

# Multivisceral transplantation in pigs: a model for research and training

Transplante multivisceral em suínos: modelo de pesquisa e treinamento

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## ABSTRACT

**Objective:** To present a model for research and training in multivisceral transplantation in pigs. **Methods:** Eight Large White pigs (four donors and four recipients) were operated. The multivisceral transplant with stomach, duodenum, pancreas, liver and intestine was performed similarly to transplantation in humans with a few differences, described below. Anastomoses were performed as follows: end-to-end from the supra-hepatic vena cava of the graft to the recipient juxta diaphragmatic vena cava; end-to-end from the infra-hepatic vena cava of the graft to the inferior (suprarenal) vena cava of the recipient; and end-to-side patch of the aorta of the graft to the infrarenal aorta of the recipient plus digestive reconstruction. **Results:** The performance of the multivisceral transplantation was possible in all four animals. Reperfusion of the multivisceral graft led to a severe ischemia-reperfusion syndrome, despite flushing of the graft. The animals presented with hypotension and the need for high doses of vasoactive drugs, and all of them were sacrificed after discontinuing these drugs. **Conclusion:** Some alternatives to minimize the ischemia-reperfusion syndrome, such as the use of another vasoactive drug, use of a third pig merely for blood transfusion, presence of an anesthesia team in the operating room, and reduction of the graft, will be the next steps to enable experimental studies.

**Keywords:** Transplants/methods; Viscera/transplants; Animal models; Professional training; Swine

## RESUMO

**Objetivo:** Apresentar um modelo de pesquisa e treinamento em

transplante multivisceral em suínos. **Métodos:** Oito porcos da raça *Large White* (quatro doadores e quatro receptores) foram operados. O transplante multivisceral com estômago, duodeno, pâncreas, fígado e intestino foi realizado a semelhança do transplante em seres humanos com algumas diferenças descritas a seguir. Foram realizadas as anastomoses de veia cava supra-hepática do enxerto com a veia cava do receptor justa diafragmática término-terminal, veia cava infra-hepática do enxerto com a veia cava inferior (suprarrenal) do receptor término-terminal e patch da aorta do enxerto com a aorta infrarenal do receptor término-lateral e reconstrução digestiva. **Resultados:** Foi possível a realização do transplante multivisceral nos quatro animais. A reperfusão do enxerto multivisceral levou a uma grave síndrome de isquemia-reperfusão, apesar do *flush* do enxerto. Os animais apresentaram hipotensão com necessidade de drogas vasoativas em altas doses, sendo todos sacrificados com a retirada dessas drogas. **Conclusão:** Alternativas para minimizar a síndrome de isquemia-reperfusão, como o uso de mais de uma droga vasoativa, uso de um terceiro porco apenas para transfusão sanguínea, presença de uma equipe de anestesia na sala de cirurgia e redução do enxerto, serão os próximos passos para possibilitar estudos experimentais.

**Descritores:** Transplantes/métodos; Vísceras/transplante; Modelos animais; Capacitação profissional; Suínos

## INTRODUCTION

The short bowel syndrome (SBS) is characterized by malabsorption due to extensive resections of the intestine. This condition results in the massive

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reduction of the enteric capacity to absorb nutrients, making artificial nutrition necessary<sup>(1,2)</sup>.

The estimated incidence of SBS is two to five per one million individuals<sup>(1)</sup>. Congenital and perinatal conditions, such as intestinal atresia, necrotizing enterocolitis, gastroschisis, and volvulus are the most frequent causes in children. In adults, the most common etiologies are mesenteric infarction, abdominal trauma, enteritis due to radiation, and Crohn's disease<sup>(1)</sup>.

Treatment of SBS may be medical or surgical, with the objective of increasing intestinal absorption. Medications for the treatment of SBS aim to increase intestinal absorption, while surgeries have the goal of elongating the rest of the intestine, allowing discontinuation of parenteral nutrition (PN)<sup>(2)</sup>. PN enables children with SBS to have satisfactory growth during the adaptation period<sup>(3-6)</sup>. PN is associated with other drugs and operations to recover normal intestinal function<sup>(7)</sup>. Some studies showed the importance of oral feeding, since it increases gastrointestinal secretion, secretion of salivary epidermal growth factor, and gall bladder motility<sup>(2)</sup>.

A growing group of patients requires long-term PN. In some cases, resulting complications make PN impossible, such as consequence of long-term catheterization (septic complications, venous thrombosis), metabolic disease of the bones, steatosis, or hepatic diseases.

The high mortality of these complications (reaching 20% in four years)<sup>(7)</sup> was the cause for establishing the indication for intestinal transplant (IT) or multivisceral transplant (MVT), both in adults<sup>(8)</sup> and in children<sup>(9)</sup>. The need for transplantation should be considered in cases of end-stage hepatic disease with failures related to long-term PN, lack of a venous access for continued PN, recurring septic episodes related to the central venous access, and inborn causes predominant in pediatric patients, such as intestinal atresia, intestinal volvulus, and congenital fissures of the abdominal wall.

Surgical treatment for SBS or functional failure of the bowel may be transplant or intestinal elongation. The latter present conflicting results and there are few techniques described<sup>(10-12)</sup>. On the other hand, short-term and long-term results of MVT, with the use of *en-bloc* liver, stomach, duodenum, pancreas, small intestine, and right colon<sup>(13)</sup>, showed significant improvements over the last 10 years<sup>(14)</sup>.

## OBJECTIVE

Bearing in mind the growing investment in research and development of MVT, this study had the goal of

describing the specialization and technical preparation of this surgical modality, still not practiced in Brazil.

## METHODS

Four experimental procedures were conducted in pigs, all performed by the same team, at the same site, and under the same conditions.

### Preoperative phase

The animals, Large White pigs, were submitted to a fluid and solid fasting for 12 hours. A deep intramuscular pre-anesthetic medication (PAM) was given, and after 10 minutes, they were washed and weighed before being taken to the operating room.

Chart 1 describes the anesthetic medications and parameters of the pigs.

**Chart 1.** Anesthetic medications and parameters of the animals

PAM (IM)	1% Acepromazine: 1 mg/Kg Midazolam: 0.2 mg/kg
IV anesthetic agent	Etomidate 2 mg/Kg
Intubation	Endotracheal tube 6.5
Isoflurane	0.5 to 1 %
Tidal volume	10 mL/Kg/h
Respiratory rate	16 rpm

Electrodes and oximeters were placed on the animals for monitoring during the entire surgical procedure.

### Surgical technique on the donor animal of the multivisceral graft

Asepsis was performed with degerming polyvinyl pyrrolidone iodine (PVPI) on the animal's skin, followed by the placement of sterile surgical drapes. Surgery began with a median xipho-pubic incision of the skin, opening of the subcutaneous tissues, hemostasis, and opening of the aponeurosis to visualize the abdominal cavity and inspect organs.

Next, the ligaments between the mesentery and mesocolon were released, then dissection was performed of the infrarenal aorta, vena cava, abdominal esophagus, and suprarenal aorta, with identification of the superior mesenteric artery (SMA) and the celiac trunk.

A furcula-xiphoid incision was made, the sternum was sectioned with a Gigli saw, hemostasis was performed, and a Finochietto retractor was placed, enabling the identification of the inferior vena cava and descending thoracic aorta. The animal received an infusion of 3 mL of heparin. The juxta ileal distal aorta was ligated, catheterized, and clamped, initiating perfusion.

The next step was the infusion of 1000 mL of Custodiol® preservation solution through the catheter of the aorta and, simultaneously, the incision of the inferior vena cava next to the right atrium and placement of sterile saline ground ice in the abdominal cavity. Blood and solution were aspirated.

Next, the esophagus was sectioned and ligated 2 cm from the cardia, followed by sectioning and ligation of the ileum 40 cm away from the ileocecal valve, splenectomy with ligation of the artery and vein abutting the splenic hilum, and the cold removal of the *en-bloc* graft with the stomach, duodenum, pancreas, liver, and intestines. The entire descending aorta to the emergence of the renal arteries was also removed.

The multivisceral graft was placed in 1000 mL of the Custodiol® solution and the skin was sutured with nylon 2-0 thread with a 3-cm curve needle.

### Operating table

Reducing the size of the intestinal graft by removal of a 2.5 meter of the ileum. The graft was reduced to approximate 3.5 m. The aorta was sutured distally to the emergence of the celiac trunk/SMA with prolene 6-0, maintaining the entire proximal thoracoabdominal aorta.

### Surgical technique on recipient of the multivisceral graft

Asepsis was performed with degerming PVPI of the animal's skin, followed by placement of the sterile surgical drapes. The operation was initiated by a median xiphoid-pubic incision of the skin, opening of the subcutaneous tissues, hemostasis, and opening of the aponeurosis and peritoneum, besides visualization of the abdominal cavity and inspection of organs.

Next, the ligaments between mesentery and mesocolon were released; the infrarenal aorta was dissected, followed by the SMA, superior mesentery vein and colic branches. The SMA was catheterized with a number 12 tube for invasive monitoring of the mean arterial pressure (MAP). Then the arteries of the celiac trunk and the SMA itself were ligated and sectioned.

The distal colon was sectioned, as well as the stomach at the level of the gastric fundus. The classic technique of clamping the infra- and supra-hepatic vena cava was performed, with the removal of the liver. Next all the peritoneal organs of the cavity were removed: liver, stomach, duodenum, pancreas, small intestine and colon.

The graft was brought to the operative field (time of cold ischemia: 4 hours and 20 minutes). With prolene 6-0 thread, a continuous suture was made with the following anastomoses:

- end-to-end supra-hepatic vena cava of the graft to the recipient juxta diaphragmatic vena cava (Figure 1);
- end-to-end infra-hepatic vena cava of the graft to the inferior (suprarenal) vena cava of the recipient (Figure 2);
- end-to-side patch of the graft aorta to the recipient infrarenal aorta (Figure 3).

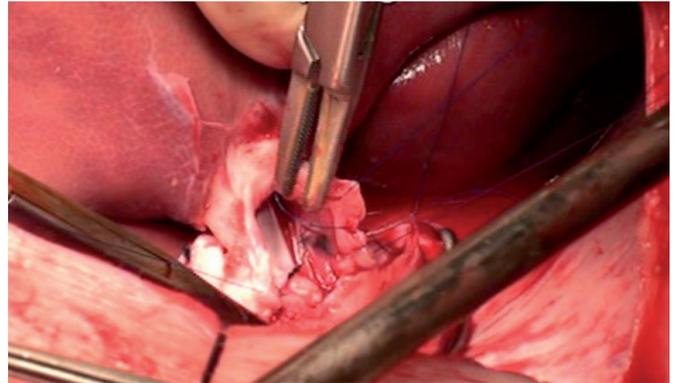


Figure 1. Anastomosis of the suprahepatic vena cava

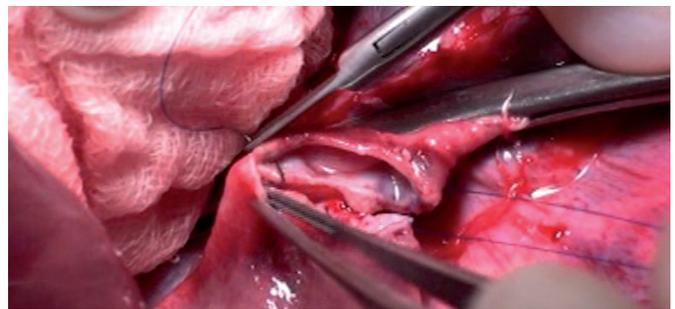


Figure 2. Anastomosis of the infrahepatic vena cava

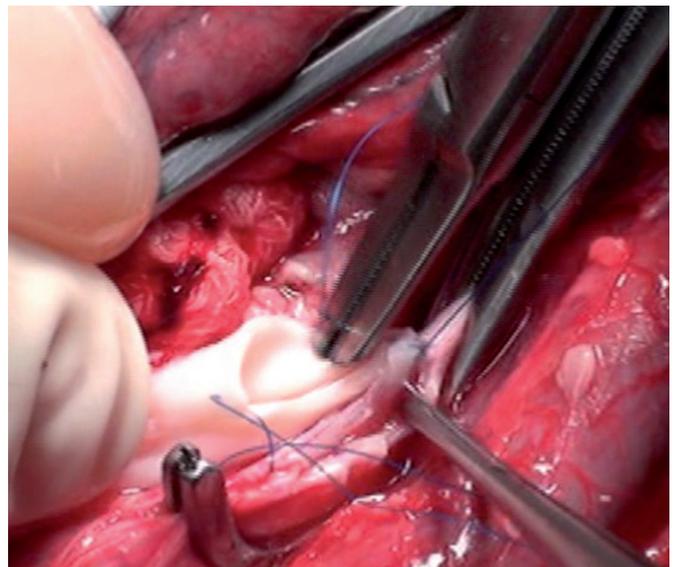


Figure 3. End-to-side anastomosis, patch of the graft aorta to the recipient infrarenal aorta

This was followed by declamping of the vena cava, and then of the aorta, with good revascularization of the graft. Inspection was made of the anastomoses, observing a point of leakage of the aorta anastomosis, which was repaired with a prolene 6-0 stitch.

The aponeurosis and peritoneum were sutured on a single continuous plan with prolene 2-0 and the skin was closed with nylon 2-0.

This project was approved by the Research Ethics Committee of the Hospital Israelita Albert Einstein (HIAE), under CEP number 534-08.

## RESULTS

The multivisceral transplant was possible in all four animals. The graft progressed with good perfusion (Figure 4), but the animals experienced tachycardia and signs of refractory hypotension (invasive MAP = 45 mmHg). Following the infusion of 2 liters of warm Ringer Lactate and 10 mL of an 8.4% sodium bicarbonate solution, there was improvement in hypotension and perfusion of the intestinal graft (invasive MAP = 63 mmHg). Five milliliters of intravenous Liquemin® were applied.

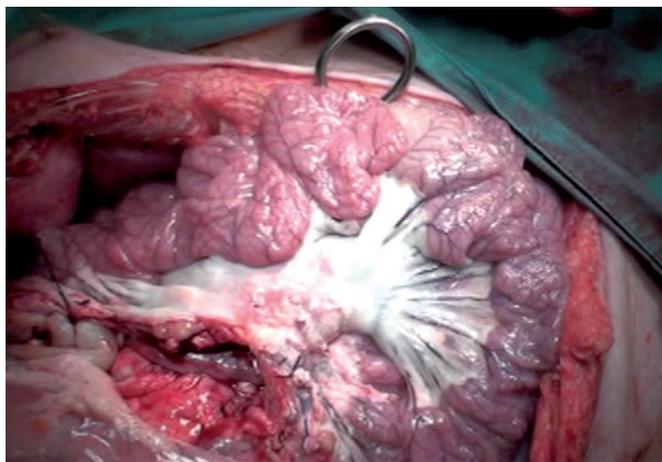


Figure 4. Reperfusion of the multivisceral graft

The animals displayed hypotension with the need for high doses of vasoactive drugs, and all were sacrificed after discontinuing these drugs.

## DISCUSSION

In the four procedures performed, there were no cases of animal survival, with death related to the ischemia-reperfusion syndrome resulting in refractory hypotension, despite flushing the graft. Depending on the intensity and time of ischemia, when oxygen is reestablished to the tissues, the damage caused by

hypoxia may be aggravated (oxygen paradox)<sup>(15)</sup>. The animals presented with hypotension requiring high doses of vasoactive drugs, and all were euthanized with discontinuation of these drugs.

Ischemia and reperfusion of the small intestine cause rupture of the mucous barrier, bacterial translocation, and activation of the inflammatory response<sup>(16)</sup>, as well as disturbances in acid-base and hydroelectrolytic balances<sup>(17)</sup>. Another factor that induces intestinal lesion after reperfusion is the generation of free radical of oxygen molecules<sup>(18)</sup>, derived from mitochondrial electron transport chains, xanthine oxidase, metabolism of endothelial cells, prostaglandins and activated neutrophils<sup>(19)</sup>.

Some alternatives to increase animal survival would be use of an additional vasoactive drug, use of a third pig merely for blood transfusion, presence of an anesthesia team in the operating room, and reduction of the graft. The use of vasoactive drugs to inhibit refractory hypotension is widely discussed in literature, especially in newborns and in situations of sepsis<sup>(20,21)</sup>. Its administration is based on the vasoconstrictive property of the drugs, which results in an increase of peripheral resistance and consequent increase of blood pressure (BP). Another form of preventing and reversing hypotension is fluid replacement. For this, a third pig could be used, merely as a donor for blood transfusions.

The presence of an anesthesia team during the operations provided great benefits in stabilizing the animal's BP. The exclusive dedication of a team and hemodynamic monitoring of the pig is indispensable to minimize the effects of the ischemia-reperfusion syndrome.

## CONCLUSION

Multivisceral transplantation was possible in four animals. Refractory hypotension occurred in all animals, which then progressed to death. Some alternatives to minimize ischemia-reperfusion syndrome, such as the use of one more vasoactive drug, the use of a third pig merely for blood transfusion, the presence an anesthesia team in the operating room, and reduction of the graft are the next steps to be taken to enable experimental studies.

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