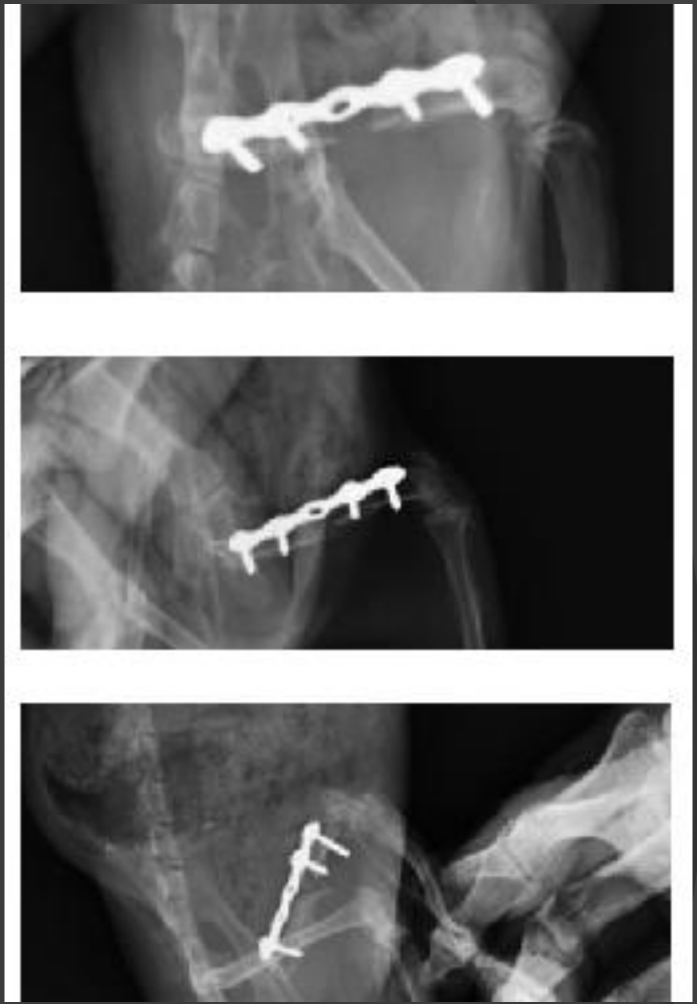


1st week X-ray examination at Group I (Sample 5, 6, and 7)

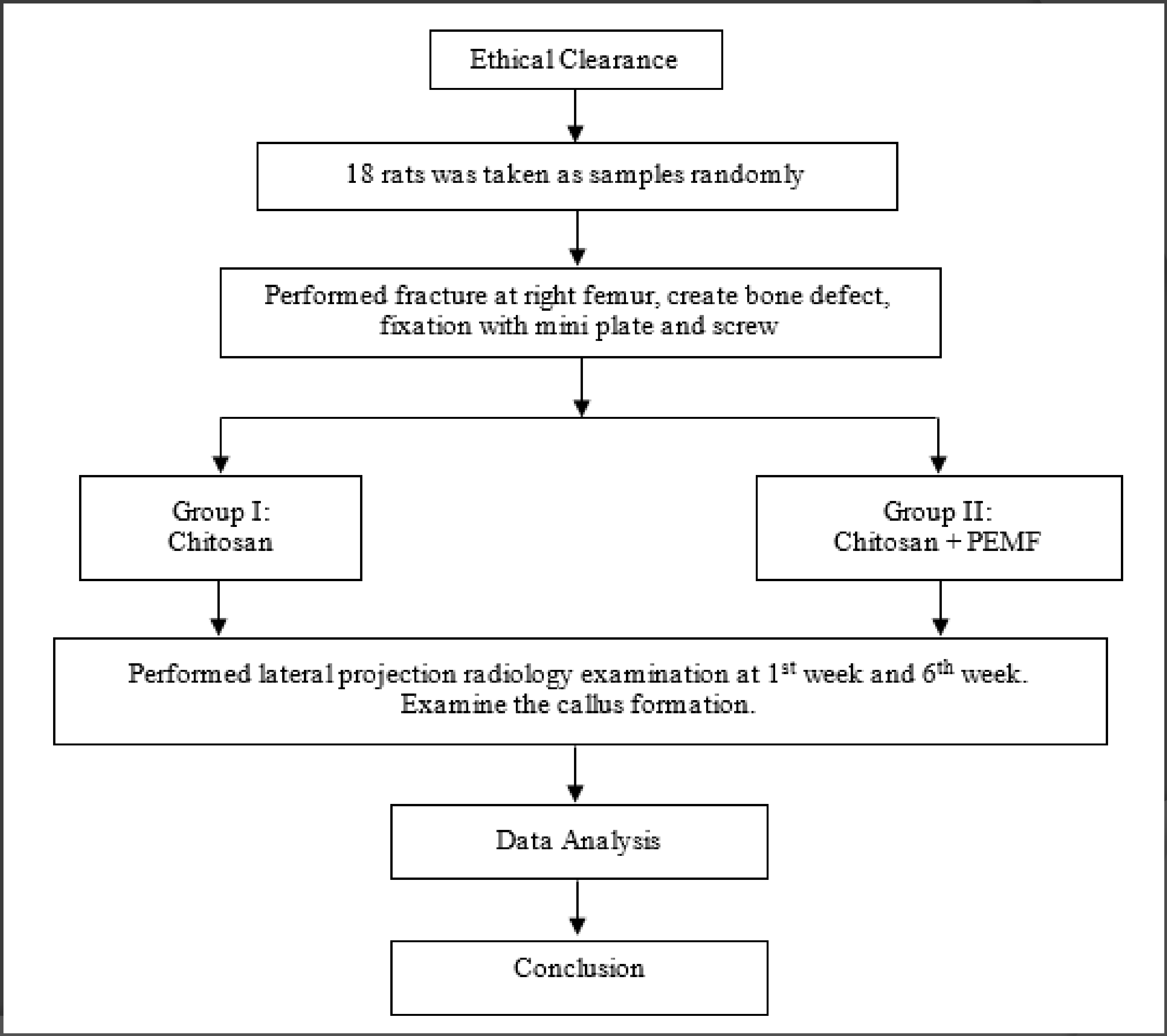
1st week X-ray examination at Group II (Sample 5, 6, and 8)

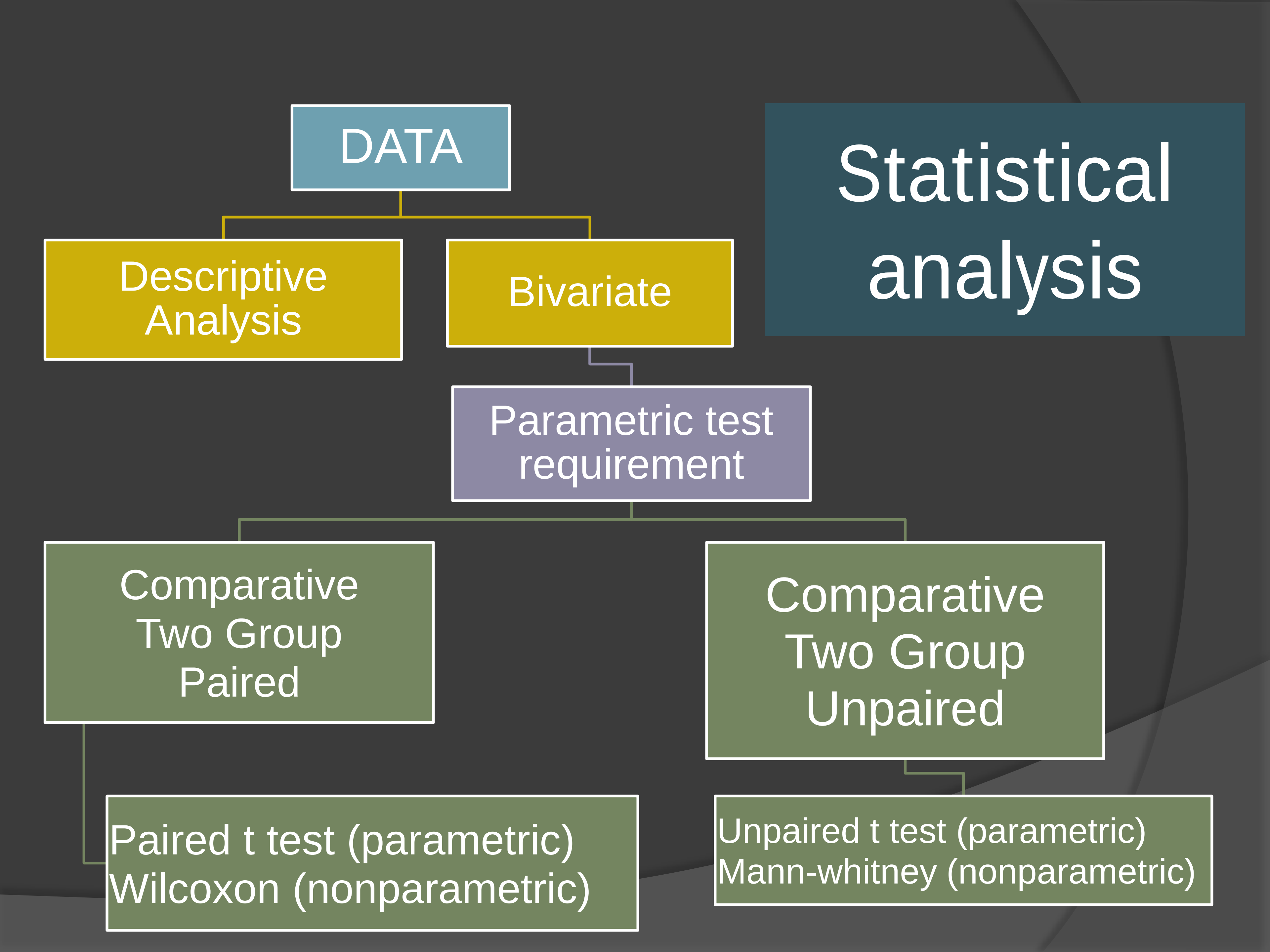


6th X-ray examination at Group I
(Sample 3, 5, and 7)



6th X-ray examination at Group II
(Sample 1, 3, and 5)


Ethical Clearance
18 rats was taken as samples randomly
Performed fracture at right femur, create bone defect, fixation with mini plate and screw
Group I: Chitosan
Group II: Chitosan + PEMF
Performed lateral projection radiology examination at 1st week and 6th week. Examine the callus formation.
Data Analysis
Conclusion

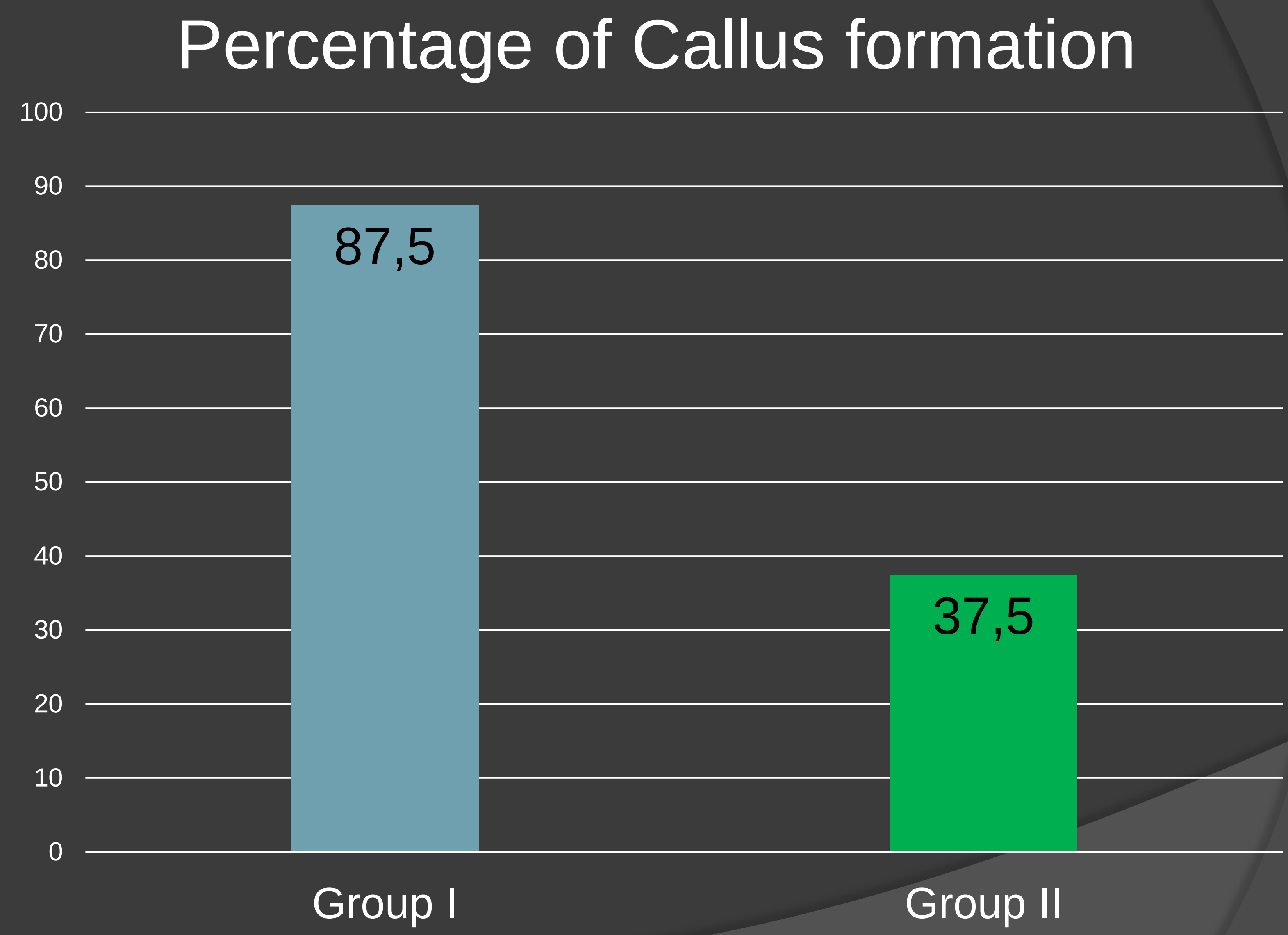

Statistical analysis
DATA
Descriptive Analysis
Bivariate
Parametric test requirement
Comparative Two Group Paired
Comparative Two Group Unpaired
Paired t test (parametric)
Wilcoxon (nonparametric)
Unpaired t test (parametric)
Mann-whitney (nonparametric)

CHAPTER IV
RESULT AND DISCUSSION

RESULT

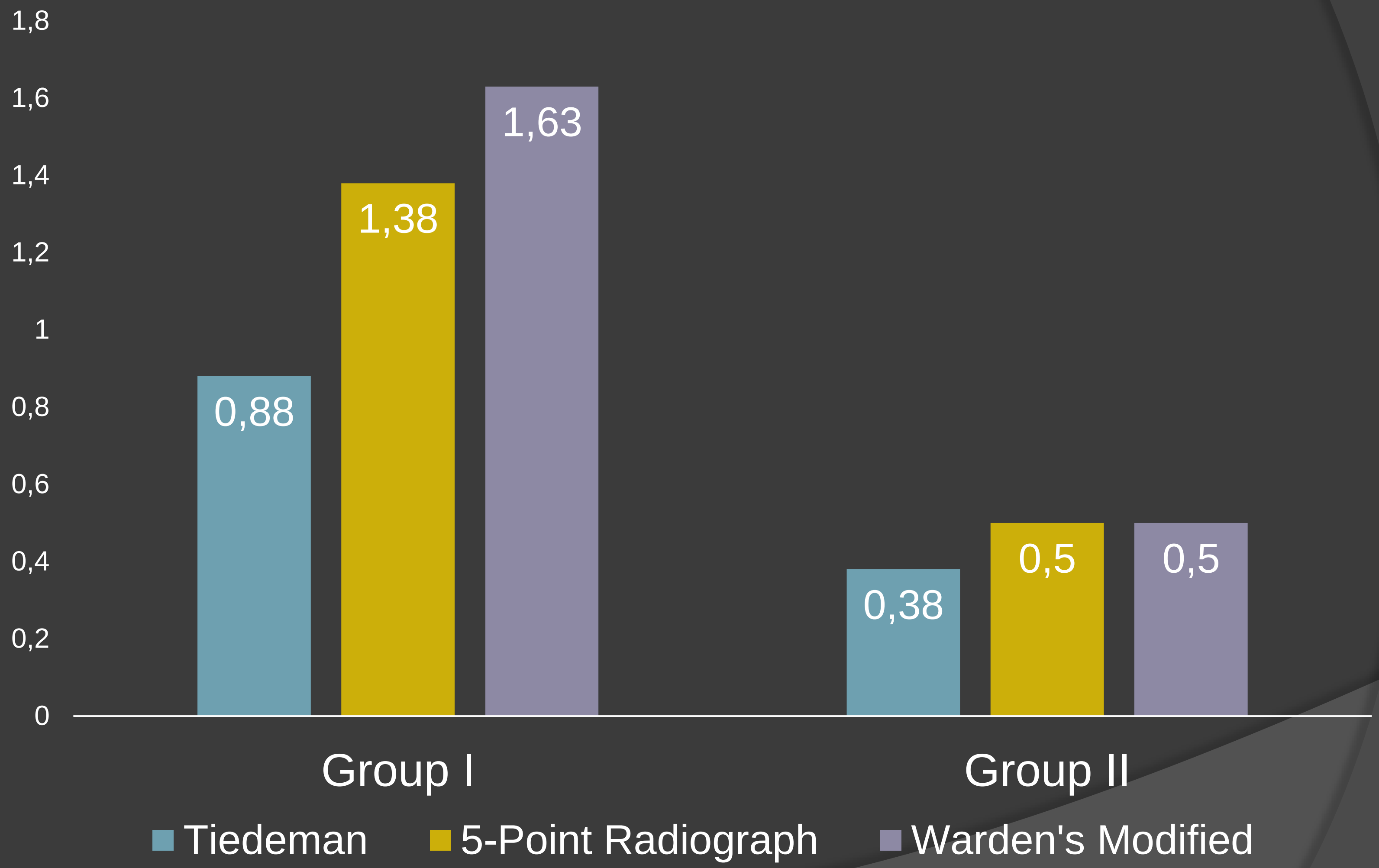
Radiology Examination Result at 6th Week

Group	Sample	Tiedeman Score	5-poin Radiograph Score	Warden's Modification Score	Callus Width (mm^2)
Group I	1	1	1	2	4,84
	2	1	2	2	5,74
	3	1	2	2	7,35
	4	1	2	2	5.20
	5	1	2	2	9,72
	6	0	0	0	0,00
	7	1	1	2	3,80
	8	1	1	1	3,69
Group II	1	0	0	0	0,00
	2	0	0	0	0.00
	3	1	1	1	3,89
	4	0	0	0	0,00
	5	1	2	2	9,11
	6	1	1	1	2,32
	7	0	0	0	0.00
	8	0	0	0	0,00

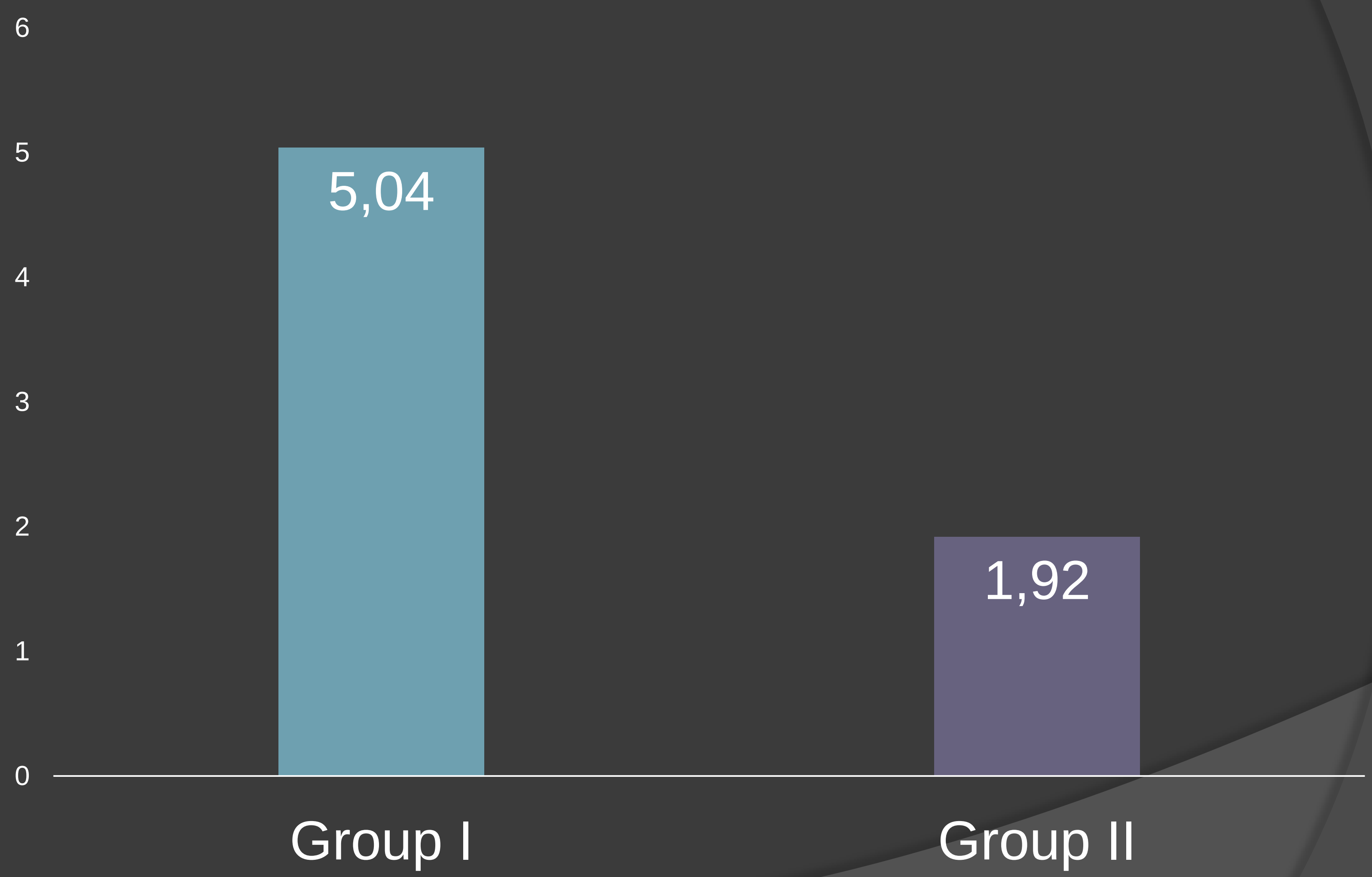


Percentage of Callus formation
100
90
80
70
60
50
40
30
20
10
0
87,5
37,5
Group I
Group II

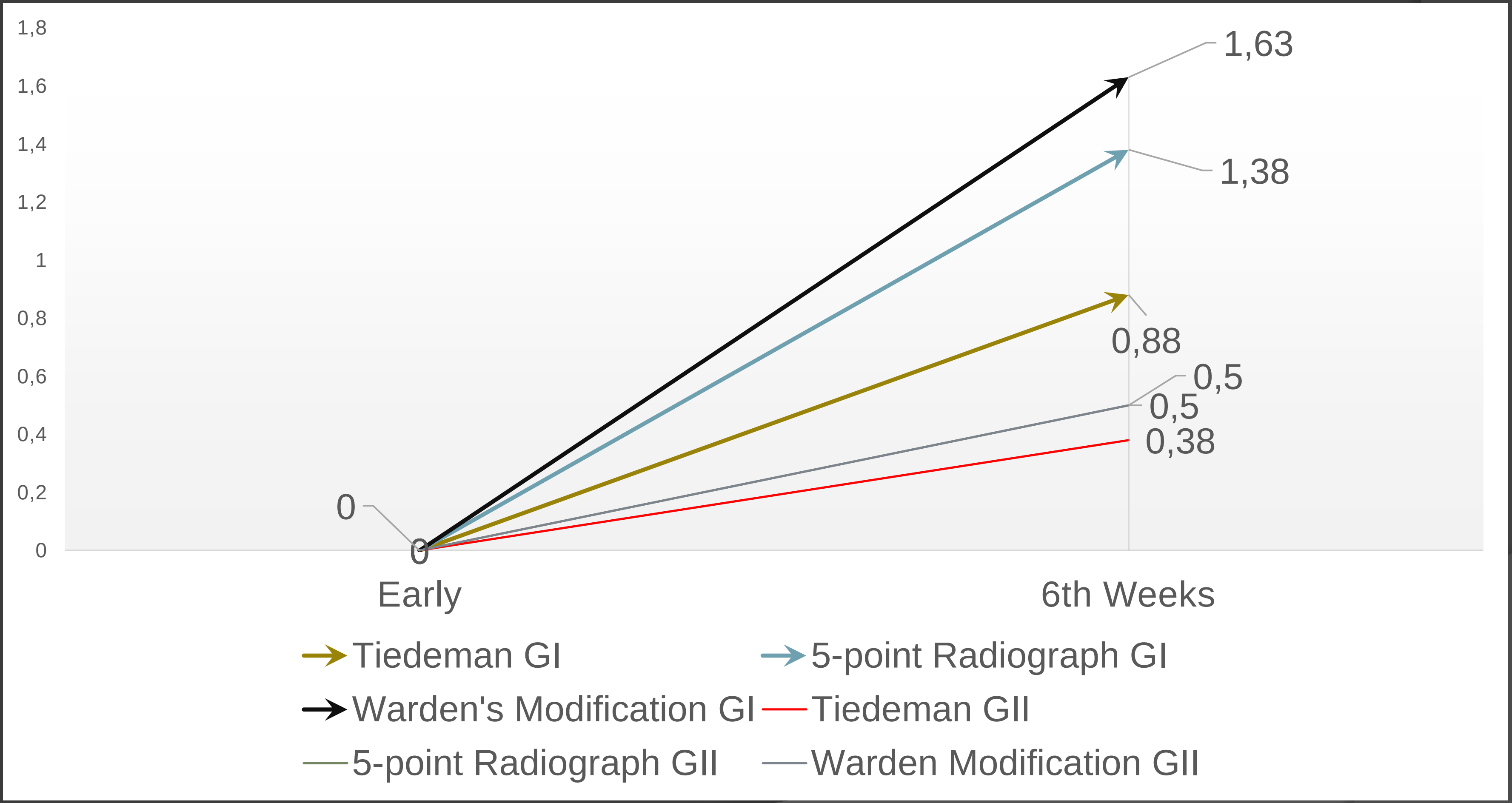
Mean Qualitative Data Between Two Group at 6th Weeks



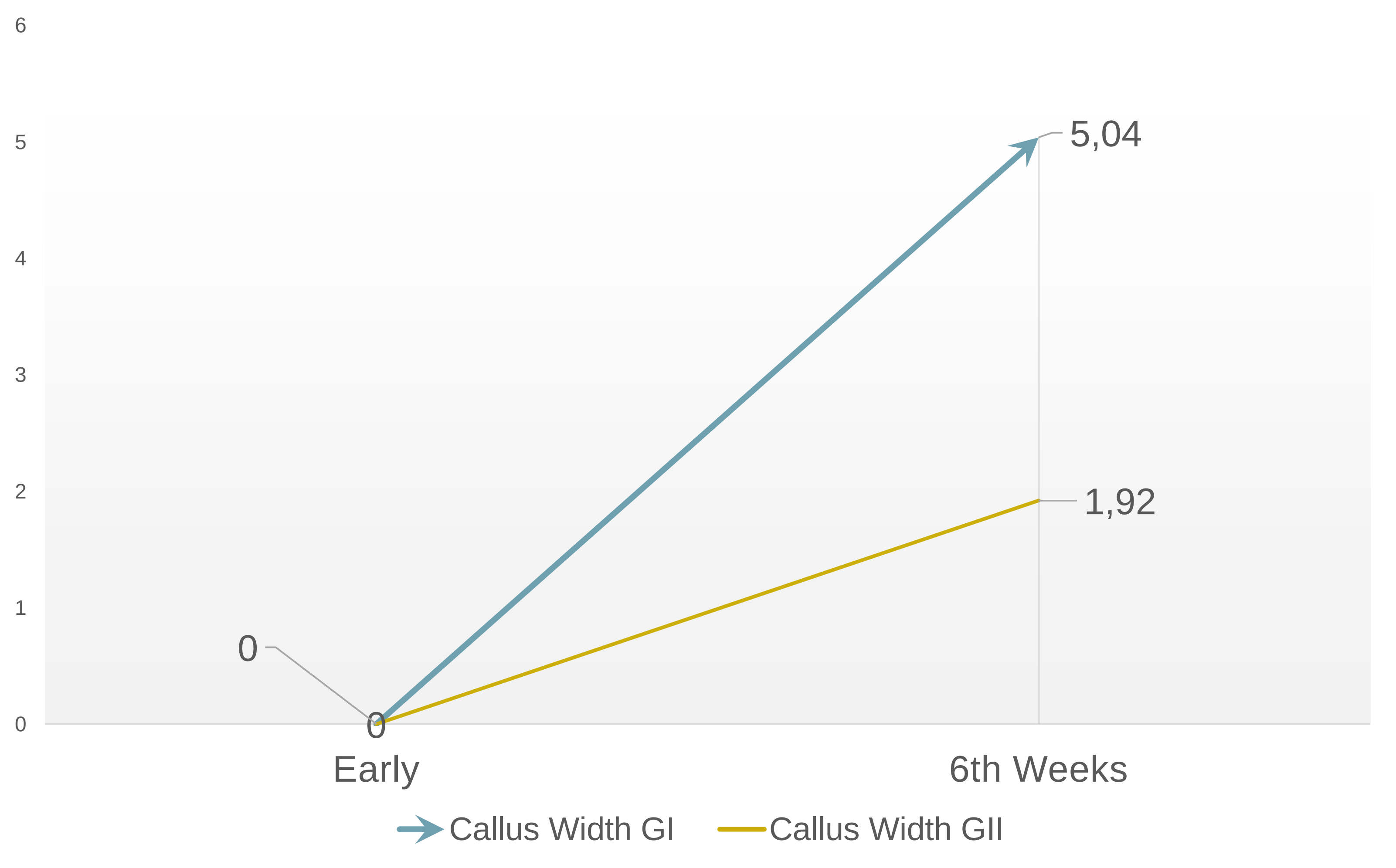
Mean Quantitative Data (Callus Width) Between Two Group at 6th Weeks (mm^2)



Qualitative Callus Formation Data Between 1st and 6th Weeks (Mean)



Quantitative Callus Formation Data Between 1st and 6th Weeks (Mean)



Qualitative and Quantitative Callus Formation Comparison Data Between 1st and 6th Weeks

Group	Score	Observation Time	N	Mean	SD	Sig. (p-value)
Group I	Tiedeman	Early 6th Weeks	8 8	0,00 0,88	0,00 0,35	0,008
	5 point Radiograph	Early 6th Weeks	8 8	0,00 1,38	0,00 0,74	0,015
	Callus Width	Early 6th Weeks	8 8	0,00 1,63	0,00 0,74	0,011
	Warden's Modification	Early 6th Weeks	8 8	0,00 5,04	0,00 2,84	0,018
Group II	Tiedeman	Early 6th Weeks	8 8	0,00 0,38	0,00 0,52	0,083
	5-point Radiograph	Early 6th Weeks	8 8	0,00 0,50	0,00 0,76	0,102
	Callus Width	Early 6th Weeks	8 8	0,00 0,50	0,00 0,76	0,102
	Warden's Modification	Early 6th Weeks	8 8	0,00 1,92	0,00 3,26	0,109

Qualitative Data Comparison Between Two Group at 6th Weeks

Qualitative Data	Group	N	Mean	SD	Sig. (p-value)
Tiedeman	Group I	8	0,88	0,52	0,046
	Group II	8	0,38	0,35	
5-point Radiograph	Group I	8	1,38	0,74	0,039
	Group II	8	0,50	0,76	
Warden's Modified	Group I	8	1,63	0,74	0,015
	Group II	8	0,50	0,76	

Quantitative Data Comparison Between Two Group at 6th Weeks

Quantitative Data	Group	N	Mean	SD	Sig. (p-value)
Callus Width	Group I	8	5.04	2,84	0,046
	Group II	8	1,92	3,26	

DISCUSSION

The goal of fracture treatment is to achieve fast fracture healing, best function restore, and low complication rate.[21]

Complications often occur in fracture with large bone defect.

In this condition → bone can't regenerate normally → the risk of delay or nonunion can be predicted[3]

3. Lan S. Chitosan-based Scaffolds for Bone Tissue Engineering. J Mater Chem B Mater Biol Med. 2014; 2: 3161-3184

21.Gomez E et all. Bone Fracture Healing: Cell Therapy in Delayed Unions and Nonunions. Bone. 2015; 70: 93-101

Closed fractures can heal in 6 weeks in normal rats.

The fractures healing at rats can be divided into 4 stages:

1. **Granulation tissue formation. (1st – 2nd weeks).**
2. **Formation of soft and hard callus. (3rd – 4th weeks)**
3. **Consolidation and remodeling. (5th – 6th weeks)[15]**

15. Estai M et all. *Piper sarmentosum* Enhance Fracture Healing in Ovariectomized Osteoporotic Rats: a Radiological Study. Clinics. 2011; 66(5): 865-872

From this study we know that there are delay or nonunion at both group.

We also know that group I (87,5%) have better chance to callus formation compare to group II (37,5%)

If we compare the callus formation between two group at 6th weeks of study, there is significant differentiation between two group qualitatively (p Tiedeman: 0,046; p 5-point radiograph: 0,039; p Warden's modification: 0,015) and quantitatively (p callus width: 0,046).

In Group I there is a synergistic function between the osteoconductive component of the chitosan and the osteoinductive component of the Pulsed electromagnetic field.

In group II only chitosan worked as osteoconductive

Although there were significant differences between the two groups, but the mean score of bone healing in both groups was still low.

For group I the mean tiedaman score 0,88±0,35 compare to 0,38±0,52 at group II (maximum callus score: 4)

For group I the mean 5-point radiograph score 1,38±0,74 compare to 0,50±0,76 at group II (maximum score: 4)

For group II the mean Warden's modified score 1,63±0,74 compare to 0,5±0,76 at group II (maximum score: 4)

For group I the mean of callus width 5,04±2,84 compare to 1,92±3,26) at group II

Citosan (CTS) has some favorable properties that can be used in the field of orthopedics. But the conductivity of chitosan itself is lacking, so it can not fulfill the natural bone properties completely.

Developing composite materials with citosan can help improve their conductivity.[8]

8. Venkatesan J et all. Chitosan Composit for Bone Tissue Engineering. Journal of Marine Drug. 2010; 8: 2252-226

Study → by Saraswathy using a composite of citosan and gelatin as bone substitution for dog → In the 9th week postoperative, union was obtained on the bone.[23]

23. Saraswathy et all. A New Bio-Organic Composite as Bone Grafting Material: In Vivo Study. Trends Biomater. Artif. Organs. 2004; 17: 37-42

Ezoddini → study using 15 male rats → make hole at tibia proximal part → given chitosan powder.

It was found that the bone healing process occurred faster in the treatment group compare to the control group.[24]

24. Ezoddini F et all. Histologic Evaluation of Chitosan as an Accelerator of Bone Regeneration in Microdrilled Rat Tibias. Dental Research Journal. 2012; 9: 694-699

Midura used a fracture model in mouse that stimulated with a frequency of 3.8 kHz pulsation, 5.56 ms duration with the maximum amplitude of the magnetic field approaches 2 mT (2 G).

In groups exposed to magnetic fields there is faster callus formation. The volume of the callus is twice as large as the control group.[25]

25. Midura RJ et all. Pulsed Electromagnetic Fields Treatments Enhance The Healing of Fibular Osteotomies. Journal of Orthopaedic Research. 2005; 23(5): 1035-1046

This study → Only lateral projection of radiology examination → Callus width.

The width of callus formed in group II was 2.63 times compared to group I.

LIMITATION

Subjectivity when performed assessment of radiological result qualitatively.

Used plate and screw as immobilization device of rat’s femur, so the observation with radiology examination is limited to one projection.

CHAPTER V
CONCLUSION AND SUGGESTION

CONCLUSION

There is positif effect of electromagnetic stimulated citosan bonegraft to increase the callus formation on femur fracture with bone loss at rats.

SUGGESTION

1. **Necessary to performed research with chitosan composite → improvement of conductivity function**
2. **Necessary to performed research with more larger samples**
3. **Necessary to performed histology examination to know the healing process microscopically**

THANK YOU