STERILISATION OF CANINE ANTERIOR CRUCIATE ALLOGRAFTS BY GAMMA IRRADIATION IN ARGON

MECHANICAL AND NEUROHISTOLOGICAL PROPERTIES RETAINED ONE YEAR AFTER TRANSPLANTATION

M. J. GOERTZEN, H. CLAHSSEN, K. F. BÜRRIG, K.-P. SCHULTZ

From Heinrich Heine University, Düsseldorf, Germany

Bone-ACL-bone allograft transplantation is a potential solution to the problem of reconstruction of the anterior cruciate ligament (ACL), but sterilisation by gamma irradiation or ethylene oxide causes degradation of the graft. We have studied the biomechanical and histological properties of deep-frozen canine bone-ACL-bone allografts sterilised by gamma irradiation (2.5 Mrad) under argon gas protection. Particular attention was paid to their collagen structure and neuroanatomy compared with those of non-irradiated allografts.

We used 60 skeletally mature foxhounds. In 30 animals one ACL was replaced by an irradiated allograft and in the other 30 a non-irradiated graft was used. In both groups the graft was augmented by a Kennedy Ligament Augmentation Device. Examination of the allografts at 3, 6 and 12 months after implantation included mechanical testing, histology, collagen morphometry, neuroanatomical morphology (silver and gold chloride stain) and studies of the microvasculature (modified Spalteholz technique).

At 12 months the irradiated ACL allografts failed at a mean maximum load of 718.3 N, 63.8% of the strength of the normal canine ACL. The non-irradiated allografts failed at 780.1 N, 69.1% of normal. All the allografts showed a well-orientated collagen structure one year after transplantation and there was no difference between the irradiated grafts and the others. The silver staining technique demonstrated Golgi

tendon organs and free nerve endings within both groups of allografts. As in the normal ACL these structures were most commonly found near the surface of the graft and at its bony attachments. At 12 months the irradiated allografts showed slight hypervascularity compared with the non-irradiated grafts.

We believe that cryopreserved ACL allografts have the potential to restore both the mechanical and the neurophysiological functions of the normal ACL.

J Bone Joint Surg [Br] 1995;77-B:205-12.

Received 23 March 1994; Accepted 18 May 1994

The treatment of injuries of the anterior cruciate ligament (ACL) is still controversial although large numbers of operations are being performed on ACL-deficient knees.

None of the current techniques has been able to restore fully the functions of the original ligament (Hey Groves 1917; Bosworth and Bosworth 1936; Coleman 1956; Slocum and Larson 1968; Matis and Schäffer 1973; Andrish and Woods 1984; Bolton and Bruchman 1985; McMaster 1985; Zichner 1985; Aichroth, Jones and Thomas 1986; Jackson et al 1987a). Even the combination of intra-articular and extra-articular procedures, although it improves stability, does not give full reconstruction (Nicholas 1973; Trillat 1973; Jäger and Wirth 1978; Ellison 1980; Clancy et al 1982). The current popular intra-articular methods which use the semitendinosus tendon (Augustine 1956; Nicholas 1973; Cho 1975; Jäger and Wirth 1978; Puddu and Ippolito 1983) or part of the patellar tendon (O'Donoghue 1963; Brückner 1966) provide adequate tensile strength but do not replicate the complicated three-dimensional architecture of the ligament or restore its neuroproprioceptive functions.

Bone-ACL-bone allografts are potentially able to restore the original anatomy (Jackson et al 1987a,b) if the forces can be reduced until incorporation has occurred and if immunological rejection reactions can be avoided. To avoid transmission of disease, particularly AIDS, allograft tissues must be sterilised but animal studies have shown that gamma irradiation or the use of chemical detergents like ethylene oxide change the histological and biomechanical properties (Parry and Wallach 1974; Paulos, Franck and

M. J. Goertzen, MD, PhD, Professor, Paediatric and Sports Medicine Division
K.-P. Schultz, MD, PhD, Professor and Head of Department, Department of Orthopaedic Surgery
K. F. Bürrig, MD, PhD, Professor, Department of Pathology
Heinrich Heine University Hospital, Moorenstrasse 5, D-40225 Düsseldorf, Germany.

H. Clahsen, MD
Department of Topographic Anatomy and Biomechanics, Anatomical Institute, Heinrich Heine University, Universitätsstrasse 1, 40255 Düsseldorf, Germany.

Correspondence should be sent to Dr M. J. Goertzen.

©1995 British Editorial Society of Bone and Joint Surgery
0301-620X/95/2885 \$2.00

Fig. 1

Fig. 2

Figure 1 – Allografts were prepared with cylindrical bone plugs at the femoral and tibial insertions of the ligament. Figure 2 – Schematic drawing of the operative procedure.

Rosenberg 1987; Haut and Powlison 1990; Gibbons et al 1991).

We have studied canine bone-ACL-bone grafts which had been deep-frozen and gamma-irradiated under argon gas protection. After transplantation we compared their mechanical, microangiographic, collagen morphological and neuroproprioceptive properties with those of non-irradiated control transplants.

MATERIALS AND METHODS

Deep-frozen bone-ACL-bone allografts (Fig. 1) were transplanted into the right knees of 60 fully-grown foxhounds (average weight 26.8 kg; range 24 to 37). In 30 animals (group A) the graft had been gamma-irradiated under argon gas protection. In the other 30 animals (group B) the ACL-allograft was not irradiated. In both groups the reconstruction was augmented with a Kennedy Ligament Augmentation Device (LAD; 3M, St Paul, Minnesota). At intervals of 3, 6 and 12 months after implantation, we performed biomechanical analysis on five dogs from each group, microangiographic studies on two dogs from each group and collagen morphological and neurohistological studies on three dogs from each group.

Operative technique

Collection of the grafts. Under aseptic conditions, the right knee was opened through a lateral parapatellar incision. The patella was dislocated medially and the knee held in full flexion. The prepatellar fat pad was retracted but not resected.

After marking the insertion of the ligament with 1.2 mm thick Kirschner wires, femoral and tibial bone blocks 2.5 cm long were removed with an 8 mm cannulated trephine. The pediculated grafts were carefully cleaned with sterile NaCl solution and placed in plastic containers for immediate deep-freezing to -80°C . The deep-frozen specimens from group A were then stored in an argon gas atmosphere and gamma-irradiated with 2.5 Mrad. During

the irradiation process the bottles were rotated in a specially manufactured device at constant speed and constant freezing at -80°C .

Transplantation. About 12 days after their collection the grafts were thawed over a period of about 45 minutes and implanted into different dogs from which they had originally been taken (Fig. 2). We were careful to replace the graft in exactly the anatomical position which had been marked at the time of removal. The Kennedy LAD was quilted on to the graft with size 2.0 absorbable sutures and a size 0 Dexon stitch was passed through the femoral and the tibial bone plugs to facilitate fixation.

The femoral bone plug was fitted first and then the tibial (Fig. 2). We tried to achieve a good 'press-fit', and fixation was secured in all cases by the additional LAD. By pulling on the LAD suture ends, the graft could be tightened. The proper tension and alignment of the collagen fibres were achieved by special clamps and repeated flexion and extension of the joint. The position was considered to be correct when the retention stitches at the insertions and the LAD were seen to pull tight at 30° to 40° flexion and at 60 N tension. Two cortical screws and serrated plastic washers were then inserted to fix the femoral and tibial ends of the LAD and the Dexon retention stitches were tied to the screw ends.

The operated leg was immobilised in a synthetic thigh cast for five weeks, to allow incorporation of the bone plugs. After removal of the casts the dogs were allowed the freedom of a large compound to exercise.

Physical examination. During the whole period of the study the dogs' walking behaviour was monitored each month and the operated joints were examined to ascertain the range of movement. Preoperatively, postoperatively, and shortly before death radiography was performed and blood samples were taken to determine haemoglobin levels, the haematocrit, and erythrocyte and leucocyte and differential blood cell counts. The retrieved knees were examined for degradation of the cartilage in any part of the joint

and the presence of osteophytes and meniscal lesions was sought.

Biomechanical analysis. The femur and tibia were cleaned of all soft tissues except the anterior cruciate ligament and fixed in special clamps with the joint segments in 30° flexion. Importance was attached to an exact vertical alignment of the ligament. Stretch tests to failure were done with a universal test machine (Type Zwick 1434) at a speed of 50 mm/min.

Microangiography. The Spalteholz microangiography technique, as modified by Arnoczky, Rubin and Marshall (1979), was used on the transplanted and the non-operated knees. Under intubation anaesthesia, the femoral arteries and veins were catheterised and heparin (5000 units) was injected intra-arterially. After irrigation of the vessels with physiological saline, a mixture of 150 ml of India ink and 10% buffered formalin was injected. The animals were then given an overdose of barbiturate and the limbs were disarticulated at the hips. After deep-freezing at -80°C, histological preparations were made by cutting 5 mm thick strips with a band-saw and preserving them in 10% neutral formalin solution. Decalcification was by the method of Spalteholz with 10% nitric acid, the preparations being kept in the solution for at least six months.

Collagen morphology. After initial fixation in formalin, specimens were decalcified in ethylene diamine tetra-acetic acid and stained with haematoxylin and eosin. Specimens were also prepared for polarised light examination. Tissue samples (1 mm × 1 mm) for transmission electron microscopy were taken from the peripheral and central parts of the allograft as well as from the normal ligament in the other knee.

After fixation in 2% glutaraldehyde and 2% formaldehyde in 0.1 M sodium cacodylate buffer (pH 7.2 to 7.4), the

specimens were irrigated with 0.1 M sodium cacodylate buffer, fixed with 1 M osmium acid and dehydrated in graded alcohol concentrations before embedding in epoxy resin. Ultrathin longitudinal and transverse sections were stained with uranyl acetate and lead citrate and examined by a Siemens electron microscope. Measurements of the collagen fibre sections were carried out using a computed IBAS morphometer at a magnification of 12 000.

Neurohistology. We used the qualitative silver stain technique of Novotny and Gommert-Novotny (1988). Other sections were stained by the gold chloride technique of Zimny, St Onge and Schutte (1985).

RESULTS

At three months, three of the 20 dogs were limping and had joint effusions but the others were walking normally. Histological and blood chemical examinations gave no indication of graft rejection in either group. Two dogs, one in each group, had a loose ligament due to a partial migration of the tibial bone plug.

Biomechanical analysis (Table I). The non-irradiated allografts had a mean maximal tensile strength of 387.2 ± 72.1 N at 3 months, 556.6 ± 67.2 N at 6 months and 780.1 ± 57.4 N at 12 months. These figures represent 34.3%, 49% and 69% of the mean maximal tensile stress measured in the non-irradiated knees at 12 months (1129.0 ± 103.3 N).

The irradiated allografts showed mean maximal tensile strengths of 580.2 ± 43.5 N (51.3%), 536.2 ± 32.2 N (47.5%) and 718.3 ± 44.9 N (63.8%) respectively. Only at three months was there a significant difference between the irradiated and the non-irradiated groups ($p > 0.01$).

Histology. At three months sections stained with haematoxylin and eosin showed some disturbance of the collagen

Table I. Results of tests to failure (mean $\pm$ sd) of the normal ACL and of non-irradiated (Allo/LAD) and irradiated (Allo/rad) allografts, with the *percentage* of normal in parenthesis

	Max tensile strength (N)	Max energy (Nm)	Elongation (mm)
Normal ACL (n = 60)	1129.0 ± 103.3	1.26 ± 0.33	2.75 ± 0.21
Allo/LAD (n = 30)			
3 mth	387.2 ± 72.1 (34.3)	0.73 ± 0.29 (57.9)	2.24 ± 0.19 (81.5)
6 mth	556.6 ± 67.2 (49.3)	1.08 ± 0.31 (85.7)	2.41 ± 0.22 (87.6)
12 mth	780.1 ± 57.4 (69.1)	1.20 ± 0.39 (95.2)	2.59 ± 0.29 (94.2)
Allo/rad (n = 30)			
3 mth	580.2 ± 43.5 (51.3)	0.91 ± 0.14 (70.5)	1.81 ± 0.13 (65.8)
6 mth	536.2 ± 32.2 (47.5)	1.01 ± 0.21 (80.1)	2.30 ± 0.21 (83.6)
12 mth	718.3 ± 44.9 (63.8)	1.14 ± 0.27 (90.4)	2.35 ± 0.19 (85.4)

Fig. 3a

Fig. 3b

Fig. 3c

Figure 3a – Cellular necrosis within an irradiated allograft three months after implantation. Figure 3b – Hypercellularity and intensive revascularisation reaction in an irradiated graft at six months. Figure 3c – Nearly normal collagen structure in an irradiated allograft 12 months after implantation (haematoxylin and eosin $\times 66$).

structure in both groups and severe vascular budding but by six months there was good remodelling of the collagen structures. This was also seen on polarised light microscopy. Remodelling was even more complete at one year in both groups (Figs 3 and 4). Accumulations of lymphocytes were sometimes found within the ligament but no monocyte cell clusters were seen.

By three months, all the bone plugs (except those in the two dogs with tibial loosening) were well incorporated. There was no ingrowth of bone into the LAD and only a few strands of loose connective tissue lay between the LAD fibres. The PAS-stained sections revealed active chondrocytes producing large amounts of mucopolysaccharides in the transitional zone between the bone and ligament (Fig. 5). No destructive effect of gamma irradiation was observed.

In all the knees the fibres of the LAD were split or ruptured and the tibial and femoral stumps were visible and stained yellow.

Electron microscopy showed no significant differences between the two experimental groups although both were different from the normal cruciate ligament with regard to the distribution of collagen fibre sizes (Fig. 6). As in the normal ligament, fibres with a diameter of 60 to 130 μm predominated. Significant differences were seen, however, in the number of fibres per μm^2 , and in the total cross-

Fig. 4

Polarised light microscopy of the transitional zone between the ligament and bone of a one-year-old irradiated allograft. The osteone structure shows osseous healing and there is normal longitudinal collagen alignment ($\times 66$).

section of the graft, which was smaller than normal in both groups, correlating well with their lower tensile strengths. The collagen crimp-wave frequency was also significantly different from normal in both groups at 12 months.

Microangiography. As in the normal anterior cruciate ligament, perfusion of the transplanted ligaments was

Fig. 5

An irradiated graft at six months showing active chondrocytes in the transitional zone between bone and ligament (PAS $\times 66$).

mainly from vessels in the retropatellar fat pad. Usually, from the sixth month, the graft was also vascularised from the femoral bone plug. Small vessels could be seen especially in the transitional zone between bone and ligament (Fig. 7a). At 12 months, the allograft usually showed a vascular pattern similar to that of the normal ligament (Fig. 7b) although there remained a slight hypervascularity in the irradiated group.

Neurohistology. The several types of staining used reflect how difficult it is to find proof of intraligamentous neural structures. We were unable to identify them in any of the grafts at three months.

At 6 and 12 months we identified isolated free nerve endings with the silver-impregnation technique and by electron microscopy (Figs 8 and 9). These were mostly near the surface in the region of the insertion of the ligament and

Fig. 6a

Fig. 6b

Computerised collagen morphometry (IBAS). The distribution of fibre sizes at 12 months is shown for non-irradiated grafts (a) and for irradiated grafts (b) and can be compared with that in the normal anterior cruciate ligament.

Fig. 7a

Thick frozen sections (5 mm) at six months (a) and 12 months (b) after transplantation of an irradiated graft (Spalteholz microangiographic technique). The six-month specimen shows the importance of the prepatellar fat pad for the initial nourishment of the allograft. At 12 months there was also vascularisation, both of irradiated and non-irradiated grafts, from their femoral and tibial insertions (F = femur; T = tibia; H = fat pad; A = ACL-allograft; P = PCL; magnification $\times 2$).

Fig. 7b

Fig. 8

An irradiated graft 12 months after implantation prepared by the silver-impregnation technique (original size $\times 170$, irradiated specimen). Free nerve endings are seen running parallel to the collagen fibres. Most are subsynovial and in the region of the insertion of the ligament to the bone.

Fig. 9

Electron micrograph of a free sensory nerve ending in a non-irradiated graft 12 months after implantation ($\times 7350$).

close to blood vessels. Their size and length were very varied and although they could be pursued microscopically by adjusting the depth of focus, it proved difficult to depict these three-dimensional structures on a two-dimensional film. Ruffini and Pacini corpuscles were not identified.

With the gold-staining technique, Golgi tendon organs were found at 12 months situated subsynovially and close to the osseous insertions (Fig. 10). No similarity between the neural structures in the grafts and those in the normal cruciate ligaments could be demonstrated because of the small numbers. For the same reason, comparative quantification of the free nerve endings in the two groups had to be abandoned. We can only say that irradiation seems not to have had any deleterious effect on the restitution of neuro-receptors in the grafts.

DISCUSSION

We have shown that, with a LAD to reduce the initial forces on it, a bone-ACL-bone allograft can restore the main biomechanical and histological characteristics of the original anterior cruciate ligament. Various studies (Friedlaender 1982; Tomford et al 1983; Tomford, Ploetz and Mankin 1986; Jackson et al 1987b, 1988; Dahners and Burroughs 1990) have shown that the best way to reduce the antigenic properties of allografts is by deep-freezing at -80°C . Apart from a few small lymphocytic nests, we found no evidence of immunological rejection reactions to our deep-frozen grafts. They became well revascularised, osseous incorporation was rapid and by 12 months collagen remodelling was complete to a nearly physiological state.

Jackson et al (1987a,b) reported that transplanted allografts had a maximum strength of 25% of the contralateral ligament, but our irradiated allografts, with augmentation, had mean values which were 63.8% of normal similar to

Fig. 10

A Golgi tendon organ (arrow) demonstrated by the gold-chloride technique in the region of the femoral insertion of an irradiated graft 12 months after implantation ($\times 800$).

the tensile strength of a semitendinosus tendon one year after implantation. The non-irradiated ligaments were not significantly stronger (69.1%).

The use of an inert argon atmosphere, and of our rotating device to allow freezing of the allograft containers during the irradiation process, reduces the development of free radicals and diminishes the deleterious effects of gamma irradiation on bone and collagen which has been reported by several authors (Paulos et al 1987; Haut and Powlison 1990; Gibbons et al 1991). Gamma irradiation initially produces a higher rate of intermolecular cross-linking in collagen which increases its tensile strength with only slight reduction of the elastic modulus.

The restoration of the neuroproprioceptive as well as the mechanical functions of the cruciate ligament may be of significance, especially in regard to early functional rehabilitation. Simple muscle-strengthening exercises are now being superseded by co-ordination-orientated and sport-

specific forms of rehabilitation (Paulos et al 1981).

A few neuroreceptors are known to be present in intact human cruciate ligament (Kennedy, Alexander and Hayes 1982; Schultz et al 1984; Schutte et al 1987), but none has been demonstrated with certainty in autogenous or allogeneic grafts. This fits with the observation that defects of co-ordination persist even when an ACL graft has successfully restored mechanical stability and full muscle strength. Our findings therefore of free nerve endings localised subsynovially and in the transition zones between ligament and joint bone as described by Schutte in intact human cruciate ligaments are important. We were only able to demonstrate these nerve endings in the 6- and 12-month specimens, perhaps because their presence was obscured at 3 months by the disorganisation of the collagen structure or because their appearance is a time-dependent feature of the regrowth of tissues into the ligament from its osseous insertions. The perivascular arrangement of the free nerve endings suggests that they may play a part in vasomotor control.

To our knowledge this is the first presentation of histological evidence of viable neuroreceptors in ACL substitutes. With the modified gold-chloride technique of Zimny et al (1985), Golgi tendon organs were also observed within the ligament, especially near its tibial insertion. It seems that gamma irradiation in argon gas does not destroy the neuroreceptors in cryopreserved ACL grafts. We saw, however, very few such receptors and have no information on the relative numbers in the intact and transplanted ligaments.

The important result of our study is that it raises the hopes that transplantation of bone-ACL-bone allografts can become a useful procedure for human ACL reconstruction.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

REFERENCES

- Aichroth PM, Jones CB, Thomas NP. Carbon fibre and Dacron composites in the prosthetic reconstruction of the anterior cruciate ligament. *J Bone Joint Surg [Br]* 1986;68-B:841.
- Andrish JT, Woods LD. Dacron augmentation in anterior cruciate ligament reconstruction in dogs. *Clin Orthop* 1984;183:298-302.
- Arnoczky SP, Rubin RM, Marshall JL. Microvasculature of the cruciate ligaments and its response to injury: an experimental study in dogs. *J Bone Joint Surg [Am]* 1979;61-A:1221-9.
- Augustine RW. The unstable knee. *Am J Surg* 1956;92:380-8.
- Bolton CW, Bruchman WC. The GORE-TEXTM expanded polytetrafluoroethylene prosthetic ligament: an *in vitro* and *in vivo* evaluation. *Clin Orthop* 1985;196:202-13.
- Bosworth DM, Bosworth BM. Use of fascia lata to stabilize the knee in case of ruptured cruciate ligaments. *J Bone Joint Surg* 1936;18:178-9.
- Brückner H. Eine neue Methode der Kreuzbandplastik. *Chirurg* 1966;37:413-4.
- Cho KO. Reconstruction of the anterior cruciate ligament by semitendinosus tenodesis. *J Bone Joint Surg [Am]* 1975;57-A:608-12.
- Clancy WG Jr, Nelson DA, Reider B, Narechania RG. Anterior cruciate ligament reconstruction using one-third of the patellar ligament, augmented by extra-articular tendon transfers. *J Bone Joint Surg [Am]* 1982;64-A:352-9.
- Coleman HM. Cruciate ligament repair using meniscus. *J Bone Joint Surg [Br]* 1956;38-B:778.
- Dahners LE, Burroughs P. The effect of exercise on ligament healing. Trans 36th meeting Orthopaedic Research Society, New Orleans, 1990:57.
- Ellison AE. The pathogenesis and treatment of anterolateral rotatory instability. *Clin Orthop* 1980;147:51-5.
- Friedlaender GE. Current concepts review: bone-banking. *J Bone Joint Surg [Am]* 1982;64-A:307-11.
- Gibbons MJ, Butler DL, Grood ES, et al. Effects of gamma irradiation on the initial mechanical and material properties of goat bone-patellar tendon-bone allografts. *J Orthop Res* 1991;9:209-18.
- Haut RC, Powlison AC. The effects of test environment and cyclic stretching on the failure properties of human patellar tendons. *J Orthop Res* 1990;8:532-40.

- Hey Groves EW.** Operation for the repair of the crucial ligaments. *Lancet* 1917;11:674-5.
- Jackson DW, Grood ES, Arnoczky SP, Butler DL, Simon TM.** Freeze dried anterior cruciate ligament allografts: preliminary studies in a goat model. *Am J Sports Med* 1987a;15:295-303.
- Jackson DW, Grood ES, Arnoczky SP, Butler DL, Simon TM.** Cruciate reconstruction using freeze dried anterior cruciate ligament allograft and a ligament augmentation device (LAD): an experimental study in a goat model. *Am J Sports Med* 1987b;15:528-38.
- Jackson DW, Grood ES, Wilcox P, et al.** The effects of processing techniques on the mechanical properties of bone-anterior cruciate ligament-bone allografts: an experimental study in goats. *Am J Sports Med* 1988;16:101-5.
- Jäger M, Wirth CJ.** Die veraltete anteromediale Kniegelenksinstabilität. *Unfallheilkunde* 1978;81: 172-7.
- Kennedy JC, Alexander IJ, Hayes KC.** Nerve supply of the human knee and its functional importance. *Am J Sports Med* 1982;10:329-35.
- Matis U, Schäffer E.** Zur Frage des Kreuzbandersatzes mit lyophilisierter menschlicher Dura beim Hund: Eine experimentelle Untersuchungen. *Berl Munch Tierärztl Wschr* 1973;86:245-52.
- McMaster WC.** A histologic assessment of canine anterior cruciate substitution with bovine xenograft. *Clin Orthop* 1985;196:196-201.
- Nicholas JA.** The five-one reconstruction for anteromedial instability of the knee: indications, technique, and the results in fifty-two patients. *J Bone Joint Surg [Am]* 1973;55-A:899-922.
- Novotny GE, Gommert-Novotny E.** Silver impregnation of peripheral and central axons. *Stain Technol* 1988;63:1-14.
- O'Donoghue DH.** A method for replacement of the anterior cruciate ligament: report of twenty cases. *J Bone Joint Surg [Am]* 1963; 45-A:905-24.
- Parry MF, Wallach R.** Ethylene glycol poisoning. *Am J Med* 1974; 57:143-50.
- Paulos L, Noyes FR, Grood E, Butler DL.** Knee rehabilitation after anterior cruciate ligament reconstruction and repair. *Am J Sports Med* 1981;9:140-9.
- Paulos L, Franck EP, Rosenberg TD.** Comparative material properties of allograft tissues for ligament replacement: effect of type, age, sterilization and preservation. *Trans Orthop Res Soc* 1987;12:129.
- Puddu G, Ippolito E.** Reconstructions of the anterior cruciate ligament using the semitendinosus tendon: histological study of a case. *Am J Sports Med* 1983;11:14-6.
- Schultz RA, Miller DC, Kerr CS, Micheli L.** Mechanoreceptors in human cruciate ligaments: a histological study. *J Bone Joint Surg [Am]* 1984;66-A:1072-6.
- Schutte MJ, Dabezies EJ, Zimny ML, Happel LT.** Neural anatomy of the human anterior cruciate ligament. *J Bone Joint Surg [Am]* 1987;69-A:243-7.
- Slocum DB, Larson RL.** Pes anserinus transplantation: a surgical procedure for control of rotatory instability of the knee. *J Bone Joint Surg [Am]* 1968;50-A:226-42.
- Tomford WW, Doppelt SH, Mankin HJ, Friedlaender GE.** 1983 bone bank procedures. *Clin Orthop* 1983;174:15-21.
- Tomford WW, Ploetz JE, Mankin HJ.** Bone allografts of femoral heads: procurement and storage. *J Bone Joint Surg [Am]* 1986;68-A:534-7.
- Trillat A.** *Chirurgie du genou.* Paris: Masson, 1973.
- Zichner L.** Kreuzbandersatz mit heterologen Bindegewebsstrukturen. *Unfallchirurgie* 1985;11:238-41.
- Zimny ML, St Onge M, Schutte M.** A modified gold chloride method for the demonstration of nerve endings in frozen sections. *Stain Technol* 1985;60:305-6.