

## Comparison Of Post-Operative Healing Speed Of Achilles Tendon Rupture Immobilized With Cast And Spalk Based On Microscopic Images In Local Rabbits (*Lepus Domestica*)

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### ABSTRACT

The Achilles tendon is the most common tendon to rupture. Immobilization after Achilles tendon rupture surgery is performed to produce functional recovery and shorten rehabilitation time. The aim of this study was to determine the comparison of the speed of healing after surgery for Achilles tendon ruptures immobilized with plaster and plaster based on microscopic images in local rabbits (*Lepus domestica*). This research method is an experimental type of research with a post-test only control group design. Thirty mice aged 3-4 months were selected for treatment of Achilles tendon injuries in the right caudal extremity. After sewing the ends of the AT together with the modified “Kessler” method (Prolene 5–0). The skin incision is cut and sutured with 1-0 thread. Rabbits were divided into two immobilization methods (cast and spalk groups), as well as controls to compare cell morphology, collagen arrangement, cellular substance, and vascularization on the tendon healing process. The study was carried out for 21 days with observations on days 7, 14, and 21. Day 7 showed plaster immobilization of  $5.87 \pm 0.76$  compared to spalk  $5.73 \pm 0.31$  and control  $4.60 \pm 0.72$ . On day 14, it was seen that the plaster immobilization treatment was still the highest at  $9.47 \pm 0.31$  compared to spalk  $9.33 \pm 0.31$  and control  $6.20 \pm 0.35$ . On day 21, the average of the cast immobilization treatment was the highest at  $10.33 \pm 0.31$  compared to spalk  $10.07 \pm 0.12$  and control  $9.47 \pm 0.50$ . The results of this study showed that cast and spalk did not have a significant effect ( $P > 0.05$ ) on the histology of archiles tendon rupture, only in the initial phase, namely cell morphology, which had a significant difference in the installation of cast and splint immobilization devices.

**Keywords:** *Achilles Tendon Rupture, Cast and Splint Immobilization, Microscopic Image.*

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### 1. INTRODUCTION

The Achilles tendon or tendo *calcaneus* is the strongest and thickest tendon in the body. The Achilles tendon consists of a combination of three muscles, namely *musculus gastrocnemius*, *musculus soleus*, and *musculus plantaris*.<sup>1</sup> The combined length of this tendon is 10-15 centimeters/cm, with a gastrocnemius component of 11-26 cm and a soleus component of 3-11 cm.<sup>2</sup>

The Achilles tendon is divided into 2 types, namely the Achilles tendon wrapped in paratenon and the tendon that is not wrapped in paratenon. Paratenone-encased Achilles tendon is better than mesothenone in the repair process when it is torn. Mesothenone connects the parietal layer on the outer side with the visceral layer on the inner side and serves as a path for blood vessels that provide nutrients to the tendon.<sup>3</sup>

The Achilles tendon is formed as a result of the composition of bundles of collagen fibers packaged by connective tissue

called the *extracellular matrix* (ECM). Collagen is synthesized by tenocytes that respond to mechanical loads. Collagen polypeptides are formed by crosslinking of collagen fibrils with a *triple helix structure* that provides tendon tensile strength. Type I collagen is the main component that makes tendons up. Collagen fibers are very thin in diameter in the early stages of healing so attractiveness is low in the early stages of healing.<sup>4</sup>

Tendons are part of the motion system in the form of connective tissue that functions as a link between muscles and bones. The Achilles tendon functions to move forces from muscle to bone to move.<sup>5</sup>

The Achilles tendon is among the most common tendons that rupture. An Achilles tendon rupture is a tear or break of a tendon connection caused by injury from a sudden change in foot position in a state of maximal passive dorsiflexion.<sup>6</sup> Achilles Tendon rupture can be caused by sudden plantar flexion of the foot, direct trauma, and longstanding tendinopathy or intratendinous degenerative conditions.<sup>7</sup>

Achilles tendon rupture injury is the most common rupture with an incidence of about 40% caused by tendon damage to the lower extremities. The global prevalence of Achilles tendon rupture is 2.1 per 100,000 people per year, the majority of which occurs in adult men 30-40 years with a male: female ratio of 3.5:1.6 Risk factors that influence the occurrence of Achilles tendon disorders are age, male sex, and obesity.<sup>8</sup>

Treatment of tendon rupture can be done nonoperatively or operatively. Operative therapy is divided into several methods, namely open repair methods, percutaneous repair, and mini-open repair. Operative treatment can strengthen muscle endurance than nonoperative, so the risk of postoperative re-injury is relatively lower.<sup>9</sup> Non-operative measures are performed by immobilization of the ankle using rigid casting or functional bracing.<sup>10</sup>

Wound healing is a process of repairing skin tissue or other organs after a wound occurs. There are three phases of wound healing, namely the inflammatory phase, the proliferation phase or fibroplasia, and the remodeling or maturation phase.<sup>5</sup> Healing of the tendon will result in adhesion to the surrounding tissue, which can affect the gliding of the tendon.<sup>11</sup> Tendon healing is carried out by immobilization using a brace for 6 weeks. Early functional and weight-bearing rehabilitation results in the best functional recovery and shortens rehabilitation time so that it can return to work or normal life as soon as possible.<sup>12</sup>

The postoperative immobilization action greatly affects the speed of tendon healing. Postoperative immobilization can be done by installing a cast and spalk using medical techniques (by passing 2 joints). Researchers are interested in conducting a study that examines the comparison of postoperative healing speed of Achilles tendon rupture with Cast and Spalk based on microscopic images in local rabbits (*Lepus domestica*) based on the background above.

## 2. RESEARCH METHODOLOGY

This type of research is an experimental study using a post-test-only control group design on local rabbits (*Lepus domestica*) as experimental animals. This design involved 2 treatment groups and 1 control. P0 as a control group were local rabbits who performed Achilles tendon rupture surgery and were given physiological NaCl, P1 was treatment group 1 in local rabbits immobilized with casts after Achilles tendon rupture surgery, and P2 was treatment group 2 in local rabbits immobilized with Casts and Spalk after Achilles tendon rupture surgery, and observations were made on days 7, 14, and 21. The research was carried out at UPT Experimental Animals, Faculty of Veterinary Medicine and Histology Laboratory, Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh from September 2023 to November 2023. The study population was male, healthy, 3-4 months old, and 800-1000 grams of body weight obtained from the Faculty of Veterinary Medicine (FKH) Syiah Kuala University (USK). The sample size was calculated using Federer's formula with the results of counting 27 rabbits. The type of data collected is microscopic data on tendon healing speed from the results of the Bonar Score with variables in the form of cell morphology, collagen, cellular substance, and vascularization.

## 3. RESEARCH RESULTS

### Results of Bonar Score on Histology Evaluation

**Table 1 Mean ( $\pm$  SD) Bonar scores on days 7, 14 and 21 postoperative Rupture of Achilles tendon of local rabbits in the control (P0), Cast (P1) and Spalk (P2) groups.**

Parameter	Treatment	Observation Time			
		Day 7	Day 14	Day 21	
Cell morphology	P0	1,13 $\pm$ 0.03 0,12	1,47 $\pm$ 0,12	0.13	2,20 $\pm$ 0,20 0.31

Comparison Of Post-Operative Healing Speed Of Achilles Tendon Rupture Immobilized With Cast And Spalk Based On Microscopic Images In Local Rabbits (*Lepus Domestica*)

	P1	1,40 0,20	±		1,93 ± 0,12		2,53 ± 0,23	
	P2	1,53 0,31	±		2,20 ± 0,20		2,47 ± 0,12	
	P0	1,20 0,35	±	0.47	1,67 ± 0,31	0.50	2,40 ± 0,20	0.16
Collagen	P1	1,53 0,31	±		2,60 ± 0,20		2,67 ± 0,12	
	P2	1,40 0,20	±		2,47 ± 0,12		2,53 ± 0,12	
	P0	1,13 0,12	±	0.55	1,33 ± 0,31	0.50	2,33 ± 0,58	0.05
Cellular Substance	P1	1,40 0,20	±		2,33 ± 0,23		2,67 ± 0,12	
	P2	1,33 0,31	±		2,20 ± 0,20		2,53 ± 0,12	
	P0	1,13 0,23	±	0.10	1,73 ± 0,23	0.05	2,53 ± 0,12	0.85
Vascularization	P1	1,53 0,12	±		2,60 ± 0,20		2,47 ± 0,31	
	P2	1,47 0,12	±		2,47 ± 0,31		2,53 ± 0,23	
	P0	4,60 0,72	±	0.91	6,20 ± 0,35	0.06	9,47 ± 0,50	0.90
Total	P1	5,87 0,76	±		9,47 ± 0,31		10,33 ± 0,31	
	P2	5,73 0,31	±		9,33 ± 0,31		10,07 ± 0,12	

**Information:** Control group (P0); cast description group (P1) and spalk (P2).

The histological evaluation in Table 1 shows the average total Bonar score combination of cell morphology, collagen, cellular substance, and vascularization scores showed the highest average score on day 7 was by immobilization with casts of  $5.87 \pm 0.76$  compared to immobilization with spalk  $5.73 \pm 0.31$  and control  $4.60 \pm 0.72$ . The evaluation on day 14 showed that the immobilization of casts was also still the highest at  $9.47 \pm 0.31$  compared to spalk immobilization of  $9.33 \pm 0.31$  and control at  $6.20 \pm 0.35$ . The evaluation on day 21 also showed that the highest average cast immobilization was  $10.33 \pm 0.31$  compared to  $10.07 \pm 0.12$  and  $9.47 \pm 0.50$  control administration. This study was conducted to evaluate histological changes in cell morphology (tenocytes) of tendon tissue rupture of the Achilles tendon in control groups and treatment of varying lengths of healing time. Day 7 evaluation showed a significant difference ( $P = 0.03$ ) in changes in cell morphology due to immobilization treatment with casts and spalk compared to controls. Casts have higher average scores than spalks and controls. Day 14 evaluation showed histological changes in cell morphology (tenocytes) in Achilles tendon rupture tendon tissue showed no significant difference ( $P = 0.13$ ) and day 21 ( $P = 0.31$ ). The overall data showed that casts and spalks affected cell morphology only in the early phases, but showed no significant differences.

The results of statistical analysis of the total scores of local rabbit Achilles tendon tissue cells on days 7, 14, and 21 in various treatment groups did not differ markedly with a significance value of  $P > 0.05$ . These results can be concluded that immobilization treatment with casts and spalks in Achilles tendon rupture did not affect the total score of local rabbit Achilles tendon tissue cells on days 7, 14, and 21 of healing of local rabbit Achilles tendon rupture. The overall data proved that the cast and spalk did not have a significant effect on the histology of Achilles tendon rupture, only in the initial phase, namely cell morphology which had significant differences in the installation of the cast and spalk immobilization apparatus.

#### Saphiro-Wilk Test Results and ANOVA Test

Data from this study were analyzed by normality test using the Saphiro-Wilk test to determine Bonar Score data on normally distributed Achilles tendon tissue. Data that are normally distributed are tested differently from the Anova test, while data that is not normally distributed is carried out by the Kruskal Wallis test. The results of the normality test on the microscopic images of local rabbits observed on days 7, 14, and 21 were normally distributed, and then the ANOVA test was carried out. The average score of microscopic images after Achilles tendon rupture based on the ANOVA test and Kruskal Wallis test can be seen in Table 2

**Table 2 Average microscopic picture scores in local rabbits on days 7, 14, and 21 after Achilles tendon rupture in the control group (P0), cast immobilization treatment (P1), and spalk (P2).**

Experimental Animal Groups	Day Microscopic Images Score					
	Ke 7	p*	Ke 14	p**	Ke 21	p**
P0	4,60 ± 0,72	0.026	6,20 ± 0,35	0.028	9,47 ± 0,50	0.033
P1	5,87 ± 0,76		9,47 ± 0,31		10,33 ± 0,31	
P2	5,73 ± 0,31		9,33 ± 0,31		10,07 ± 0,12	

**Information:**

\* = Based on Anova test

\*\* = Based on the Kruskal Walls test

P0 = Negative control group, i.e. local rabbits with Achilles tendon rupture were given NaCl

P1 = Treatment group 1, i.e. local rabbits with Achilles tendon rupture given a cast

P2 = Treatment group 2, i.e. local rabbits with Achilles tendon rupture given Spalk

The results of the analysis showed that the average microscopic image increased highest in spalk immobilization compared to casts and controls on day 7 but on day 14 the highest in casts compared to controls and spalks. The administration of casts and spalks for the 21st time showed a decrease in average. The results of the analysis of the effect of casts and spalk found that there were significant differences  $p = 0.026$ ,  $p = 0.028$ ,  $p = 0.033$  on days 7, 14, and 21. On day 7 of the Duncan Test, there was no significant difference between groups, so in this result, it can be concluded that in the treatment of casts and spalk there was no significant difference in the Achilles tendon rupture of local rabbits on days 7, 14 and 21.

**Results of histological picture of Achilles tendon tissue**

Observation on day 7 after injury treatment and operative action, histological features of tendon tissue showed an increase in cellular substance with a mixture of spindle-shaped fibroblasts, mononuclear cells, fibrin, and neovascularization in all three treatment groups, however, no increase in collagen fiber density was seen in all three treatment groups. In the cast and spalk groups, fibroblast spread was less random, and more neovascularization formed compared to the control group.

Observation on day 14, qualitatively cellular substance levels, with a mixture of spindle-shaped fibroblasts, mononuclear cells, fibrin, and neovascularization in all three treatment groups, and already There is an increase in collagen fiber density. In the cast group, there is a display of active granulation tissue and a large hemorrhagic area. However, the spalk group compared to the control group showed a further process of neovascularization and implantation infiltration that was reduced without granulation. Qualitative differences were observed between the cast and spalk immobilization groups. The casting group showed tissue disorganization, abundant implant infiltration, hemorrhagic areas, and mononuclear and polymorphonuclear cells, while the formation of new blood vessels was more common in the spalk group. In the control group, the density of collagen fibers was seen to be rare, while in the cast and spalk groups, there was an increase in collagen fiber density and dense capillaries were present in the cell gap.

Observations on day 21 of cellular substances with a mixture of spindle-shaped fibroblasts, mononuclear cells, fibrin, and neovascularization in all three treatment groups have decreased, but there is an increase in collagen fiber density. The casting group and the control group showed no difference in cellular enhancement with large numbers of young fibroblasts, as well as fewer collagen, fibrocyte, and fibrosis fibers. The spalk group showed more organized tissue, large numbers of fibrosis, and low fibrolase counts characterizing partially healed tissue, bundles of collagen fibers aligned in one direction parallel to the long axis of the tendon and close to normal tendon collagen fibers. Based on the presence of different cell types, the number of blood vessels, and the arrangement of collagen fibers on days 7, 14, and 21, it can be indicated that there is more progressive tissue improvement in the spalk and control groups compared to the cast group.

#### 4. DISCUSSION

Histological evaluation showed that the average total Bonar score combined with cell morphology, cellular substance, collagen, and vascularization scores showed the highest average score on day 7 with cast immobilization treatment of  $5.87 \pm 0.76$  compared to spalk  $5.73 \pm 0.31$  and control  $4.60 \pm 0.72$ . Furthermore, the evaluation of day 14 showed that the immobilization treatment of casts was also still the highest at  $9.47 \pm 0.31$  compared to spalk at  $9.33 \pm 0.31$  and control at  $6.20 \pm 0.35$ . On day 21, the highest average cast immobilization treatment was  $10.33 \pm 0.31$  compared to  $10.07 \pm 0.12$  and  $9.47 \pm 0.50$  controls. Although there was an increase in casts compared to spalk and control, the results of the analysis showed that casts and spalks did not have a significant difference where  $P > 0.05$ . There is a tendency to increase some components in the Bonar score, although not significantly.

Research conducted by Tianliang on the effectiveness of the use of cast and spalk immobilization evaluated 28 days postoperatively showed that there was no significant difference in the tendon healing process and the same complication rate of 15%. The advantage of immobilization cast plaster (plaster) is a good braking effect and does not need frequent replacement or strengthening, but it is difficult to understand its tightness. Too loose can easily cause the plaster to come off, and too tight can easily disrupt the feeding and digestion process of the experimental animal, thus causing the death of the animal. This splint is simple, easy, and convenient to change the incision dressing and does not affect the diet and digestion of experimental animals, causing fewer cases of death in experimental animals, but its tightness is also difficult to understand. The splint (spalk) is too loose and easily loose, too tight, and easily causes necrosis of localized skin or foot. Over time, the adhesive tape will decrease, thus requiring timely reinforcement and frequent operation.<sup>13</sup>

Research conducted by Kari Kauranen showed that there was no statistically significant difference in outcomes between operated lower extremities and non-operated contralateral 12 and 24 weeks after surgery in both groups. When the results were compared between the cast group and the active support group, no statistically significant differences were seen in reaction time, movement speed, stroke speed and anterior-posterior coordination on both sides, but the lateral coordination scores of the operated leg were higher. In the cast group compared to the active buffer group 12 weeks after surgery ( $p < 0.05$ ). By 24 weeks after surgery, this unique difference had disappeared. It appears that the recovery of the above-mentioned foot motor performance function does not depend on whether the foot is fitted with a cast with AT in a strained state or active support during the initial postoperative period following the repair of AT rupture. The function of this operated leg has recovered to the level of the non-operated contralateral leg within 12 weeks after surgery.<sup>14</sup>

Griffith, et al.'s research developed a method for percutaneous repair as a compromise between open and nonoperative surgical methods. In 18 patients using this technique, only two developed non-infectious skin complications and no recurrent ruptures. Rowley and Scotland, describe 24 patients with Achilles tendon rupture; 14 were treated with immobilized casts only, with ankle position equinus, and 10 were treated with percutaneous repair. One patient who had percutaneous repair had spinal nerve entrapment, but no other complications were encountered. Patients who had sutures performed were more likely to obtain near-normal plantar flexi strength and they also returned to activity more quickly than those in the group done with casts alone.<sup>15-16</sup>

The results of the study Kerkhoffs, et al according to the modified Rupp score found that 91.3% of patients in the walking cast use group got good or very good results, as well as 93.8% in the cast use group ( $p = 0.9$ ). Mild atrophy in calf muscles was reported in 3 patients in the walking cast group (13.0%) and in 4 patients in the cast group (25.0%). One re-rupture was reported in the cast use group (4.3%). Functional care after Achilles tendon repair surgery is safe, and there is no increased risk of re-rupture or wound healing problems.<sup>17</sup>

Research shows that in the recovery progression of Achilles tendon rupture, both the control group and the cast and spalk immobilization treatment groups show good recovery perspectives. This condition shows that the operation has been carried out with good and correct operating standards, and there is no infection or tissue damage. The results of the histological evaluation in this study proved that the repair process of cell proliferation and tissue reading began from day 7 to day 21 with the discovery of an increase in basic substance, tenocytes, collagen, and tissue vascularization in both the control group and the cast and spalk immobilization treatment groups.

## 5. CONCLUSION

This study concluded that there was no difference in postoperative healing of Achilles tendon rupture given the installation of cast and spalk immobilization devices in local rabbits based on microscopic images.

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