EFFECT OF THE GRAFTING SECTION AREA ON ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION SURGERY – HISTOLOGICAL STUDY ON DOGS

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ABSTRACT

Objective: To correlate the initial grafting section area with the outcomes from anterior cruciate ligament (ACL) reconstruction surgery. Eight dogs underwent operations, divided into two groups according to graft size: Group A, 25% and Group B, 40% of the patellar ligament (PL) width. Methods: After eight months, the dogs were sacrificed for macroscopic and histological analysis on the reconstructed ligaments. Each dog's contralateral knee was used as a control. Results: In both groups, all the reconstructed ligaments were seen to be viable and hypertrophied. The morphology of the PL grafting had changed, which was observed by measuring the crimp and cellularity, and it resembled that of the ACL. Conclusion: The grafting section area did not influence the histological outcomes from ACL reconstruction surgery in dogs.

Keywords – Anterior Cruciate Ligament; Grafting; Histology; Dogs

INTRODUCTION

Since the introduction of anterior cruciate ligament (ACL) reconstruction surgery, the technique has evolved such that knee stability and function have become restored more rapidly and efficiently, and less traumatically[1]. This has occurred not only through advances in surgical technique and instruments, but also through studying the following factors: graft types and properties, positioning, tensioning, fixation methods and postoperative rehabilitation[2]. Among all these variables, the one that has received least attention in the dimensions of the graft, i.e. the sectioned area.

Today, autologous grafts are the type most used for ACL reconstruction, and the middle third of the patellar ligament and the knee flexor tendons are the most popular graft sources[3]. Both of these are prepared in such a way that they have a sectioned area that is similar to or greater than the ACL, since it is commonly accepted that after intra-articular implan-tation, their maximum breaking load reduces during the ligamentization process, which is characterized by graft modification in four stages: avascular necrosis, revascularization, cell proliferation and remodeling. Several experimental studies have demonstrated that the maximum breaking load becomes significantly reduced immediately after surgery and then gradually increases with time, until reaching only 30 to 50% of the contralateral ACL, seven to twelve months after the operation[4,5]. Beynnon et al[6] reported a human case in which the maximum failure loading of a patellar ligament graft was 87% of that of the ACL, after eight months. Thus, it has been recommended that grafts of diameter greater than or equal to that of the native ACL should be used in order to compensate for the inevitable loss of graft resistance after implanta-tion. At time zero, the resistances relative to the ACL presented by a 14 mm graft from the central portion of the patellar ligament, a quadruple graft from the

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semitendinosus ligament and a double graft from the semitendinosus plus double graft from the gracilis are respectively 168%, 206% and 238%[7,8].

Although it is generally accepted that grafts of greater diameter have better postoperative stability, the effect of graft dimensions on the result from ACL reconstruction has not yet been defined. Several authors have not found any correlation between grafts of different sizes and the postoperative stability or function, through clinical or experimental studies[2,9-11]. On the other hand, better results have been found with grafts of smaller dimensions, given that less sound donor tissue was sacrificed, thereby diminishing the pain and improving postoperative function[12-14]. Yet other studies have shown diminished risk of complications consequent to graft hypertrophy during the ligamentization phase, or limitation of knee extension, or even graft injury due to impact on the intercondylar wall or roof[9,15].

The present study had the aim of correlating the effect of the initial sectioned area of the graft with the final result from ACL reconstruction surgery, by means of a histological analysis on the reconstructed ligaments.

MATERIAL AND METHODS

After approval from the Ethics Committee for Animal Experimentation (protocol no. 486), eight dogs were subjected to ACL reconstruction surgery using autologous grafts from the patellar ligament. In four of them (group I), the graft size used corresponded to one quarter (25%) of the width of the patellar ligament; and in the other four (group II), the graft corresponded to two fifths (40%) of the width of the patellar ligament. The sectioned area of the grafts and the sectioned area of the ACL were obtained during the operation, by multiplying the width by the thickness, as measured using a digital pachymeter.

All the dogs were adults, without defined breed, weighing between 13 and 24 kg (mean of 17.35 kg). They had stable knees, with a good range of motion, and did not present any intra-articular abnormality, as observed during the operation. The right knees were operated, and the left knees were left as controls.

Both groups of dogs were observed for eight months. They remained confined to the kennels and no specific exercise was done either before or after the operation. After this period, the dogs were sacrificed by means of an intravenous injection of potassium

chloride. The sectioned area was then measured, and histological analyses were conducted on the original ACL, the reconstructed ACL (rACL) and a segment of the patellar ligament of the same dimensions as used in the graft, for comparison.

The change in the graft area from the time of initial implantation to the final evaluation was always calculated in the following manner: 100 (final area – initial area) / initial area (%). A comparison of the results between the largest and smallest graft diameters was done using Student's t test for independent samples with a 5% significance level. The study was complemented with the coefficient of variation as a percentage for each of the samples[16].

Student's t test for independent samples was also used to determine the nuclear area and crimp, with a 5% significance level.

Surgical technique

Using an aseptic technique, after administering anesthesia, applying a tourniquet to the limb on the proximal third of the thigh and making an anterior longitudinal skin incision of approximately 5 cm in length, the patellar ligament was located and posterior medial parapatellar arthrotomy was performed. After measuring the total length of the patellar ligament, a graft was harvested from the medial portion of the patellar tendon without bone blockage, in accordance with the standard established between the groups. Following this, the graft was prepared using Kracov stitches at the extremities, using nylon 2-0 thread.

During the arthrotomy, the fatty pad was divided only in the most proximal portion, and the patella was dislocated laterally. The ACL was completely removed, and both the origin and the insertion were used as reference points for positioning the bone tunnels. The tibial tunnel was constructed by initially passing a Kirschner wire through from the medial cortical bone of the proximal tibia, to emerge at the tibial insertion of the ACL. Over this guidewire, a drill was used with a bit size in accordance with the graft size. The femoral tunnel was constructed through the tibial tunnel, by initially passing the guidewire through from the origin of the ACL in the femur. Then, over this, the tunnel was drilled using the same bit size as in the tibia, and ended in a cul-de-sac.

The joint was washed with physiological serum to remove any bone particles.

After the tunnels had been constructed, the graft

was guided into them, using suturing thread, and was attached using screws and washers that were fixed in the metaphyses of both the femur and the tibia, under appropriate tension.

The surgical wound was closed in layers using nylon 3-0 thread and was occluded using a dressing and compressive bandaging. No immobilization was used, and no restriction was placed on loading the operated limb.

Macroscopic and histological analysis

After the dogs had been sacrificed, the anteroposterior stability and range of motion of both knees were tested manually. Following this, after making an inventory of the joint cavity, the reconstructed ACL was removed from the operated knee, and the ACL and a segment of the medial portion of the patellar tendon, of the same dimension as the material that was used as a graft in the other knee of the same dog, was removed from the non-operated knee.

The tendons and ligaments were fixed in 4% formaldehyde dissolved in 0.1M phosphate buffer (pH 7.2), for 48 hours. After fixation, the material was washed in running water, dehydrated in an ascending ethanol series, clarified in xylol and embedded in Paraplast Plus. Sections of 5 μm in thickness were produced using a rotary microtome and were placed on silanized slides and stored until the time of use.

The sections embedded in Paraplast were stained using hematoxylin-eosin, for a general analyses on the structure of the tendons and ligaments; using picrosirius, for an analysis on the collagen fibers; and using 0.025% toluidine blue at pH 4.0, for glycosaminoglycans. The sections were observed under normal and polarized light, and photomicrographs were produced under a Leica DMLB microscope.

RESULTS

Before completing the eight-month period, one of the dogs had to be sacrificed because of disease that was unrelated to the surgical procedure. Thus, four dogs remained in group I and three dogs in group II. The seven remaining dogs all presented good evolution, and most of them started to place weight on the operated paw, on the ground, within the first postoperative week.

Clinical evolution

None of the dogs presented any abnormality of gait or limitation of range of motion. However, all of them presented some degree of laxity (positive anterior drawer test), in comparison with the contralateral knee. This was more evident in three dogs in GI and one in GII.

Macroscopic examination

Despite using grafts from different sectioned areas, it was observed that both in GI and in GII, all the grafts used had a sectioned area that was smaller than the original ACL at the time of the surgery, and that after the eight-month period, hypertrophy occurred in these grafts (Table 1). The grafts were viable and covered with an abundant quantity of synovial tissue. No degenerative joint alteration was found.

From the mean and standard deviation results of the groups, it was seen that the coefficient of variance in group I (CV = 97.7%) was three time bigger than the coefficient in group II (CV = 31.1%), thus indicating that the behavior of the second group was more homogenous than that of the first group, although the difference between the areas was not shown to be significant.

Table 1 – Descriptive measurements on the percentage variation of the areas (hypertrophy) in relation to the start and end times of the assessment.

Observation unit	Group I (%)	Group II (%)	Test result
1	79.6	157.4	
2	21.7	90.9	
3	175.0	100.0	
4	389.0		
Mean ± standard deviation	165.7 ± 161.8	116.1 ± 36.1	(p > 0.05)
Coefficient of variation	(97.7%)	(31.1%)	

Microscopic examination

We observed that after eight months, the patellar ligament grafts had acquired morphological characteristics similar to those of the ACL (Figures 1 and 2). We quantified this similarity through measuring the crimp (i.e. the undulating pattern of collagen fibers presented in the tendons and ligaments) and the area occupied by cell nuclei (i.e. the cellularity of the tissue), in the samples from the patellar ligament graft, ACL and rACL, as shown in Tables 2 and 3. In these, it can be seen that with regard to these two parameters, the characteristics of the patellar ligament became modified over the eight months since the implantation (rACL), to a pattern very close to that of the ACL.

Nuclear area and crimp

Tables 2 and 3 presented the results relating to the nuclear area and crimp, respectively, in groups I and
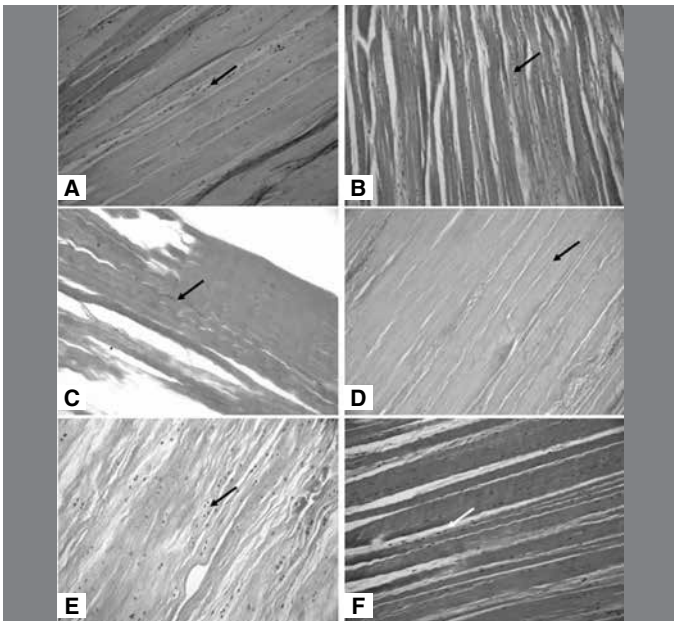
Figure 1 – Histological sections from the ligaments, stained with hematoxylin-eosin and observed under conventional light. (A) ACL of control dog, group I; (B) ACL of control dog, group II; (C) Patellar ligament of control dog, group I; (D) Patellar ligament of, control dog, group II; (E) rACL of dog, group I; (F) rACL of dog, group II. Note nuclei forming lines between the collagen fibers (arrows). Note greater number of nuclei in the ACL and rACL, and that the morphology of the rACL comes close to that of the control ACL, independent of the thickness of the graft.
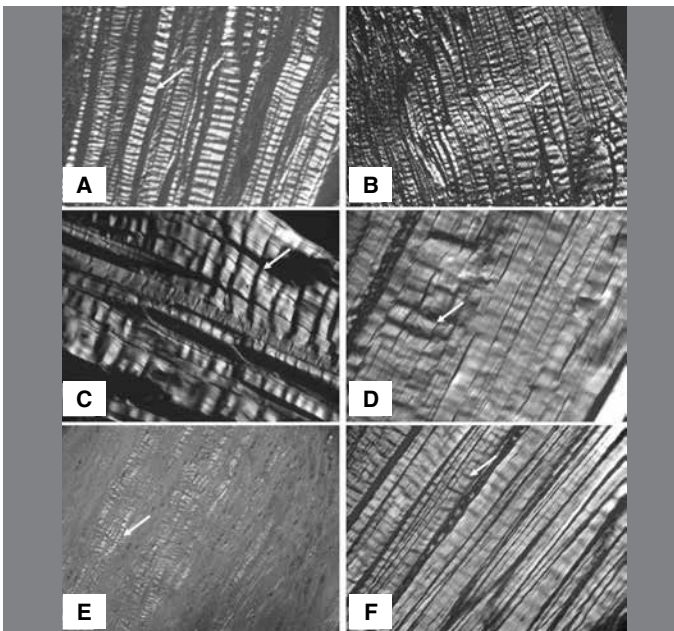

Figure 2 – Histological sections from the ligaments, stained with picrosirius and observed under polarized light. (A) ACL of control dog, group I; (B) ACL of control dog, group II; (C) Patellar ligament of control dog, group I; (D) Patellar ligament of control dog, group II; (E) rACL of dog, group I; F) rACL of dog, group II. Note the alternation of light and dark areas along the collagen fibers, which represent the crimp undulations of the different materials. Note that the size of the crimp is greater in the patellar ligament and that both rACLs have crimp morphology similar to that of the control ACL, independent of the thickness of the graft. Magnification 200x.

II, and in the variables studied, accompanied by the statistical analysis.

No significant difference was observed between the grafts in groups I and II for the ACL, patellar ligament and rACL ($p > 0.05$).

Table 2 – Mean and standard deviation of the nuclear area according to graft type (%).

Variable	Graft		p value
	Group I	Group II	
ACL	2.07 ± 0.18	2.10 ± 0.09	p > 0.05
Patellar Ligament	1.10 ± 0.20	1.08 ± 0.15	p > 0.05
rACL	2.10 ± 0.18	2.07 ± 0.19	p > 0.05

Tabela 3 – Média e desvio padrão do comprimento de crimp segundo tipo de enxerto (micrômetro).

Variable	Graft		p value
	Group I	Group II	
ACL	28.24 ± 2.76	33.47 ± 4.92	p > 0.05
Patellar Ligament	72.08 ± 8.91	72.77 ± 13.84	p > 0.05
rACL	33.96 ± 6.42	32.06 ± 5.12	p > 0.05

DISCUSSION

Our study demonstrated that after the eight-month period, the grafts used became hypertrophied and showed histological characteristics similar to those of the ACL. Group II presented behavior that was more homogenous than shown by group I, i.e. by using grafts of greater diameter, with dimensions closer to those of the native ACL, the results were more constant. Neither group showed any intra-articular degenerative alterations (a characteristic of ACL insufficiency in dogs) after intra-articular analysis on the knees operated, thus suggesting that even in the cases that presented a greater degree of laxity on clinical examination, the rACL was viable. The modification to the morphology of the patellar ligament graft, as seen through cell proliferation, remodeling and reorganization of the collagen fibers to a pattern similar to that of the ACL, is another factor favoring the functionality of the grafts in the two groups.

Although it is recommended that grafts of dimensions similar to or greater than those of the original ACL should be used, we did not find any histological study in the literature comparing the effects of grafts with different sectioned areas, in ACL reconstruction surgery.

Shino et al[17] compared the use of auto and allografts for reconstructing the ACL in dogs, using grafts of different dimensions. However, despite finding that after 52 weeks, the allograft had a histological pattern similar to that of the ACL and mechanical resistance that was 30% of the level in the control ACL, they did not make comparisons of the difference from the initial size of the graft, given that this was not the objective of their study.

Several other studies have also demonstrated that after a one-year period, the graft resembled the ACL

from a histological point of view, and that it underwent some degree of hypertrophy[18,19]. Alm and Strömberg[20] showed that a graft from the central third of the patellar ligament had a histological and vascular pattern that was similar to that of the ACL, four to five months after the operation.

Although several studies have demonstrated that after a period of ligamentization, grafts have histological similarities to the ACL, the correspondence between these similarities and their functional properties remain a matter of controversy. Several experimental studies have shown that the structural properties of grafts are significantly inferior to those of the original ACL[17,18,21,22], but it cannot be affirmed that this is due partly or totally to the initial dimensions of the graft used. Cummings *et al*[9] performed ACL reconstruction in sheep using grafts of different sizes and tension and observed six months later that the initial differences had disappeared, i.e. there was no difference in the sectioned area of the graft even though the initial measurements had been different, and that there was no difference in anteroposterior translation, despite the initial laxity of the graft. The initial dimensions of the graft did not seem to have any effect on the mechanical properties studied and, moreover, it was also observed that larger grafts were associated with worse postoperative results (limitation of exten-sion and greater damage to the joint cartilage).

There are other characteristics that should be taken into consideration in analyzing studies on animals, such as: joints of smaller sizes (implying greater technical difficulty), biomechanical differences with human knees and lack of control over ensuring adequate rehabilitation. These factors are significant, given that it is well known that the forces applied to the graft during the ligamentization phase play an important role in the final result from remodeling[9,23]. In clinical studies, in which these factors can be better understood and controlled, no functional differences have been found between grafts of different sizes, in humans[2,10,11].

CONCLUSION

The sectioned area of the graft did not influence the result from ACL reconstruction surgery in dogs, from analyzing the results from a histological point of view. However, use of grafts of dimensions closer to those of the native ACL presented results of greater homogeneity. Further research will be necessary in order to understand the role of the initial dimensions of the graft in ACL reconstruction surgery. In this manner, the smallest sized graft possible for safe surgery with lower morbidity will be attained.

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