LIMB REPLANTATION IN RATS - EXPERIMENTAL MODEL

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INTRODUCTION

The idea of replantation is not a new one. The replantation (reattaching all structures important to the function of a complete amputated body part, providing arterial inflow and venous outflow) was a challenge for surgeons for a long time, but better results started to appear after using the surgical microscope.¹⁻³ First experimental replantation was performed by Höpfner in 1903 on dog. Ronald Malt performed the first replantation on May 23, 1962 at Massachusetts General Hospital on a 12-year-old boy who had his right arm amputated in a train accident (The Journal of the American Medical Association, 1964). Since Malt’s first replantation, technological advances and the use of the microscope have made possible the replantation of other body parts, including thumbs, fingers, ears, scalps, facial parts, and genitalia.⁴⁻⁵

Replantation in limb amputations (especially upper limb) is mandatory in the actual technical developmental conditions. Social, economical and psychological implications of limb amputation determine medical responsibilities in emerging conservative surgery instead amputation. The procedure is very complex with critical microvascular stage and difficult to teach with conventional surgical instructive methods.⁶⁻⁷ We describe a teaching model of replantation in rat hind limb.

MATERIAL AND METHODS

Preoperative preparation

We have used 7 Sprague Dawley adult rats, weighing between 250-300g of weight. Under i.m. anesthesia
(xylazine and ketamine) the medial and lateral thigh sides were shaved. The rat was positioned in decubitus with the posterior limbs immobilized in extension with elastic bands. The anterior limbs were free in order to permit respiratory movements. Wet swabs were used to protect the subcutaneous tissue and the exposed organs during surgery.

Together with the usual microsurgical instruments, for replantation we used a few osteosynthesis tools: portable motor drill, an 18G needle and a steel 3-0 suture for cerclage.

**Anatomy**

The proximal segment of the hind limb in rat includes:
- skeleton, represented by the femoral bone;
- the muscles of the thigh, grouped into anterior femoral muscles, medial femoral muscles, muscles of the gluteal region and posterior femoral muscles;
- the vessels: femoral artery, femoral vein and their branches;
- the peripheral nerves: femoral, obturator and sciatic nerves and their branches. (Fig. 1)

**Surface landmarks**

The limit between the middle and the proximal third of the thigh were marked, using as surface landmarks the inguinal flexion fold and the knee joint. We drew a transverse curve line with its concavity towards the cranial extremity. This incision line creates a cranial skin and subcutaneous flap based on the inferior epigastric artery that will protect the anastomosis.

**Amputation**

The skin of the medial and lateral aspect of the thigh was incised, on the previously marked line. The dissection began by preparing the elements of the vasculo-nervous femoral bundle. The muscular branches of the femoral artery and vein were ligated, to obtain a greater mobility of the vessels to be anastomosed. Once prepared, they can be easily protected while cutting the muscle and performing osteotomy. We cut the gluteus maximus muscle on the lateral aspect of the thigh, dissected and cut the sciatic nerve. Hind muscles were sectioned, carefully protecting the vasculo-nervous pedicle; the bleeding was controlled with the bipolar. (Fig. 2)

Preparing of the femoral vessels for the future anastomosis started before cutting them. First microclamp was put on the vein in a proximal position, thus causing its dilatation, and performed adventicectomy in good conditions. Then we clamped the distal artery, obtaining a partially dilated artery, and perform also the adventitial stripping. Now we cut the vasculo-nervous femoral bundle.

The distal segment is now in ischemia, and the time until revascularisation should not exceed 60 minutes from this moment. We cleaned the femur for about 1 cm and cut it with the circular saw, shortening it by 0.5 cm. Bone shortening will allow performing vascular anastomosis without any tension. (Fig. 3)

**Bone fixation**

We washed the femoral medullar channel with saline. We adjusted a fragment of suitable length from the 18G needle and use it for centro-medullar osteosynthesis. We performed a hole in frontal plane into the distal and proximal femoral segments and put the cerclage wire. We put the needle into the medullar channel and made a knot of the wire. A stable bone coaptation was obtained, without permitting bone fragments movements to put the anastomosis at risk. (Fig. 4)
**Muscle and nerve suture**

The muscles were sutured with absorbable 4-0 sutures, starting on the medial aspect of the thigh: vastus medialis, adductors, semimembranosus, semitendinosus, gracilis and then on the lateral aspect: biceps femoris, caudofemoris and partially the gluteus medius.

This is the best moment for sciatic nerve coaptation. Epiperineural suture with a 10-0 stitch is good enough in this exercise. Then we continued suturing on the lateral aspect with gluteus medius, gluteus maximus and tensor fasciae latae. Finish the muscle suture on the internal aspect: rectus femoris, vastus lateralis and intermedius. All muscles are now sutured. We performed coaptation of the femoral nerve, also using a 10-0 stitch. If the time runs up, we first performed the suture of the femoral vessels.

**Vascular suture**

The vascular step of the surgery started with the end to end artery anastomosis, which is situated in the distal plane, then suture the vein by separate stitches. 10-0 nylon suture was used. The clamps were removed in the following order: first the proximally clamp of the vein, second the distally clamp of the vein, third the distally clamp of the artery and last the proximally clamp of the artery. (Fig. 5)

After performing the patency tests, we covered the anastomosis with the inguinal fat pad irrigated by the inferior epigastric vessels and suture the skin.

**Skin suture**

The skin suture started on the internal aspect of the thigh, with separate non-absorbable 4-0 stitches. We checked the viability of the replanted segment by inspecting the color (pink), the turgor (full, elastic) and by pulsoxymetry. (Fig. 6)
Postoperative care
No immobilization of the hind leg or other special treatment is necessary. For a longer term follow-up, the rat should be protected from hurting his anesthetized leg by using a plastic collar.

RESULTS
All the animals had a good postoperative evolution, with the survival of the replanted segment, minimal edema and no trofic or infectious complications.

DISCUSSIONS
Since the first successful digit replantation with microvascular repair (reported by Komatsu and Tamai, 1968), the laboratory microsurgical training importance was obvious, because of surgical procedure complexity and critical microvascular stage being difficult to teach using conventional surgical instructive methods.7,10

Most models from literature use similar technique. Some authors describe techniques very similar with the clinical approach, first detaching the limb by clear cut amputation and than debridement, dissection of the vessels, nerves, muscle and bone, bone fixation, muscle suture, vessels and nerve repair skin suture.6,8 We consider that nerve and vessels dissection and clamping before amputation is mandatory for limited bleeding and ischemic time shortening. Also, the bone fixation by pine and intraosseous wiring will provide better bone alignment and stability than using pine only.

The rat is very convenient animal for teaching microsurgery. They are relatively easy to obtain, manipulate and anesthetize and the size of their vessels is equivalent to digital vessels. They also provide real limb structures handling (bone, muscles, etc.), different from other models.11,12,14 Our model can be used for junior surgeons training to improve their instruction and practice and avoid many possible pitfalls. This model teaches also microsurgical technique and limb structure handling but also surgical steps in replantation and postoperative manage of the replanted segment.

CONCLUSIONS
Considering limb replantation a very important surgery, with high risk level, the laboratory training is a primordial step to avoid pitfalls, achieve good survival rate and functional results. Our model of rat hind replantation insures all surgical steps of the replantation. It can be considered the ideal experimental model for training in replantation to improve clinical proficiency.

REFERENCES
5. Jones NF. Replantation in the Upper Extremity, Ch. 82, Grabb and Smith’s Plastic Surgery. 5th Ed., Lippincott-Raven, 1997