Annals of Gastroenterology (2012) **25,** 147-161

Surgical text for orthotopic liver transplantation model with small-for-size graft in the pig: key techniques and pitfalls

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Abstract

Background Challenges with small-for-size grafts are a critical issue in the liver transplantation field, and a reliable and reproducible animal model is required.

Method We performed 50 orthotopic liver transplantations in pigs with a 30% graft, and retrospectively investigated the learning curves. We modified our surgical procedures according to our experience. Here, we describe our current procedures in detail with retrospective evaluation of our experience. The artery to the right lateral lobe crosses the portal vein trunk. A 30% graft is taken using the right lateral lobe attached to a sufficient length of aorta. Hepatic venous plasty is undertaken on the back table to attach a venous patch to the anterior wall of the suprahepatic inferior vena cava, which has no extrahepatic margin. To minimize hypoperfusion to the digestive tract, an aorta-to-aorta anastomosis is performed in a side-to-end fashion in a minimal surgical field before suprahepatic inferior vena cava and portal vein reconstruction. A temporary transjugular portosystemic shunt is also inserted before suprahepatic inferior vena cava reconstruction. The recipient suprahepatic inferior vena cava is clamped at the intramediastinal level, including the margins of the diaphragm in the clamp.

Results Although survival rate during first forty cases were under 0.2, a reasonable survival rate of 0.6 had been achieved after the experiences of forty cases.

Conclusion Precedent arterial reconstruction using an aorta-to-aorta anastomosis minimizes congestive damage and shortens operative time. Hepatic venous reconstruction should be completed without any outflow block, by using venous plasty and adequate clamping.

Keywords porcine model, pig, split liver, small-for-size graft, surgical technique

Ann Gastroenterol 2012; 25 (2): 147-161

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Conflict of Interest: None

Financial disclosures: This work was supported by grants to T. Hori from the Japan Society for the Promotion of Science (No. C20591523) and from the Uehara Memorial Foundation, Tokyo, Japan (No. 200940051)

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Received 20 July 2011; accepted 6 February 2012

Introduction

Clinically, liver transplantation (LT) is made possible by the phenomenon of immunological tolerance after allograft transplantation and the ability of the liver to regenerate even after initial insufficient volume. Use of experimental animal models has given new insights into LT procedures. Rodent models of orthotopic LT (OLT) have been established, and samples from rodents are particularly suitable for laboratory assays due to the growing availability of gene-altered or knockout animals and the development of specific agents and antibodies. However, OLT is technically difficult in rodents, even when reconstruction of the hepatic artery is omitted. OLT models are also available in larger animals such as dogs [1,2] and pigs [3,4], and these can provide clinically relevant and reliable data.

Transplantation of small-for-size (SFS) grafts is a critically important procedure used in living donor LT to enhance donor safety in Japan, as well as in cadaveric donor LT because of donor shortages in the United States and Europe. The main focus of study in the liver regeneration field is patients with SFS syndrome and liver reperfusion injury. Reliable and reproducible models of split OLT (SOLT) are important for undertaking clinically relevant studies [5]. Porcine OLT with a 30% graft is an important model for SOLT with SFS graft.

Pseudo models of fake OLT/SOLT, such as temporary clamping or simple hepatectomy, are still used experimentally for assessing reperfusion injury and/or SFS syndrome [6,7], because the LT model demands more advanced skills and experience [5,8]. Cold ischemic time (CIT) is a critical factor in producing reliable data using the LT model, and plays an important role in the mechanisms of reperfusion injury and SFS syndrome [5]. Data obtained from pseudo models are not clinically relevant, and should not be translated into the LT field [5]. The development of clinically relevant SOLT models is more useful in the study of liver regeneration [9]. In this paper, we describe the detailed surgical procedures of our SOLT model in the pig. Furthermore, we discuss the key points and pitfalls of porcine SOLT models, based on our experience.

Materials and Methods

Animals

Outbred female Yorkshire pigs with body weight of approximately 30 kg are kept in the animal facilities of Mie University for one week before surgery (Fig. 1A). This size is chosen because general anesthesia is technically difficult in a smaller swine, and the strength of a larger swine increases the difficulty of postoperative care. All experimental protocols have been approved by the ethics committee of the Mie University Graduate School of Medicine. Animal handling and care meet the requirements of our institutional guidelines for animal welfare.

Sedation, intravenous access, intubation and general anesthesia

Each pig is starved for 12 h before surgery, though enough water is provided to prevent dehydration. The animal is then sedated with ketamine hydrochloride (150 mg) and atropine sulfate (1 mg) via deep intramuscular injection with an 18-gauge needle. Once sedated, the pig is weighed and placed in the supine position (Fig. 1B), and an electrocardiography monitor is attached.

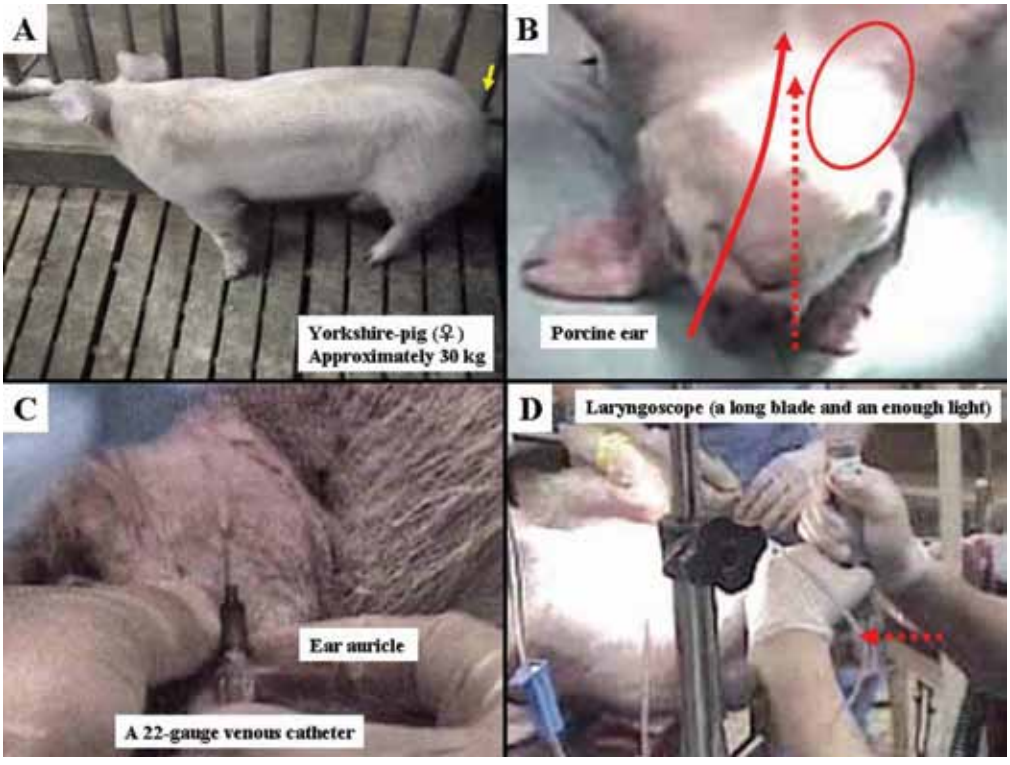

Figure 1 (A) We used female Yorkshire pigs weighing approximately 30 kg. The tail is not curled yet (arrow). (B) The pig is placed in the supine position. The neck should be completely straight during intubation. In the case shown, the cervicothoracic spine is curved (solid arrow), and should be moved so that it is straight (dotted arrow). Venous and arterial cannulas are inserted in the neck (circle). (C) Initial intravenous access is established via an ear vein with a 22-gauge cannula. (D) A long-bladed laryngoscope and a good light source are used for intubation. The neck should be straight (arrow)

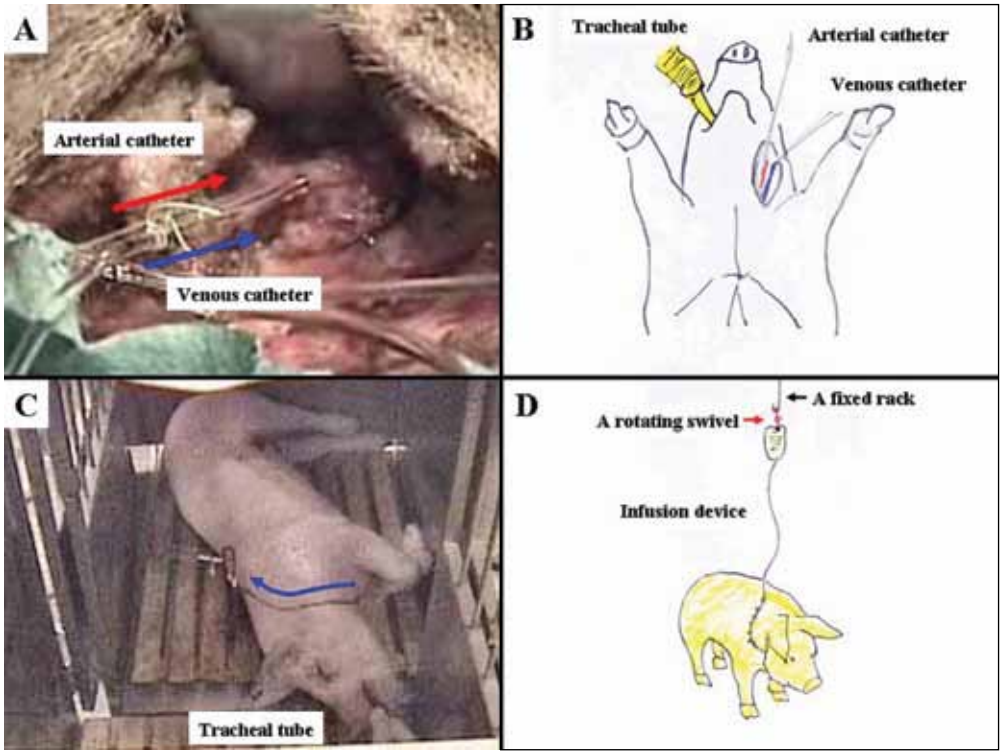

Figure 2 (A) An intravenous line is placed in the external jugular vein (blue arrow) and an arterial line is placed in the carotid artery (red arrow). (B) The pig is placed in the supine position with positive pressure mechanical ventilation, central venous cannulation and arterial monitoring. (C) The recipient pig is placed in a cage with temporary clamping of the cannulas at the first signs of recovery. The cannulas are fixed to the back (blue arrow). (D) The length of infusion tubing should be carefully adjusted. A rotating swivel (red arrow) protects against twisting of the tubing

Intravenous access is established via an ear vein using a 22-gauge cannula (Fig. 1C). This access is well protected due to its importance for subsequent procedures, including the induction of general anesthesia, until central venous access is available.

Intravenous 5% phenobarbital is administered (2 mL). Due to the long distance between the snout of the pig and the trachea, a long laryngoscope blade (at least 20 cm) may be required together with a good light source, though a Macintosh laryngoscope can sometimes be used. The neck should be straight to increase ease of vocal cord visualization (Fig. 1D). After intubation, positive pressure mechanical ventilation is started and general anesthesia is maintained with oxygen, nitrous oxide and isoflurane inhalation. Intravenous muscle relaxant (vecuronium bromide, 2 mg) and opioid analgesic (buprenorphine, 0.4 mg) are administered hourly. Blood gas analysis and blood cell count are undertaken regularly (iSTAT, Abbott Laboratories, Inc., Princeton, NJ, USA). Metabolic acidosis is corrected with bicarbonate as needed.

The carotid artery is identified between the internal and external jugular veins. Central venous access for adequate fluid administration and real-time monitoring of hemodynamic state is indispensable in this model. An intravenous cannula is placed in the external jugular vein and an arterial cannula is placed in the carotid artery (Fig. 2A). This completes preoperative preparation of the pig, with supine positioning, positive pressure mechanical ventilation, central venous cannulation and arterial monitoring (Fig. 2B). Before donor surgery, blood is collected from the recipient pig with close monitoring, in case transfusion is required during the recipient operation. A transfusion bag containing acid citrate dextrose is used. Collection of 400-600 mL is usually possible with maintenance of hemodynamic stability.

After closure of the abdominal wall in the recipient, atropine sulfate (1 mg) and neostigmine (4 mg) are administered intravenously. A warmer is used immediately after surgery, because mild hypothermia is always present. To prevent problems due to pig behavior during recovery from anesthesia, the recipient pig is placed in a cage with catheters temporarily clamped at the first signs of recovery (Fig. 2C). Once return to a normal level of consciousness is confirmed, the pig is weaned from ventilation and fluids are administered.

Technical modifications of surgical procedures in the porcine SOLT model with a 30% graft

In our first series, we performed a 70% hepatectomy in 40 pigs [10]. Subsequently, we performed 50 cases of porcine SOLT. During these 50 cases, we modified our surgical procedures

according to our experience. Here, we describe our current surgical procedures with retrospective evaluation of our experience. Porcine OLT model has been performed in many laboratories since the beginning of the nineties [11-13], and their reports state that groups of 6 or 7 subjects are sufficient in order to learn this model. We retrospectively evaluated our learning curves for successful OLT with 30%-SFS graft.

The piggy-back technique is difficult in the porcine OLT [14], although few researchers reported this technique in the pig [15].

Postoperative care of the recipient

As the pig moves around a lot inside the cage, the length of infusion tubing should be carefully adjusted. A rotating swivel system is useful to protect against twisting of the tubing (Fig. 2D). Intermittent intravenous administration of antibiotics (cefmetazole, 1 g/100 mL every 8 h) and analgesics (buprenorphine, 0.4 mg every 6 h) is important for a successful outcome. Administration of an intravenous histamine-2 receptor antagonist or proton pump inhibitor is also important, because a stressed pig may easily develop a lethal peptic ulcer.

Anatomy of the porcine liver

The porcine liver is divided into four lobes: right medial lobe (RML), right lateral lobe (RLL), left medial lobe (LML) and left lateral lobe (LLL). The RLL accounts for 30% of the liver volume. The gallbladder is adjacent to the RML. The suprahepatic inferior vena cava (SHIVC) is completely covered by liver parenchyma and diaphragm, and therefore has no extrahepatic margins (Fig. 3). The infrahepatic inferior vena cava (IHIVC) extends into the RLL. There are no structures behind the liver except for a thin membrane. The typical appearance of the hepatic hilus is shown in Fig. 4. The bilateral hepatic ducts join to form the common bile duct (CBD). The portal vein (PV) bifurcates into left and right branches. The common hepatic artery (CHA) divides into the gastroduodenal artery and the branches for each lobe. The artery to the RLL is located separately on the PV trunk. The right gastric artery arises from the artery to the LLL.

Donor operation

The abdomen is prepared and draped in a sterile fashion and a long midline skin incision is made (Fig. 5A). Care is taken to avoid damage to the liver, digestive tract, bladder and urethra during laparotomy. The xiphoid process of the sternum is exteriorized and may have to be removed to improve the surgical field (Fig. 5B). Homeostasis is achieved, especially around the umbilicus and the xiphoid process. An adequate surgical field is exposed using retractors to hold the bilateral

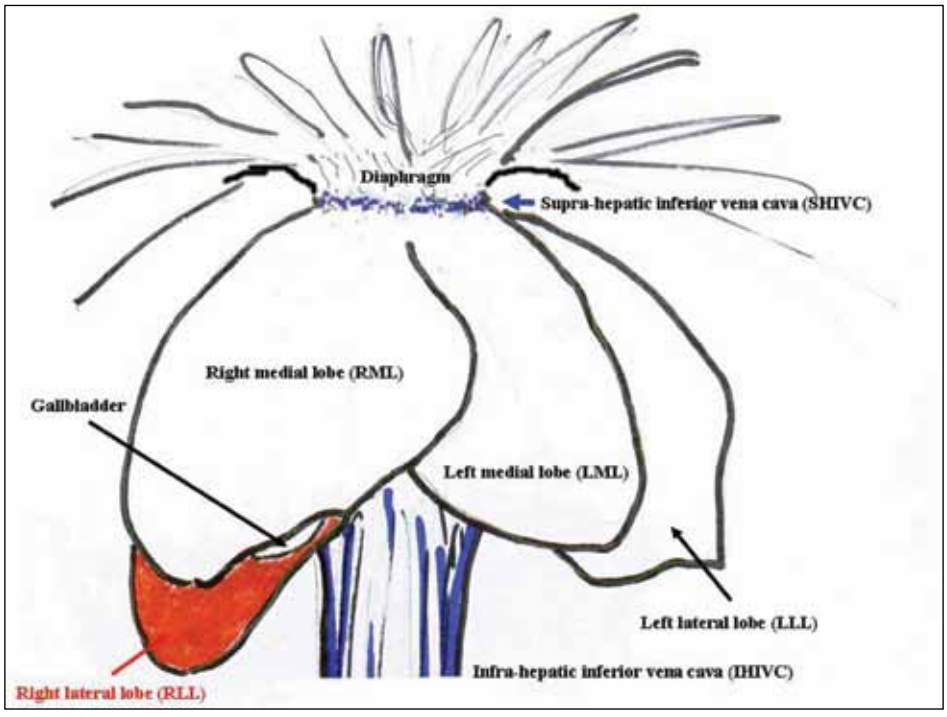

Figure 3 The liver is divided into four lobes: RML, RLL, LML and LLL. The 30% graft is harvested using the RLL (red). The SHIVC has no extra-hepatic margins (blue arrow)
IHIVC, infrahepatic inferior vena cava; LLL, left lateral lobe; LML, left median lobe; RLL, right lateral lobe; RML, right medial lobe; SHIVC, suprahepatic inferior vena cava

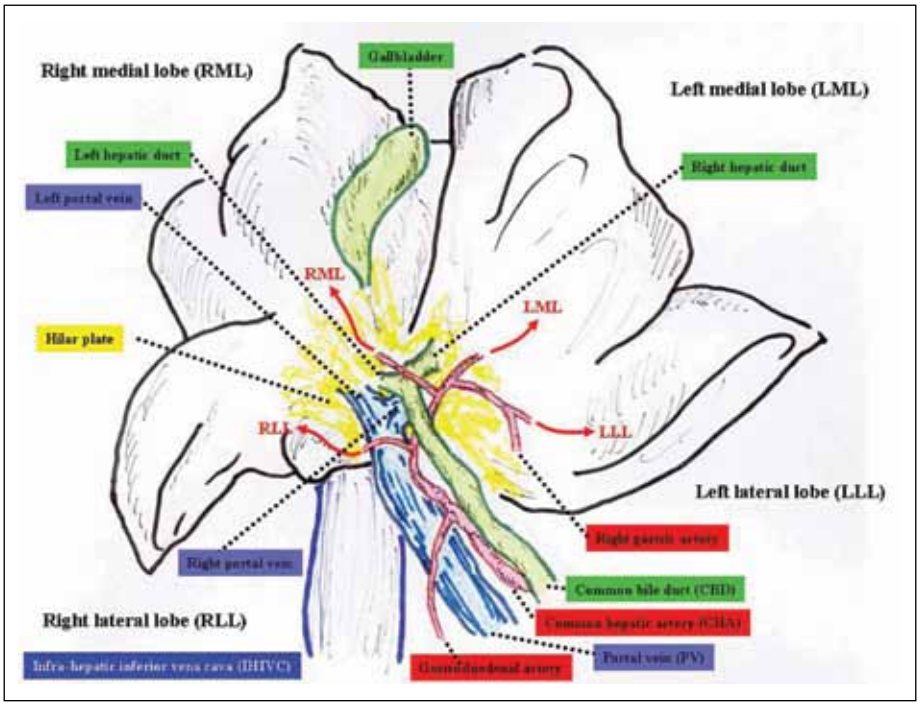

Figure 4 Typical appearance of the hepatic hilus of the pig. The arterial branch to the RLL is located separately on the PV trunk, and the right portal vein supplies portal flow to the RLL
CBD, common bile duct; CHA, common hepatic artery; IHIVC, infrahepatic inferior vena cava; LLL, left lateral lobe; LML, left medial lobe; PV, portal vein; RLL, right lateral lobe; RML, right medial lobe

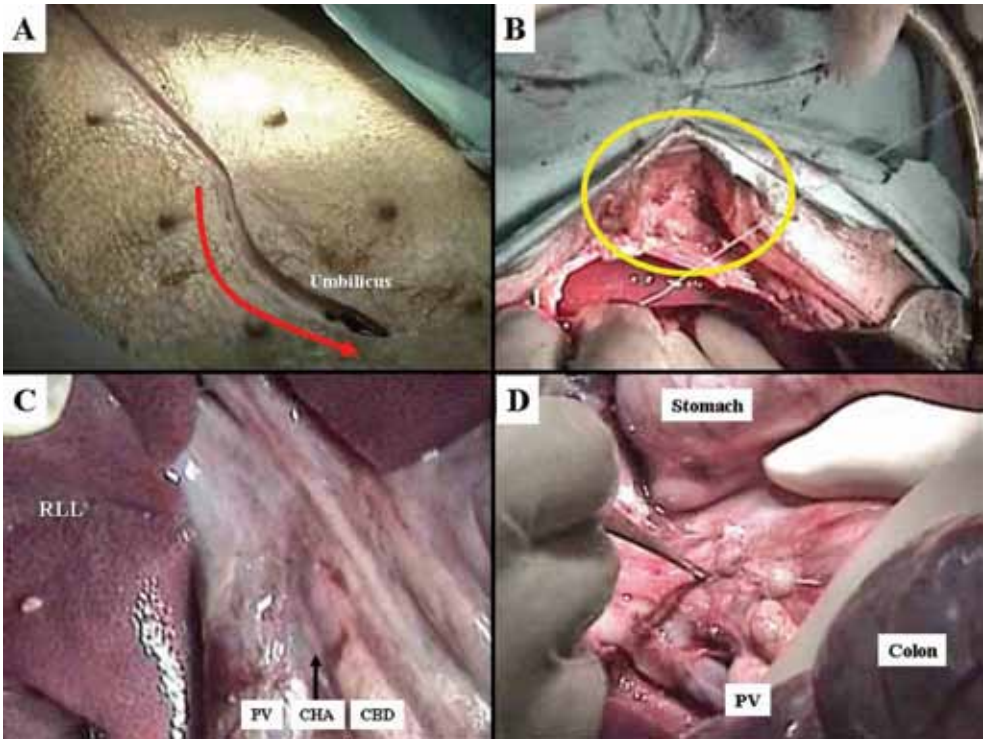

Figure 5 (A) A long midline skin incision is made (arrow). The areas around the umbilicus and the xiphoid process bleed easily. (B) The xiphoid process of the sternum can be removed if required (circle). (C) View of the hepatic hilus. (D) The hepatoduodenal and hepatogastric ligaments are cut
CBD, common bile duct; CHA, common hepatic artery; PV, portal vein

costal margins aside.

The appearance of the hepatic hilus is shown in Fig. 5C. The hepatoduodenal and hepatogastric ligaments are sharply cut by a pinch-and-burn cutting technique (Fig. 5D), and the right gastric artery arising from the artery to the LLL is ligated. The CBD is skeletonized and cut, and a stent tube is inserted. The stent tube is fixed in place with a suture, and bile discharge is observed (Fig. 6A). The periportal lymph nodes are removed (Fig. 6B), and the PV and CHA are skeletonized (Fig. 6C). The PV and CHA should be adequately separated from one another. The PV trunk includes the splenic vein and three or four branches of the superior mesenteric vein at the pancreas. The hepatic artery is dissected away from surrounding tissues from the hepatic hilus to the root of celiac artery, including the dense connective tissue around the celiac and superior mesenteric arteries. The artery to the RLL is carefully identified, and the proper hepatic artery is ligated distal to the artery to the RLL. This immediately changes the color of the liver except for the RLL (Fig. 6D). The left PV is completely skeletonized (Fig. 7A), and is clamped with a Pott's clamp (Fig. 7B). This increases the change in liver color. The left PV of the graft is closed with a continuous bilateral retention suture using 6-0 monofilament polypropylene suture (MPS) (Fig. 7C). Glisson's capsules for the RML, LML and LLL are dissected and ligated *en bloc* (Fig. 7D). Hepatic resection is started at the hepatic margin near the SHIVC (Fig. 8A), and vessels and ducts crossing the line of transection are carefully ligated (Fig. 8B). Because bleeding or oozing from the cut surface after graft recirculation is a potentially fatal problem, hemostasis is carefully confirmed (Fig. 8C). An electrocautery scalpel and bipolar forceps with saline irrigation are very useful for ensuring hemostasis. The transparent membranes around the liver, which fix each lobe to the surrounding organs, are cut, and the retroperitoneum is incised to the left of the aorta and to the right of the IHIVC (Fig. 8D). The abdominal aorta is dissected from above the celiac artery to the common iliac artery (Fig. 9A). The renal arteries and superior and inferior mesenteric arteries are ligated. The IHIVC is dissected to below the right renal vein, and both renal veins are ligated (Fig. 9B). The right adrenal gland is removed from the IHIVC later on the back table. Identification of the vessels to the right adrenal gland at this time is often risky. With adequate retraction, the dorsally located lumbar branches of the aorta and IHIVC are identified (Fig. 9C). Immediately before the graft is harvested, it remains attached only by vessels and the diaphragm (Fig. 9D).

Intravenous heparin (3000 IU) is administered before graft harvesting. The abdominal aorta is ligated above the celiac artery, and a tube is inserted into the common iliac artery and fixed in place with a suture. Another tube is inserted into the PV trunk as proximally as possible, and is also fixed in place with a suture. Perfusion of the graft via both tubes is started *in situ*, using cold preservation solution (histidine-tryptophan-ketoglutarate solution, 4°C) (Fig. 10A). The IHIVC

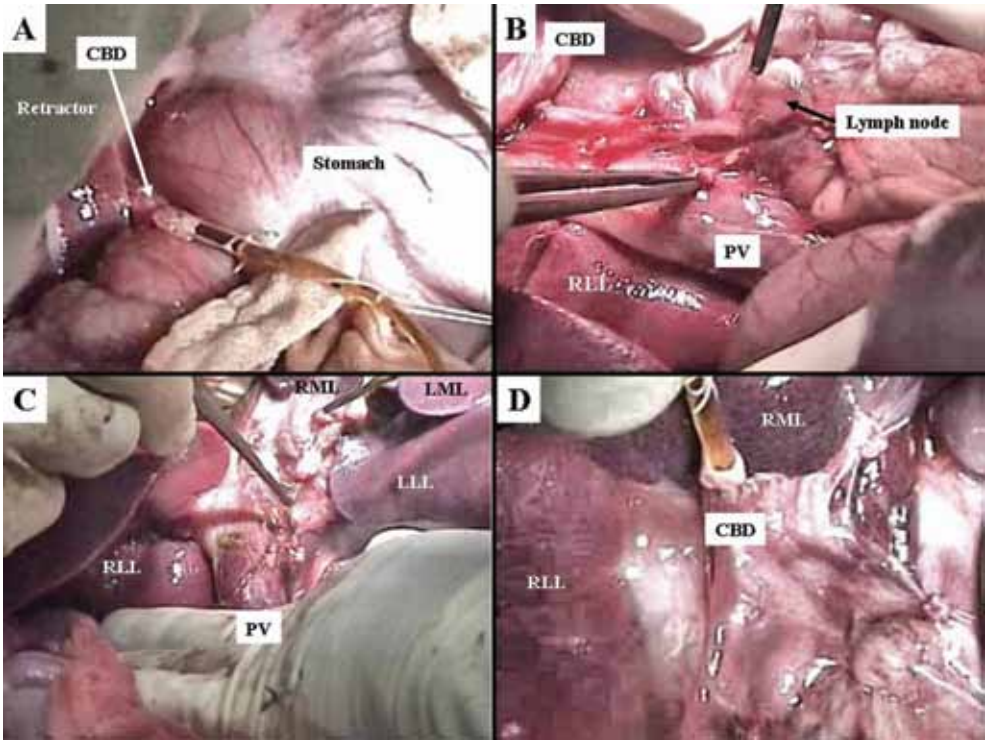

Figure 6 (A) The CBD is cut, and the stent tube is inserted. Bile discharge is observed. (B) Periportal lymph nodes are removed. (C) The PV and CHA are skeletonized. (D) The proper hepatic artery is ligated distal to the branch to the RLL. Liver color changes immediately, except for the RLL

CBD, common bile duct; LLL, left lateral lobe; LML, left median lobe; PV, portal vein; RLL, right lateral lobe; RML, right medial lobe

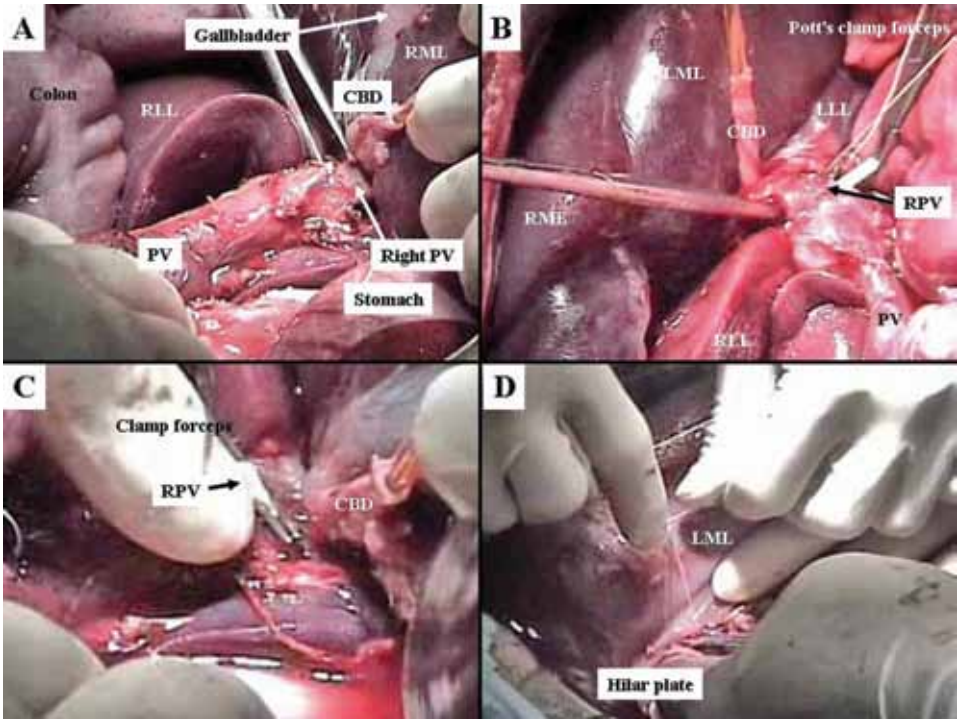

Figure 7 (A) The left PV is completely skeletonized. (B) The left PV is clamped with a Pott's clamp. The difference in liver color becomes more marked. (C) Forceps are tunneled behind the left PV. The left PV of the graft is closed with a continuous suture under the bilateral retention suture. (D) Glisson's capsules for the RML, LML and LLL are dissected and ligated *en bloc*
CBD, common bile duct; LLL, oleft lateral lobe; LML, left medial lobe; PV, portal vein; RLL, right lateral lobe; RML, right medial lobe

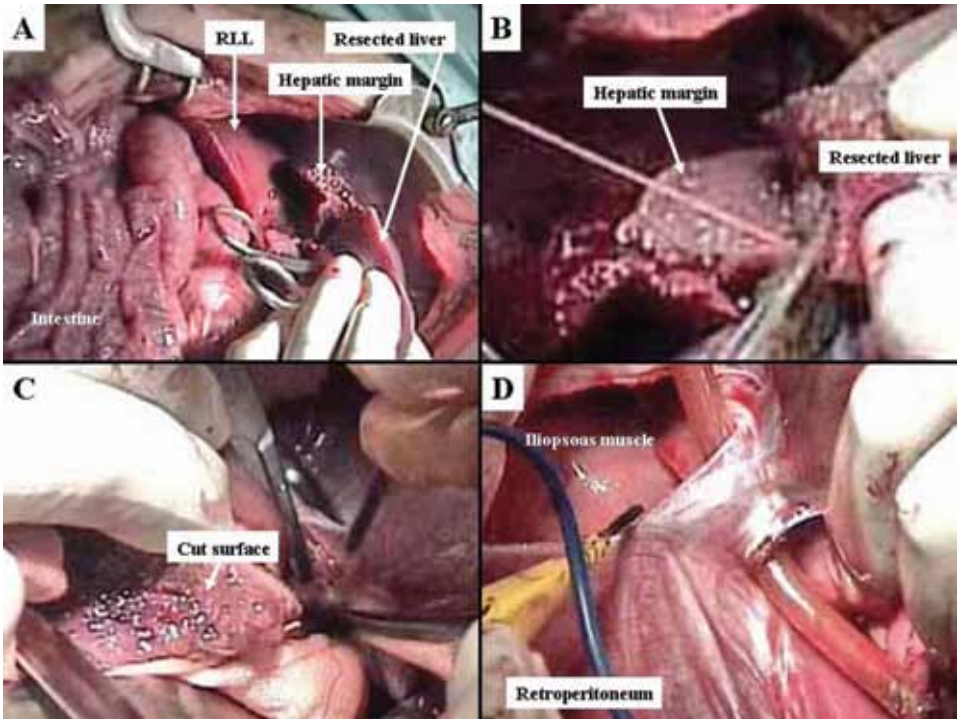

Figure 8 (A) Hepatic resection is started at the hepatic margin near the SHIVC. (B) Vessels and ducts crossing the line of transection are ligated. (C) Hemostasis of the cut surface is carefully confirmed. (D) The retroperitoneum is incised to the left of the aorta and to the right of the IHIVC
IHIVC, infrahepatic inferior vena cava; RLL, right lateral lobe

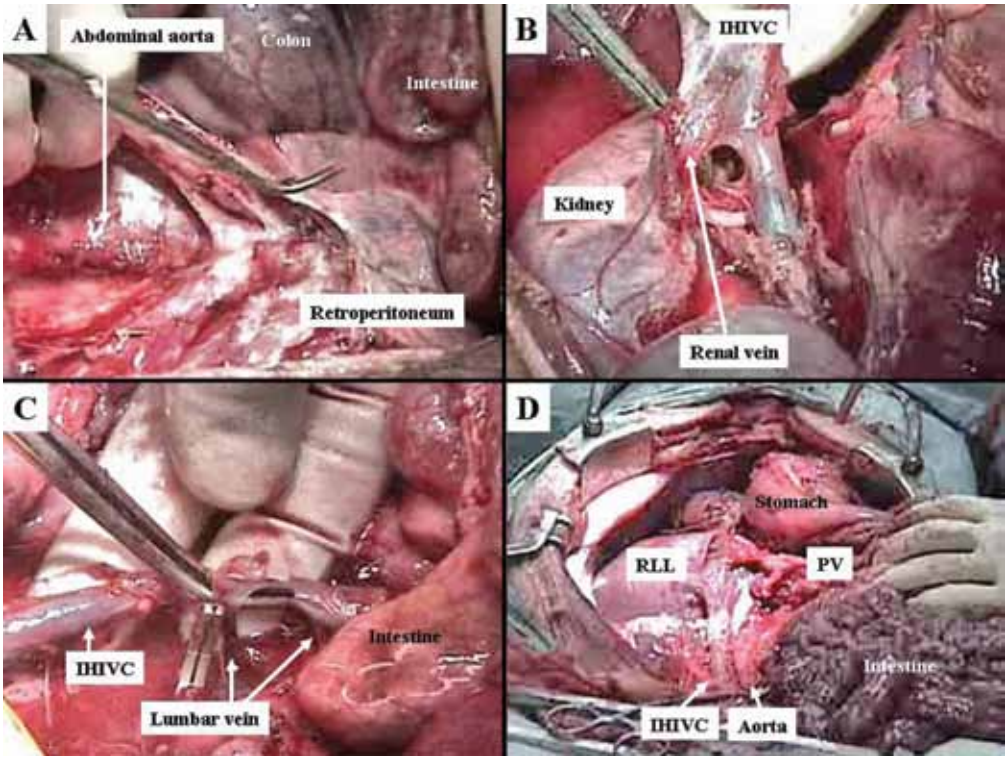

Figure 9 (A) The abdominal aorta is dissected from above the celiac artery to the common iliac artery. (B) The IHIVC is dissected to below the left renal vein. (C) Lumbar branches are identified with retraction of the aorta and IHIVC. (D) The donor graft is only attached by vessels and the diaphragm
IHIVC, infrahepatic inferior vena cava; PV, portal vein; RLL, right lateral lobe

is cut below the left renal vein. The thoracic cavity is opened, and the intramediastinal IVC is cut. The 30% graft with the attached abdominal aorta is then harvested (Fig. 10B).

Back table procedure

The graft is transferred to a basin of crushed ice covered by an isolation bag. Under perfusion on the back table, the graft is carefully examined for any obvious injuries (Fig. 10C). Adequate repair is undertaken of any detected injury. The closures of the portal and arterial branches are also examined. The right adrenal gland is removed from the IHIVC, and the arterial and venous branches to the right adrenal gland are carefully ligated using 6-0 MPS (Fig. 10D). After perfusion with a total of 1000-1200 mL (at least 3 mL/g of graft weight), the graft is weighed. Leakage tests are performed for the PV, IHIVC (Fig. 11A) and aorta (Fig. 11B). The bifurcation of the aorta is cut in a branch patch-fashion using the bilateral common iliac arteries. The anterior and posterior diaphragm is completely trimmed from the SHIVC. A venous patch is added to the anterior wall of the SHIVC, because the SHIVC has no extrahepatic margins. A continuous 5-0 MPS suture is placed beneath the bilateral retention suture for patch attachment. There are usually bilateral inferior phrenic veins, and these are sutured closed (6-0 MPS). These sutures become the marker for graft placement before SHIVC reconstruction. From the inside of each hepatic vein, other extra-hepatic branches are identified (Fig. 11C). Closure of these small branches is very important for the prevention of massive hemorrhage immediately after graft reperfusion. The graft is then preserved until implantation (Fig. 11D), with the preservation solution and CIT selected based on the aims of the individual experiment.

Recipient operation

Induction of anesthesia and laparotomy are the same in the recipient operation as in the donor operation. The procedures for placement of the venous and arterial cannulas are also the same, and the venous cannula is tunneled subcutaneously to exit at the back of the neck to prevent postoperative removal of the catheter by a pig foot. This line can also be used for blood sampling.

An adequate surgical field is easily achieved by retracting the digestive tract (Fig. 12A). This retraction sometimes causes congestion and inadequate perfusion in the retracted organs (Fig. 12B). In the recipient operation, surgeons intensively take care of all organs, to ensure survival. To avoid hypoperfusion to the digestive tract, an aorta-to-aorta anastomosis is performed in a side-to-end fashion in the minimal surgical field available

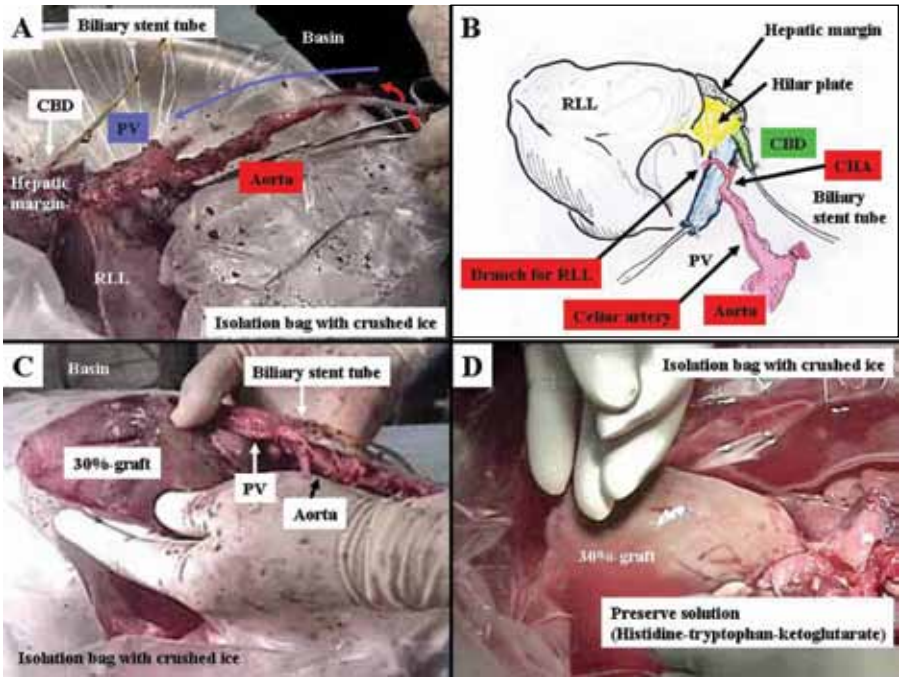

Figure 10 (A) Graft perfusion with cold preservation solution is started immediately via the PV (blue arrow) and aorta (red arrow). (B) Schematic diagram of the 30% graft with the attached abdominal aorta. (C) The graft is carefully examined to identify any obvious injuries. (D) Repair of any detected graft injuries is undertaken. The right adrenal gland is removed from the IHIVC, and arterial and venous branches to the right adrenal gland are ligated
CBD, common bile duct; CHA, common hepatic artery; PV, portal vein; RLL, right lateral lobe

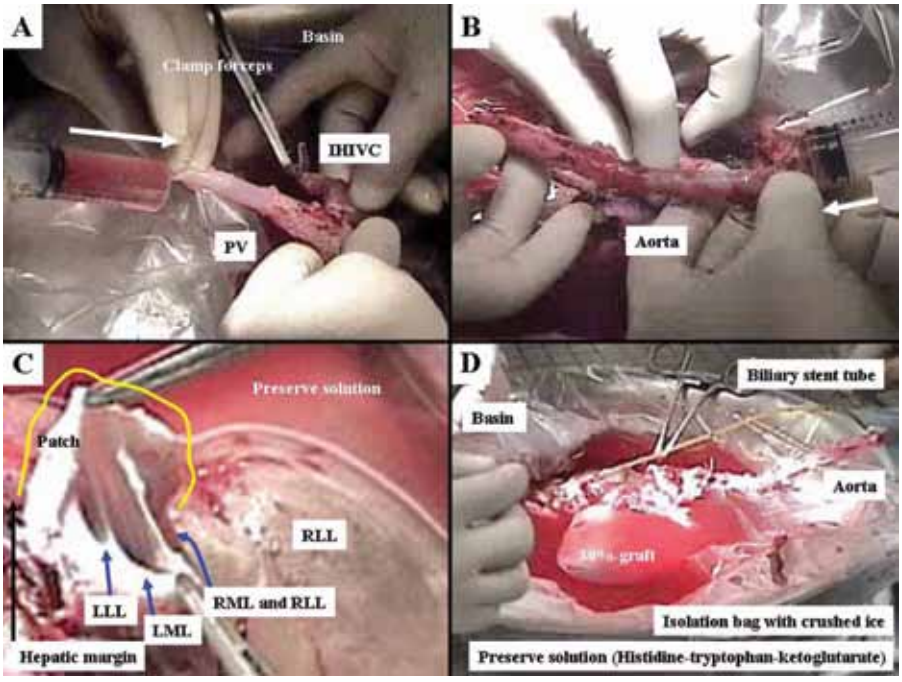

Figure 11 (A) Leakage tests are performed for the PV and IHIVC under pressure (arrow). (B) Leakage test for the aorta is performed under pressure (arrow). (C) A venous patch (yellow line) is sutured to the anterior wall of the SHIVC using 5-0 MPS. The inferior phrenic veins are carefully skeletonized and ligated. From inside each hepatic vein (blue arrow), other small branches are identified. (D) The graft is preserved until implantation
IHIVC, infrahepatic inferior vena cava; LLL, left lateral lobe; LML, left medial lobe; MPS, monofilament polypropylene suture; PV, portal vein; RLL, right lateral lobe; RML, right medial lobe; SHIVC, suprahepatic inferior vena cava

when using flexible retractors (Fig. 12C). The recipient aorta is partially dissected without injuring the para-aortic lymphatic duct, and partially clamped. An opening is made in the anterior wall of the recipient aorta and stay sutures are placed bilaterally (Fig. 12D). An aorta-to-aorta anastomosis is performed in a side-to-end fashion using a continuous suture (5-0 MPS) (Fig. 13A). This procedure maintains perfusion to the digestive tract (Fig. 13B), and drastically shortens the operative time compared with the microsurgical procedures required for hepatic artery reconstruction. After clamping of the graft aorta, the partial clamp on the recipient aorta is released.

Although the procedures used to mobilize the whole liver are basically the same as for the donor operation, the vessels are cut at different locations. The PV, hepatic artery and CBD are cut as close to the hepatic hilus as possible. The IHIVC is cut as close to the RLL as possible. The SHIVC is cut at the edge of the liver parenchyma. To maintain perfusion to the retraced organs, skeletonization and dissection of the PV, CBD, CHA, IHIVC and SHIVC are completed with as little retraction as possible (Fig. 13C). Intravenous heparin (1,000 IU) is administered. After 3 min, any clot is removed with gauze, and the peritoneal cavity is cleaned. A careful check for bleeding points is undertaken, as hemostasis of bleeding points after liver implantation is very difficult. An antithrombotic- and antibiotic-coated tube is prepared (Fig. 13D) and is sutured to the PV trunk and the jugular vein to form a temporary transjugular portosystemic shunt. An anhepatic phase is then started. The HA is ligated, the IHIVC and SHIVC are clamped, and the native liver is removed (Fig. 14A). Systolic arterial pressure usually decreases 20-30 mmHg at this time.

During liver removal, the IHIVC and SHIVC clamps should be carefully placed, especially the SHIVC clamp. In the recipient operation, the Satinsky clamp should be placed on the intramediastinal IVC making sure to grasp sufficient margins of the diaphragm in the clamp, because the SHIVC does not have a sufficient extrahepatic margin for anastomosis (Fig. 14A-D). Ventrally- and caudally-directed retraction of the Satinsky clamp exposes a good surgical field for anastomosis. The graft SHIVC is placed in position with correct axial alignment using the hepatic margin as a marker (Fig. 14B, 14D, 15A). Each lobe has its own drainage vein near the level of the SHIVC, and the vein from the RLL lies in a common channel with the vein from the RML (Fig. 14C). Stay sutures are placed bilaterally (Fig. 15A, 15B). The posterior wall of the graft SHIVC lies straight and the anterior wall is curved (Fig. 15A). The first suture is placed from the outside of the graft SHIVC to the inside, and the posterior wall is sutured from the left side with a continuous suture (Fig. 15B). The graft SHIVC suture bites may include liver parenchyma. Secure posterior suturing is important, because hemostasis of any bleeding points in this area is impossible after graft reperfusion. The last suture is tied on the outside of the recipient SHIVC, and this thread is tied to a stay suture from

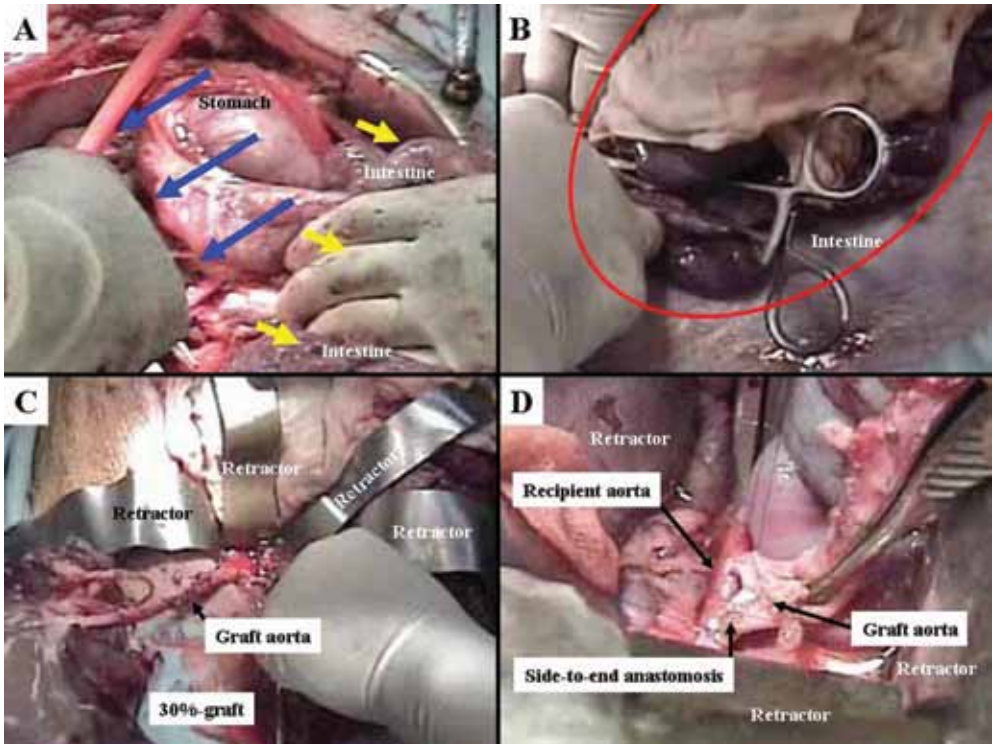

Figure 12 (A) An adequate surgical field (blue arrow) is easily achieved using optimal retraction of the digestive tract (yellow arrow). (B) Retraction sometimes results in hypoperfusion and congestion of the shifted organs (circle). (C) A side-to-end anastomosis is performed between the aortas in a minimal surgical field using flexible retractors. (D) Stay sutures are placed bilaterally

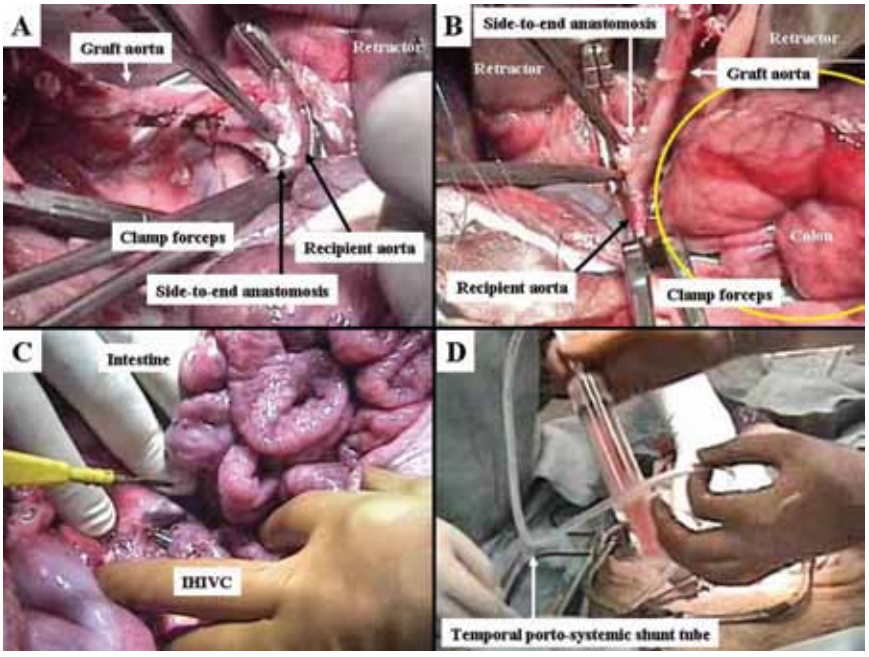

Figure 13 (A) An aorta-to-aorta anastomosis is performed in a side-to-end fashion using a continuous suture. Suturing on the left side is shown. (B) Suturing of the aortic anastomosis on the right side is shown. Perfusion to the digestive tracts is maintained (circle). (C) To maintain perfusion to the shifted organs, surgical procedures before the anhepatic phase are completed with the minimal possible retraction. (D) Temporary portosystemic shunt tubing is prepared
IHIVC, infrahepatic inferior vena cava

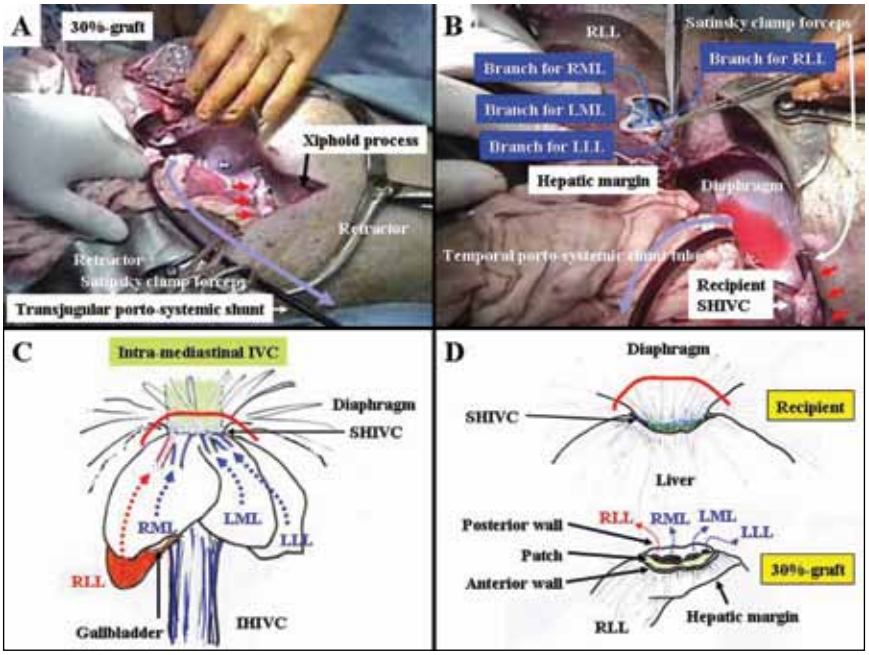

Figure 14 (A) Transjugular portosystemic shunt is placed (purple arrow). (B) Transjugular portosystemic shunt is in place (purple arrow). In the recipient pig, the SHIVC clamp is placed on the intramediastinal IVC and should grasp sufficient margins of the diaphragm (red arrow). Each lobe has its own drainage vein near the level of the SHIVC (light blue arrows). (C) Each lobe has its own drainage vein near the level of the SHIVC. The vein from the RLL lies in a common channel with the vein from the RML. The SHIVC does not have enough extrahepatic margin for anastomosis. In the recipient pig, the SHIVC clamp is placed on the intramediastinal IVC and should grasp sufficient margins of diaphragm (red line). (D) The SHIVC does not have an enough extrahepatic margin for anastomosis (red line). The graft SHIVC is placed in position with correct axial alignment using the hepatic margin as a guide
IVC, inferior vena cava; IHIVC, infrahepatic inferior vena cava; LLL, left lateral lobe; LML, left medial lobe; RLL, right lateral lobe; RML, right medial lobe; SHIVC, suprahepatic inferior vena cava

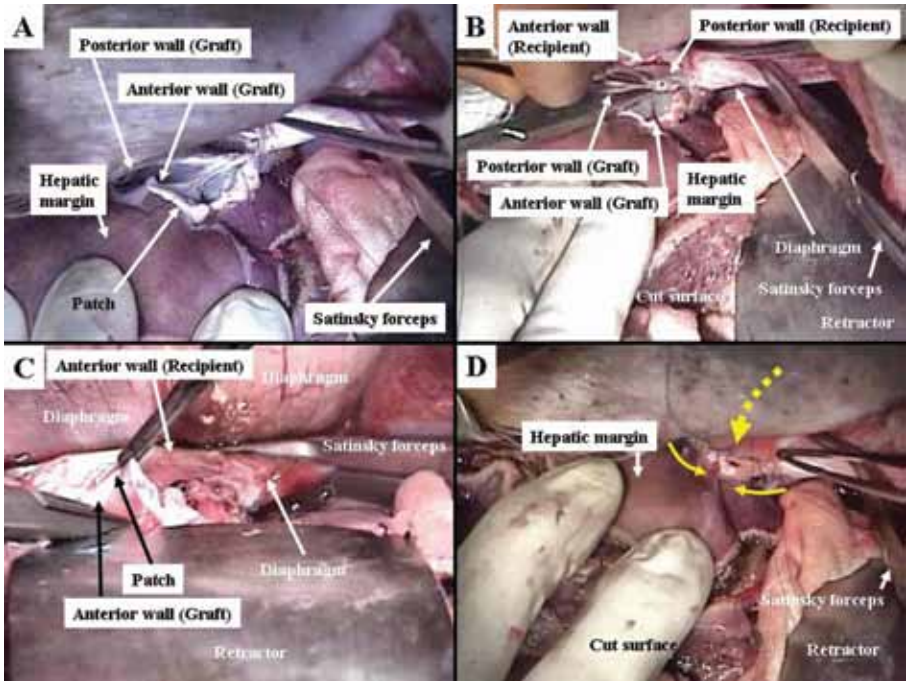

Figure 15 (A) The graft SHIVC is placed in position with correct axial alignment, using the hepatic margin as a guide. Stay sutures are placed bilaterally. The posterior wall lies straight and the anterior wall is curved. (B) Stay sutures are placed bilaterally. The posterior wall is sutured from the left side using a continuous suture. The suture bites of the graft SHIVC usually include liver parenchyma. (C) The anterior wall is sutured from both sides using a continuous suture (solid arrow). (D) Before completion of the anterior wall suture, the SHIVC is filled with heparinized saline to expel the air (dotted arrow)
SHIVC, suprahepatic inferior vena cava

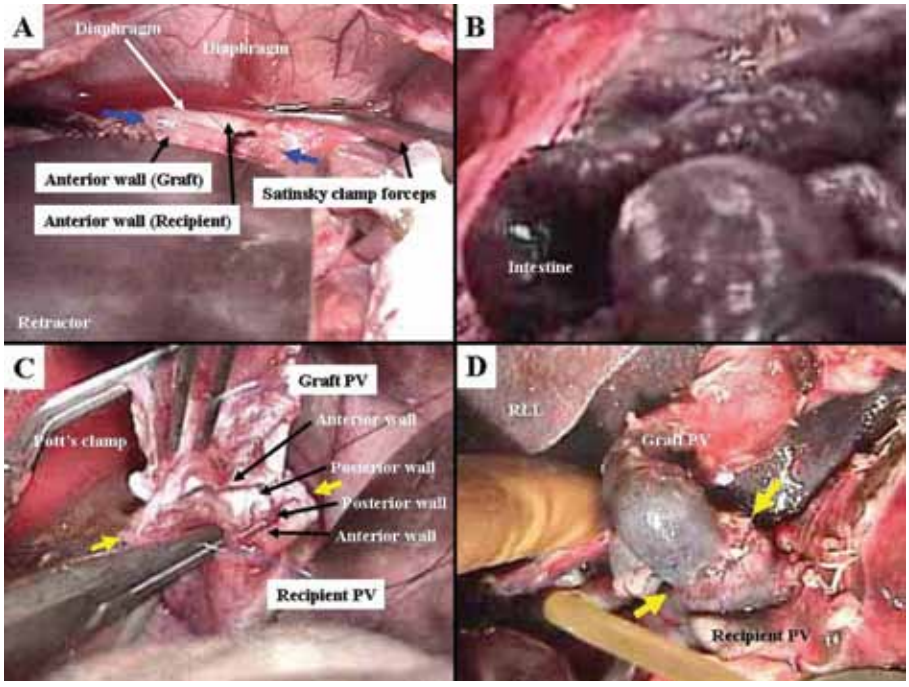

Figure 16 (A) The SHIVC is reconstructed without twisting or over-tightening (blue arrow). (B) The recipient PV trunk is clamped using a Pott's clamp. The digestive tract becomes congested immediately, and changes color. (C) Using the stay sutures for retraction, the posterior and anterior walls are sutured (arrow). (D) After the PV anastomosis (arrow), the SHIVC clamp is released, and then the PV clamps are removed followed by the clamp on the graft aorta. The graft color changes immediately, without any segmental defects
PV, portal vein; SHIVC, suprahepatic inferior vena cava

the right side, avoiding over-tightening. The anterior wall is then sutured from both sides using a continuous suture (Fig. 15C). Before completion of the anterior wall suture, the SHIVC is filled with heparinized saline to expel the air (Fig. 15D). The anterior suture is completed, and this thread is used to form a stay suture, avoiding over-tightening (Fig. 16A). A growth factor is not required. The retractors are released.

The temporary portosystemic shunt tube is removed, and the recipient PV trunk is clamped with a Pott's clamp. Perfusion of the digestive tract is indicated by an immediate color change (Fig. 16B). Bilateral stay sutures are placed at the recipient PV trunk. Using the stay sutures for retraction, the posterior and anterior walls are sutured (Fig. 16C). The PV anastomosis is completed using a continuous suture (6-0 MPS). To avoid over-tightening, a growth factor is usually left. The SHIVC clamp is released, followed by the PV clamps and then the graft aorta clamp. This completes graft reperfusion and the graft color changes immediately, without any segmental defects (Fig. 16D). The color of the digestive tract also improves, and recovery from the congestive damage takes place (Fig. 17A). Systolic blood pressure usually increases by 10-20 mmHg at this time. Bilateral stay sutures are placed at the recipient and graft IHIVCs. Using the stay sutures for retraction, the posterior and anterior walls are sutured (Fig. 17B). A growth factor is not required. IHIVC anastomosis is completed using a continuous suture (5-0 MPS) (Fig. 17C). The bulldog clamps are released first from the graft and then from the recipient. Systolic blood pressure usually increases by 20-30 mmHg after IHIVC reperfusion. There are three options for reconstruction of the biliary tract: external choledochostomy using the CBD stent tube, duct-to-duct reconstruction or choledochojejunostomy. We suggest that an external choledochostomy is enough for the short-term observation after OLT.

The peritoneal cavity and organs are irrigated with warm saline. No drains are placed. The peritoneum is closed with a continuous absorbable suture. The fascia and skin layers are closed individually with interrupted sutures. Blood transfusion may be administered if required (Fig. 17D).

Results

Orthotopic liver transplantation with a 30% graft is a critical animal model for split orthotopic liver transplantation with small-for-size graft. Previous documents demonstrated that the survival rates ranged from 0.3 to 0.6 after surgery with 30% graft [5,8-14]. Although survival rate during initial forty cases were under 0.2, the survival rate of 0.6 had been achieved after the experiences of forty cases. After the experience of 40 cases, a reasonable survival rate had been achieved (Fig. 18).

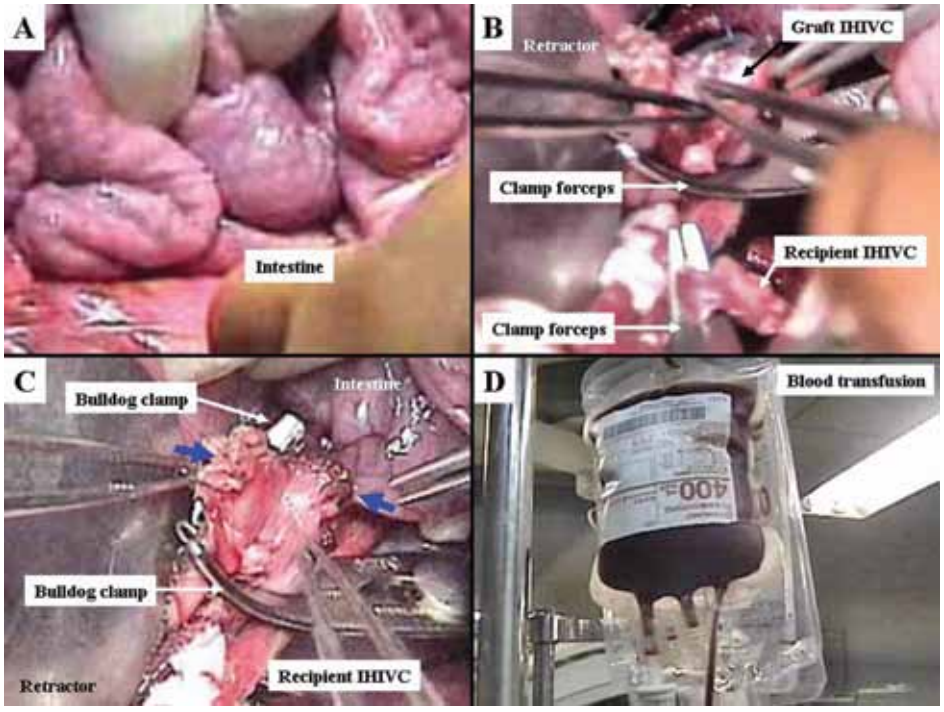

Figure 17 (A) After graft reperfusion, the digestive tract recovers from the congestion and its color improves. Systolic blood pressure usually increases 10-20 mmHg after graft reperfusion. (B) Using the stay sutures for retraction, the posterior and anterior IHIVC walls are sutured. (C) The IHIVC anastomosis is completed with a continuous suture (arrow). Systolic blood pressure usually increases 20-30 mmHg after IHIVC reperfusion. (D) Blood transfusion is administered if required
IHIVC, infrahepatic inferior vena cava

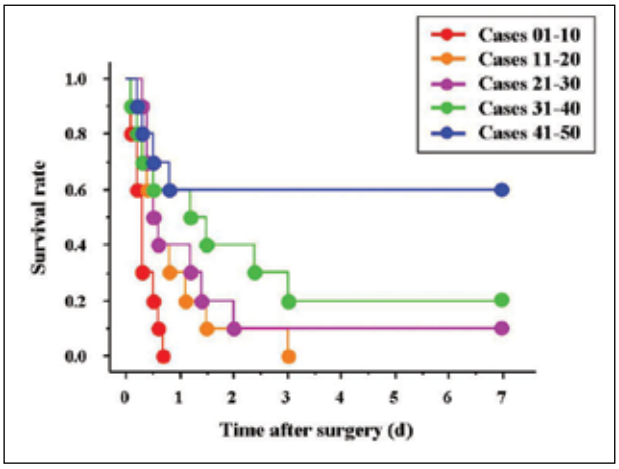

Figure 18 Learning curves for porcine OLT with 30% graft
OLT, orthotopic liver transplantation

Discussion

Stable general anesthesia and optimal fluid administration are important for successful porcine SOLT. Adequate fluid administration is required immediately after the placement of an intravenous cannula, as a fasted pig may be dehydrated. Even subtle dehydration may cause unexpected drug effects. Optimal hydration and a stable systemic hemodynamic state should be obtained before arterial reconstruction, removal of the native liver or graft implantation. Blood pressure may decrease drastically, especially during the anhepatic phase. Even with low blood pressure, an intensive volume load during the time without a transjugular portosystemic shunt (i.e., the operative time for PV reconstruction) may worsen the hemodynamic state, because a pig easily develops right cardiac failure with subtle overload.

During donor operation, we encountered the anatomical variations mainly in the hepatic artery. We should be careful for the isolation of the hepatic arterial branch for RLL. Hepatectomy for SOLT can be performed during the donor operation or on the back table. We have experienced unexpected bleeding from the cut hepatic surface immediately after graft reperfusion. It is therefore useful to confirm hemostasis of the cut surface after donor hepatectomy, and the back table often allows a more flexible surgical approach. Our sampling and autopsy findings indicate that nonperfused tissue at the hepatic margin will quickly atrophy.

Good hepatic venous outflow should be ensured in LT [16,17]. Reliable SHIVC reconstruction is important, as a block to outflow will result in a poor outcome. The porcine SHIVC does not have a sufficient extrahepatic margin for venous anastomosis, making the piggy-back method anatomically impossible. Then, we employed a classical method of OLT, not a piggy-back technique for SHIVC reconstruction. We therefore fashion a venous patch on the back table [18,19]. The vein from the RLL lies in a common channel with the vein from the RML at the level of the SHIVC. A venous patch is attached to the entire anterior length of the SHIVC wall. This procedure has the advantage of preventing any twisting, torsion or kinking in the recipient, as bilateral stay sutures can easily be placed. During SHIVC plasty, small extrahepatic branches should be identified to prevent intractable bleeding after graft reperfusion. During the recipient operation, a Satinsky clamp is placed on the intramediastinal IVC, which also grasps the margins of the diaphragm, and ventrally- and caudally-directed retraction of the clamp provides excellent exposure of the surgical field for anastomosis. The graft SHIVC is placed in position with the correct axial alignment by using the hepatic margin as a guide. Preoperative imaging is difficult in the pig, and a mismatched physical size may mean a difference in size of the subphrenic space, causing a 30% graft to move into a subphrenic recess far from the original position. The graft SHIVC should be carefully placed based on the alignment of the hepatic margin and graft, and fixed to the recipient SHIVC using stay sutures. A subtle shift in axis can easily cause torsion, twisting or kinking of the SHIVC anastomosis. If there is other no way to prevent a shift in axis due to movement of the graft into the subphrenic space, gauze can be placed behind the graft. A shift in axis of the SHIVC reconstruction causes outflow block, and the resulting complications can destroy the experiment. Care should be taken with all SHIVC reconstruction procedures, including adequate initial placement, smooth suturing and optimal ligation. Secure posterior suturing is required even if the suture bites of the graft SHIVC include liver parenchyma, because hemostasis of any bleeding points in this area after graft reperfusion is impossible.

The swine has a long digestive tract and spiral colon. Repeated and prolonged tractions of digestive tracts for vessel anastomoses easily triggered the congestive damage during vessel anastomoses. The congestive damage of digestive tracts during the recipient operation is the main problem in this model, based on our experience. The damage due to congestion and temporary ischemia from repeated retraction during surgery can be so severe that it becomes irreversible. One possible explanation is that the complete skeletonization easily causes torsion, twisting and kinking during retraction. Adequate skeletonization is paradoxically an important procedure for successful anastomosis. A temporary portosystemic shunt is indispensable in porcine SOLT models to preserve the circulation and minimize congestive damage [20-22]. We also have the clear impression that placement of a transjugular portosystemic shunt shortens the total PV clamp time. Clamping of the PV during reconstruction cannot be altogether avoided, but we currently achieve an anhepatic phase of only 40 min. The conventional order of vessel reconstruction is: SHIVC, PV and HA. Complete clamping during these procedures necessarily involves damage to the digestive tract. Microsurgical CHA reconstruction requires adequate traction to expose the surgical field, and we experienced a case that did not recover from the resulting congestive damage. We now achieve hepatic arterial reperfusion first by using an aorta-to-aorta anastomosis, which has the advantage of needing minimal retraction and a short operative time. The question arises whether aorta-to-aorta anastomosis also has an advantage after graft reperfusion. Our impression is that it does not. After reperfusion, flow to the anastomoses is easily

affected by traction, even if an aorta-to-aorta anastomosis is employed. The disadvantage of aorta-to-aorta anastomosis is that a massive ascites will occur after surgery if the para-aortic lymphatic duct is injured.

In conclusion, investigation of SFS syndrome is critical in the LT field. It is useful to develop an experimental 30% graft model for the split grafts used with cadaveric donors for pediatric cases and due to donor shortage in the United States and Europe, and for the shift to left-lobe living donor grafts to enhance donor safety in Japan. The described porcine SOLT model with initial aorta-to-aorta reconstruction has been established and provides clinically relevant data. We hope that our surgical guide will be informative for researchers with an interest in the LT field.

Acknowledgments

We are grateful to Prof. Kagemasa Kuribayashi (Mie University Graduate School of Medicine, Mie, Japan) for his technical support of the equipment used in porcine care.

This work was supported by grants to T. Hori from the Japan Society for the Promotion of Science (No. C20591523) and from the Uehara Memorial Foundation, Tokyo, Japan (No. 200940051).

Summary Box

What is already known:

- OLT models are available in larger animals such as dogs and swine, and these can provide clinically relevant and reliable data
- Small-for-size (SFS) grafts is a critically important procedure used in living donor LT to enhance donor safety in Japan, as well as in cadaveric donor LT because of donor shortages in the United States and Europe. The main focus of study in the liver regeneration field is patients with SFS syndrome and liver reperfusion injury
- Reliable and reproducible models of split OLT (SOLT) are important for undertaking clinically relevant studies

What the new findings are:

- Although porcine OLT with a 30% graft is an important model for SOLT with SFS graft, the details of surgical procedures were still not well-described
- At first, we showed the details of surgical procedures for orthotopic liver transplantation model with small-for-size graft in the pig
- Next, we discussed the key techniques and pitfalls in our experiences

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