Transplant International

ORIGINAL ARTICLE

Successful pancreas allotransplantations after hypothermic machine perfusion in a novel diabetic porcine model: a controlled study

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SUMMARY

The standard technique for pancreas preservation for transplantation is static cold storage (SCS). In this experimental study, we compare SCS to hypothermic machine perfusion (HMP) of the pancreas to assess if the latter could safely prolong the ischaemia period prior to transplantation. We worked in two phases, first with organ preservation for 24 h and second, preservation for either 2 or 6 h before allotransplantation. In phase 1, exocrine injury markers were found to be nonsignificantly lower, in the HMP group ($n = 3$) vs. SCS ($n = 3$) after 24 h of preservation; amylase ($P = 0.2$), lipase ($P = 0.3$) and lactate dehydrogenase ($P = 0.1$). In phase 2, 14 recipient diabetic pigs (after total pancreatectomy) received allotransplantations with $n = 4$ and $n = 4$ pancreases after HMP for 2 and 6 h vs. $n = 3$ and $n = 3$ pancreases after SCS for 2 and 6 h, respectively. There were no differences in recipient survival ($P = 0.7$), and mean survival was 14 days (0–53 days). All recipients had allograft function defined as detectable C-peptide and independent normoglycemia. We have not highlighted vascular thrombosis in all allotransplantations. This study reports the first successful pancreas allotransplantation after HMP preservation for up to 6 h with no evidence of graft thrombosis.

Transplant International 2021; 34: 353–364

Key words
diabetic porcine model, hypothermic perfusion, pancreas allotransplantation, static cold storage

Received: 4 October 2020; Revision requested: 21 October 2020; Accepted: 30 November 2020

Introduction

Pancreas transplantation is one of the known most effective treatments for type 1 diabetes mellitus and is associated with improved survival and quality of life for patients [1,2]. Typically, it is offered to patients with type 1 diabetes with end-stage renal disease (ESRD) as a simultaneous pancreas and kidney (SPK) transplantation, occasionally as a pancreas after kidney transplant with proven long-term improvement in recipient morbidity and mortality. Pancreas transplantation is also offered to a much smaller group of pre-uraemic patients as pancreas alone transplantation, who suffer from the devastating effects of hypoglycaemic unawareness.

Despite the very good outcomes of pancreas transplantation for patients, an important concern remains of a having a small donor pool necessitating the utilization of nonstandard, expanded criteria donors (ECD)

doi:10.1111/tri.13797

[3] and donors after circulatory death (DCD) [4,5] to support transplant activity.

The pancreas is highly susceptible to oedema and ischaemic injury during both organ retrieval and preservation leading to damaging effects on the graft microcirculation [6]. Microcirculatory dysfunction is a key contributor to the sequela of graft thrombosis, pancreatitis and eventual graft failure. Also due to the inability of cells to generate adenosine triphosphate (ATP) as a necessary energy source under the anaerobic conditions encountered during static cold storage (SCS), the desired endocrine component of the pancreas, the Islets of Langerhans are negatively affected by the resultant hypoxia and ischaemia reperfusion injury (IRI) which leads to irreversible damage and associated poor clinical outcomes postislet transplantation [7].

hypothermic machine perfusion (HMP) was first developed by Alexis Carrel in experimental transplant models over a century ago [8]. Belzer *et al.* [9] subsequently introduced this procedure in the clinical setting of kidney transplantation and since then HMP has grown in its utility.

Many studies have demonstrated the benefit of HMP not only for renal allograft preservation but particularly for its significant reduction of the incidence of delayed graft function for DCD, and specifically for extended criteria or marginal donors [10].

The earliest published experimental *ex vivo* perfusions of pancreases were described in 1926 in a canine model by Babkin and Starling. Florack *et al.* [11,12] after HMP perfusion of canine segmental pancreases using Ringers solution with a 30 mmHg perfusion pressure performed autotransplantation by anastomosing the splenic pedicle to the iliac vessels. All perfused pancreases demonstrated oedema, haemorrhage and venous congestion after revascularization. Due to these poor results, there was a decline in whole pancreas perfusion for transplantation research with a subsequent shift in focus towards islet isolation after HMP [13–16].

Despite these early hurdles, factors such as the growing shortage of optimal donor pancreases for transplantation, the encouraging results of HMP in kidney transplantation, the development of new perfusion solutions and successful solid organ perfusion technologies for the lung, heart, kidneys and liver has led to a resurgence of research in machine perfusion for whole pancreas preservation.

The pancreas is a low flow organ with complex vascular anatomy and without the end arteries found in other established *ex vivo* perfused abdominal solid organs namely the liver and kidney. The *ex vivo* perfusion protocols for liver and kidneys involve high mean perfusion pressures which is not well tolerated by pancreases and can lead to endothelial injury and severe oedema and conversely if mean perfusion pressures are too low it may not be sufficient enough to support the extracorporeal viability of the pancreas. These unique features of the pancreas encourage work focusing on specific perfusion parameters for this organ. Defining these optimal parameters can be challenging probably explaining why to date there are no reports of pancreas transplantations following successful machine perfusion.

Nonetheless, there have been some encouraging recent progresses made in pancreas perfusion research. Our group previously recently reported that HMP was feasible and safe for a period of 24 h for human discarded pancreases [17] and for 12 h on healthy nonhuman primates' pancreases [18].

Materials and methods

The aim of this work is to compare the traditional preservation technique of SCS to HMP to assess if the latter is a potential strategy to safely prolong the ischaemia period prior to successful transplantation and we demonstrate this in a novel diabetic porcine allotransplantation model.

The primary outcome is evidence of pancreas viability postpreservation assessed by levels of pancreas injury markers in the perfusate including amylase, lipase and lactate dehydrogenase (LDH) as well as allograft function postallotransplantation. Secondary outcomes include histological assessment of serial biopsies, oedema assessment and perfusion parameters, recipient survival, recipient morbidity and post-mortem assessment of the rate of vascular thrombosis after transplantation.

This research was performed in two phases:

Phase 1 (Fig. 1)—Feasibility phase; comparison of two pancreas preservation strategies, static cold storage (SCS) with $n = 3$ pancreases vs. HMP of $n = 3$ pancreases for 24 h without allotransplantation.

Phase 2 (Fig. 2) Allotransplantation phase; 14 recipient diabetic pigs (nonstreptozotocin, insulin-dependent after total pancreatectomy) were divided into four experimental groups and had allotransplantation after graft preservation of 14 donor pancreases preserved in either SCS for 2 h ($n = 3$), SCS for 6 h ($n = 3$), HMP for 2 h ($n = 4$) and HMP for 6 h ($n = 4$).

Experimental animals

The study protocol was approved by the French Ministry of Research (APAFiS # 18 169). Male pigs "large white"

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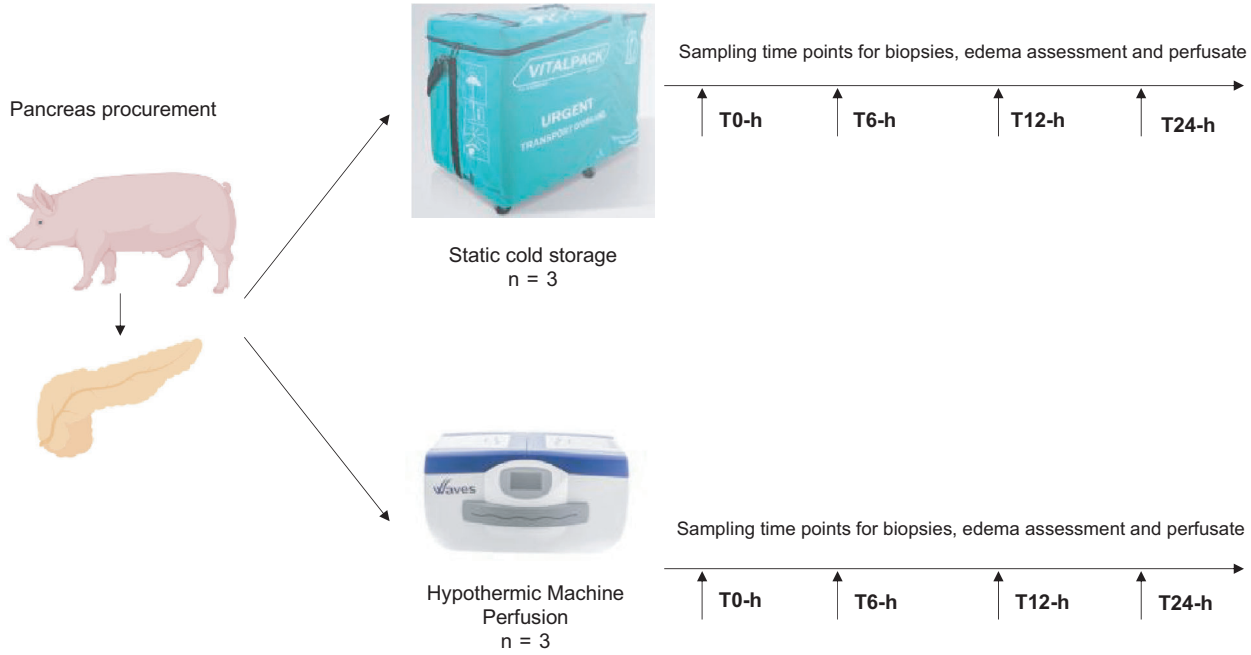

Figure 1 Phase 1; Feasibility phase. Comparison of two pancreas preservation strategies, static cold storage (SCS n = 3) versus hypothermic machine perfusion (HMP n = 3) for 24 h without allotransplantation.

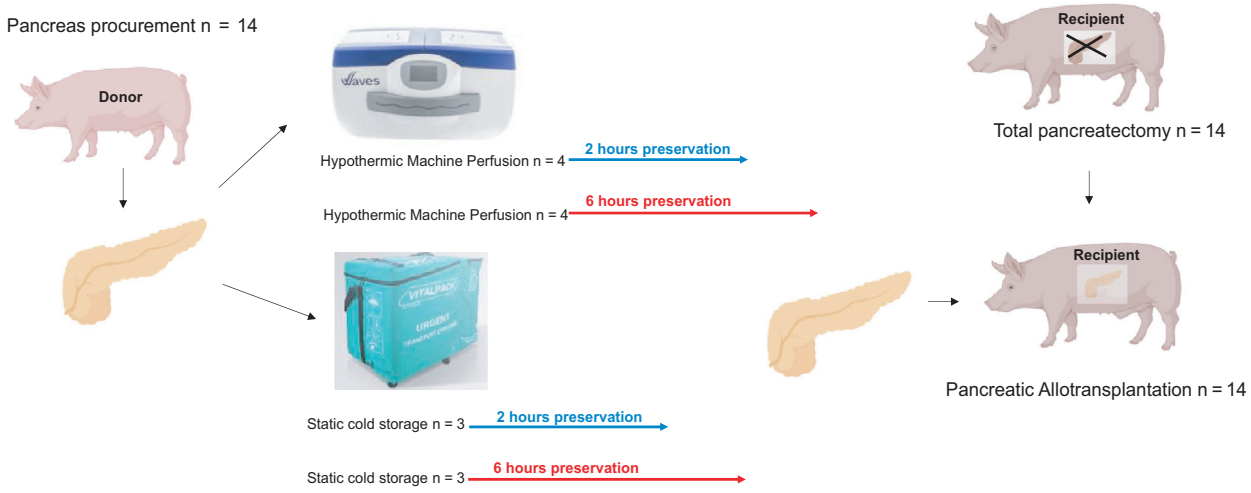

Figure 2 Phase 2; Allotransplantation phase. Fourteen recipient diabetic pigs (insulin-dependent after total pancreatectomy) were divided into four experimental groups and had pancreas allotransplantation after graft preservation of 14 donor pancreases preserved in either SCS for 2 h (n = 3), SCS for 6 h (n = 3), HMP for 2 h (n = 4) and HMP for 6 h (n = 4).

Sus scrofa pig, average weight of 80 kg were used. The ABO group of the pigs were determined using test cards with pre-applied antibodies (Serafol, Berlin, Germany). On completion of the study protocol which was when the animal showed any early signs of pathology including rejection or suffering, the pigs were euthanized under general anaesthesia by intravenous injection of pentobarbital (140 mg/kg). All experiments were in accordance with ARRIVE guidelines and were carried out according to EU Directive 2010/63/EU for animal experiments.

Anaesthesia, intraoperative and postoperative monitoring

Anaesthesia and intraoperative monitoring protocols were similar for all surgical procedures, namely total

pancreatectomy alone for organ procurement in donor pigs or total pancreatectomy to induce diabetes with subsequent pancreas allotransplantation in recipient pigs with both procedures being performed in one session. Prior to surgery, the pigs were fasted for food and drink for 12 h to ensure an empty stomach prior to anaesthesia. The pigs were premedicated with Zoletil© (Virbac, Carros, France; Zolazepam/Tiletamine) 15 mg/kg IM and were subsequently intubated and ventilated with a mixture of isoflurane (2%), nitrous oxide (49%) and oxygen (49%). The gas flow rate was 2 l/min, and respiratory rate was 20 breaths/min. Cardiovascular and respiratory parameters measured included mean arterial pressure, pulse rate, heart rate and oxygen saturation, and for monitoring temperature, a thermal probe was sited in the oesophagus. Central venous lines were placed in the internal jugular vein to enable delivery of intravenous hydration, analgesia and to allow regular blood sampling.

Postoperative analgesia for recovered pigs was given as intravenous injections of opioids, Nalbuphine (Nubain©, Mylan, Canonsburg, PA, USA) and paracetamol dose of 25 mg/kg IV. Prophylactic antimicrobial therapy was administrated as a single dose of Cefazolin 250 mg (Cefovet©, Dopharma, Ancenis, France). Postsurgery the pigs were allowed unlimited access to drinking water but diet was reintroduced 72 h post-total pancreatectomy and allotransplantation to reduce stress on the intestinal anastomoses.

No immunosuppressive drug regime was started postallotransplantation. Allograft rejection in the porcine models usually occurs beyond 3 weeks, and any evidence of rejection was an indication for euthanasia.

Surgical technique for porcine duodeno-pancreas procurement

We simulated a donation after brainstem death (DBD) model for the procurement of pancreases from the anesthetized pigs to use in phase 1 (Feasibility) and as pancreas donors in phase 2 (Allotransplantation). After the induction of general anaesthesia, a midline xipho-pubic laparotomy incision is made to expose the pancreas. A splenectomy is then performed after ligation of the splenic artery and vein. The proximal segment (D1, postpylorus) and third segment (D3) of the duodenum are mobilized and stapled off using a GIA© stapler (Medtronic, Minneapolis, MN, USA) in order to free the head of the pancreas with a cuff of duodenum. Then dissection of the mesentery was continued at a distance from the pancreas to allow stapling and disconnection of the root of the mesentery from the body of the pancreas with the GIA© stapler. The renal arteries and veins were dissected out and ligated to decrease the volume of in situ perfusion fluid required. An intravenous injection of 300 IU/kg of heparin is given before the placement of arterial and venous cannulas.

Both the supra-coeliac aorta and the infra-hepatic inferior vena cava are ligated to isolate perfusion to the region of the pancreas and perfusion is started using cold IGL-1 solution© (Institut Georges Lopez, Lissieu, France). Crushed ice is poured into the abdominal cavity to facilitate further cooling. The inferior hepatic vena cava is cut to allow venting of blood during perfusion. When it is observed that the pancreas appears pale due to adequate perfusion and effluent from the cut infrahepatic inferior vena cava is clear and no longer bloody, the hepatic pedicle is dissected out. The common bile duct, hepatic artery proper are both identified, dissected out and ligated. A careful dissection of the portal vein is carried out, and the portal vein was divided away from the pancreas (>1 cm) to provide sufficient vein length necessary for subsequent transplantation. Proximally the supra-coeliac aorta and distally the infrarenal aorta are both divided containing the necessary blood supply to the pancreas.

The tail of the pancreas is then dissected of the retroperitoneum in a left lateral to medial direction to free the whole duodeno-pancreatic block. The pig is then euthanized under general anaesthesia as described in Section 2.1 above at the end of the procedure.

Surgical preparation and preservation technique of pancreases for HMP and SCS

The pancreases in the HMP group are prepared by suture ligating the lumbar and renal branches coming off the excised tube of aorta supplying the pancreas with nonabsorbable monofilament Prolene© 5/0. The distal end of the aortic tube is cannulated with a 10 mm cannula (the proximal supra-coeliac was tied off during procurement). The pancreas is flushed using a syringe filled with IGL-1 via the arterial cannula, and any leaks observed are suture ligated. The cannulated pancreas is then placed in and connected to the organ chamber cassette in the Waves© machine (Waters Medical System, Rochester, MN, USA), which provides hypothermic (4 °C) pulsatile organ perfusion. Although this machine was originally designed for HMP of kidneys, the size and shape of the organ chamber cassette accommodate the pancreas well and the device allows adjustment of perfusion pressures which was set to a

low systolic perfusion of pressure (15 mmHg) in this study. The Waves© machine continuously records temperature, flow rate, perfusion pressure and resistance index during the pancreas perfusion which is recorded for analysis.

The pancreases in the SCS group required no special preparation and were stored in a bag filled with IGL-1© solution and placed on ice at a temperature of 4 °C.

Feasibility phase (phase 1); SCS versus HMP without allotransplantation

The control group (SCS) included pancreases ($n = 3$) that were preserved for 24 h in IGL-1© (Institut Georges Lopez) preservation fluid (1 l) and placed in ice at 4 °C (Fig. 1).

The intervention group included pancreases ($n = 3$) that were placed on a device that provided hypothermic, pulsatile perfusion at 4 °C for 24 h. Perfusion fluid was IGL-1© (Institut Georges Lopez; 1 l) without oxygenation. For both groups, no subsequent transplantation was performed.

Biochemical analysis of the preservation liquid

Preservation fluid samples from both the HMP and SCS groups were collected at the start of preservation (T0h), 6 h (T6h), 12 h (T12h) and at 24 h, the end of preservation (T24h). The samples collected were analysed for amylase, lipase and lactate dehydrogenase (LDH).

Macroscopic oedema assessment

Pancreas macroscopic oedema was assessed using the visual macroscopic oedema assessment scale (ES; 0 = no oedema; 1 = mild oedema; 2 = moderate oedema; 3 = severe oedema [19]).

Monitoring of pancreas perfusion parameters on HMP

During HMP perfusion, parameters of flow (unit = ml/min) and resistance index (RI) (unit = ru) were monitored and recorded by the Waves© machine, and pressure was set at 15 mmHg.

Histological and immunohistochemical analysis

Two pancreatic biopsies were taken with a biopsy gun (Bard Biopsy, Tempe, AZ, USA) at the start of preservation (0 h) and at 6, 12 and 24 h. The biopsies were immediately fixed in 10% formalin and subsequently embedded in paraffin, and the produced slides were stained with haematoxylin and eosin (HE), anti-insulin (monoclonal Ab Immunotech clone E2E3 ref. 0593 1/400), anti-glucagon (polyclonal Ab Eurodiagnostica ref. 2263P-GLU-1/250) and anti-somatostatin (polyclonal Ab Dako ref. A0566 – 1/4000). A blinded pancreas pathologist performed the histological evaluation of the slides.

Allotransplantation phase (phase 2); pancreas allotransplantation after preservation by SCS or HMP in insulin-dependent diabetic pigs

The four experimental groups (Fig. 2) described above had a total pancreatectomy followed by pancreas allotransplantation after preservation either by HMP or SCS for 2 or 6 h. The pancreas grafts for preservation and subsequent allotransplantation for the recipient pigs were procured from 14 donor pigs.

The surgical technique for duodeno-pancreatic procurement and preparation of the allograft for either SCS or HMP is the same technique used in phase 1 (Section 2.3).

Induction of diabetes and subsequent pancreatic allotransplantation

The recipient pig general anaesthesia is initiated before the end of the graft preservation period. A midline xiphopubic laparotomy incision is made, and dissections of visceral attachments are performed to expose the pancreas. A total pancreatectomy is then carried out to create an experimental model of an insulin-dependent diabetic pig without the use of streptozotocin. Serial intraoperative blood tests confirm hyperglycaemia >20 mmol/l with a need for exogenous insulin. An intravenous injection of 25 IU/kg of heparin is given before the recipient inferior vena cava and infrarenal aorta are clamped for anastomoses. An end to side anastomosis between the donor portal vein and the recipient's inferior vena cava and an end to side anastomosis between the aortic tube of the allograft and the recipient aorta are performed with non-absorbable 5/0 suture (Prolene©). A side to side enteric anastomosis between the duodenum of the pancreas graft and the recipient jejunum is performed with absorbable suture 4/0 (PDS©). A drain is placed alongside the vascular anastomoses to monitor for intraabdominal haemorrhage. The midline incision is closed with absorbable suture (Monocryl©), and then, the animals are woken up, recovered, provided analgesia and transferred to their housing.

If any sign of intraperitoneal haemorrhage is observed, a surgical exploration under general anaesthesia is performed to ensure hemostasis. Allograft function is defined by the detectable C-peptide levels (>0 pmol/l), and independent normoglycaemia without need of exogenous insulin requirement and graft survival is evidence of allograft function up to euthanasia. An autopsy is performed after euthanasia of all animals that received pancreas allotransplantation to investigate that status of the pancreas transplant and to investigate the cause of pathology.

Postoperative blood tests

Blood glucose levels were checked three times a day using a glucometer (Accucheck©; Roche, Meylan, France). Daily venous blood samples were taken and analysed for C-peptide and nonfasting glucose.

Statistical analysis

Quantitative data were expressed as means and ranges. We compared means between two groups, using Student *t*-tests, and values are expressed as the mean in its unit. All reported p-values were two-sided with a significance level at $P < 0.05$. Statistical analyses were performed using S Prism 7.0a (GraphPad Software Inc, La Jolla, CA, USA).

Results

Feasibility phase (phase 1); SCS versus HMP for 24 h without allotransplantation

Perfusate analyses

Amylase, LDH and lipase were chosen as markers for pancreas injury and were observed to increase steadily during SCS. At T0h, these markers were higher in the HMP group, without statistical differences. Throughout the perfusion, although these markers remained lower than the SCS group at 24 h (Fig. 3), no significant differences were observed between both groups, amylase ($P = 0.2$), lipase ($P = 0.3$) and lactate dehydrogenase ($P = 0.1$).

Macroscopic oedema assessment

After 12 h of SCS, no evidence of macroscopic oedema (ES = 0) was observed in the three pancreases, and at

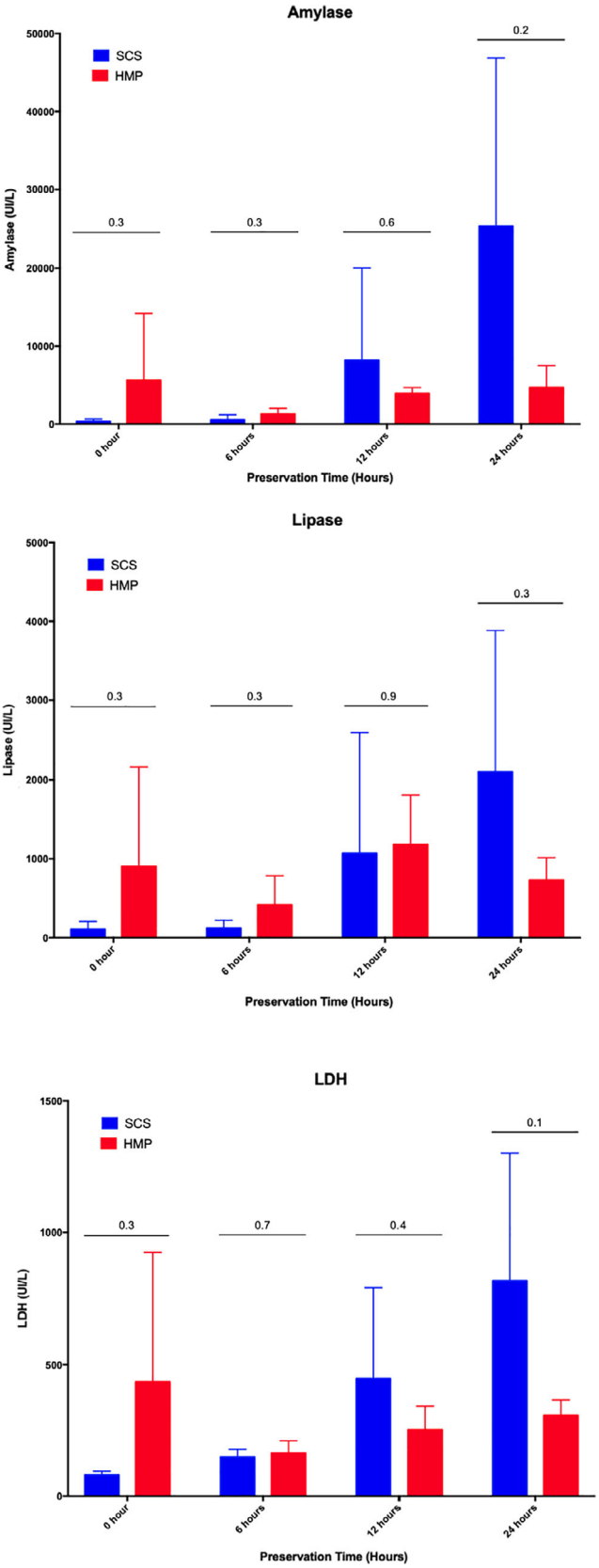

Figure 3 Perfusate analyses for amylase, lipase and LDH at 0, 6, 12 and 24 h in blue for SCS (static cold storage) versus red for HMP (hypothermic machine perfusion) groups.

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24 h, only one pancreas was observed to have a mild oedematous appearance (ES = 1).

In the HMP group, after 12 h perfusion, all three pancreases were observed to have moderate oedematous appearances (ES = 2). After 24 h of HMP, all perfused pancreases appeared severely oedematous (ES = 3; Fig. S1).

HMP perfusion parameters

The resistance index (RI) in pancreas 1 and pancreas 3 steadily decreased in the first 5 h of perfusion and then reached a stable plateau (Fig. S2). Pancreas number 2 was mobilized during perfusion to perform a biopsy and this inadvertently led to a twist of the aorta (inflow) resulting in a transient rise of the RI which subsequently gradually deceased.

The flow rate in pancreases number 1 and 2 gradually increased during perfusion suggestive of good microcirculatory compliance, the flow rate of pancreas number 3 remained stable for the duration of HMP.

Immunohistochemistry

After 6 and 12 h of preservation, histological appearances were similar in both the SCS and HMP groups and reported as absence or slight inter-acinar oedema, some areas of peri-lobular multifocal necrosis and focal ischaemic necrosis of adipocytes (Fig. 4).

After 24 h of preservation, the histological appearances of the HMP group exhibited higher grades of interstitial oedema with several moderate perineal and centro-lobular inter-acinar enlargements, significant acinar necrosis with several areas of multifocal centro-lobular necrosis, steatonecrosis and multiple areas of diffuse ischaemic necrosis of adipocytes and the islets of Langerhans compared to the SCS group. Beyond 12 h of preservation, higher grades of interstitial oedema, ischaemia and necrosis were observed in the HMP biopsies compared to the SCS samples.

Histology samples from both groups showed normal and similar appearances up to 24 h for insulin, glucagon and somatostatin immunohistochemistry (Fig. S3).

Pancreas evaluation in the HMP group

In order to evaluate the relationship between macroscopic appearance and histological lesions of pancreases preserved by HMP, we presented the data of each pancreas' perfusion data, macroscopic appearance and histological grading.

Concerning macroscopic oedema assessment, as reported, after 12 h of HMP, all pancreases presented moderate oedematous appearances (ES = 2). After 24 h of HMP, all pancreases all perfused pancreases appeared severely oedematous (ES = 3; Figs. S1 and S4).

Concerning immunohistochemistry evaluation, the histological appearances of each pancreases were presented in Table S1. HMP perfusion parameters were presented in Fig. S2.

There does not seem to be a relationship between macroscopic appearance and histological grading after HMP (Fig. S5).

Allotransplantation phase (phase 2); SCS versus HMP for 24 h with allotransplantation

Clinical outcomes, survival, postallotransplantation glucose and C-peptide levels

Mean recipient survival post-transplantation was 14 days (range 0–53 days), without statistical difference between the SCS and HMP groups ($P = 0.7$; Fig. 5). All recipients in both groups had allograft function. Figure 5 shows postallotransplantation C-peptide and glucose levels after 2 h preservation by SCS and HMP and Fig. 5 shows similar graphs post 6 h preservation.

There were a total of 8 early postoperative deaths (occurring in less than 24 h post-transplantation) and were associated with surgical or anaesthetic complications.

In the remaining six pigs, graft survival rate was higher in SCS group, without statistical differences [100% (3/3) vs. 67% (2/3) in SCS and HMP groups respectively, ($P = 0.9$)]. Indeed, the AlloTx-6h HMP n°4 presented an acute rejection as of the 5th day postoperatively. Duration of graft survival was similar, SCS 22.6 days (8–30) vs. HMP 21.3 days (14–30) ($P = 0.7$).

Post-mortem examinations were performed for all allotransplantations and there was no evidence of pancreas vascular thrombosis.

Discussion

Since the first pancreas transplantation performed in 1966 [20], static cold storage (SCS) has remained the most common preservation method for pancreases after retrieval due to its simplicity and low cost. Since University of Wisconsin (UW) solution was developed in 1986 by Belzer and Southard, it has been the most common preservation solution, reducing pancreatitis and thrombosis rates [21,22].

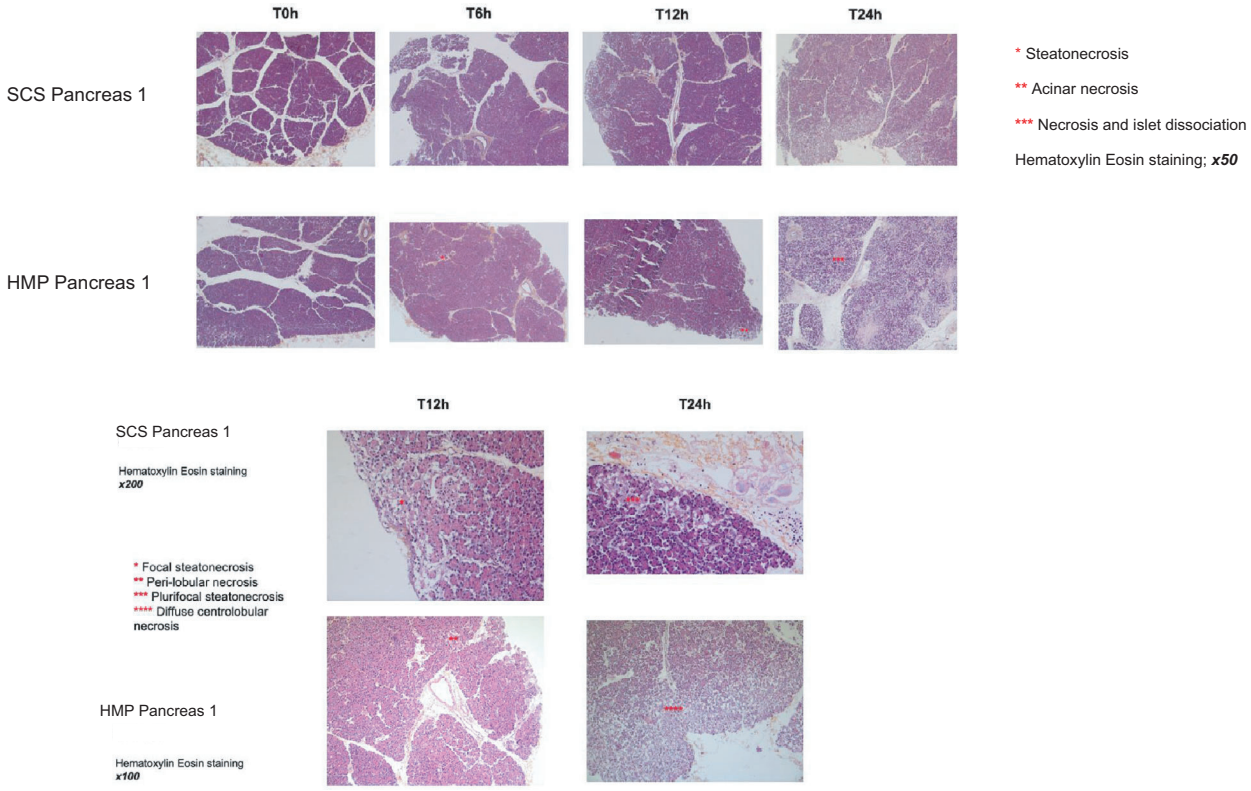

Figure 4 Histological analysis of static cold storage (SCS) versus hypothermic machine perfusion (HMP) groups.

All hypothermic preservation techniques are based on the principle that cooling an organ reduces the cellular metabolic rate and the demand for oxygen and adenosine triphosphate (ATP) required for cellular energetic maintenance. Lowering the organ temperature to 4–5 °C reduces the metabolic rate to about 10% which helps to minimize ischaemic injury due to hypoxia [23].

The pancreas, however, is extremely sensitive to both warm and cold ischaemia and these have a considerable impact on preservation duration and technique [24]. Warm ischaemia (WI) is particularly time critical as deleterious effects are observed beyond 30 min, with significant loss of islet yield and beta-cell function after islet transplantation [25,26].

Static cold storage is currently sufficient for standard criteria DBD pancreases, but it is becoming clear that organs retrieved from DCD and ECD are even more sensitive to cold ischaemia and have more profound ischaemia reperfusion injury (IRI) [27]; therefore, these are the groups that may benefit from alternative preservation methods such as machine perfusion.

In the UK in 2019, 253 (54%) retrieved pancreases were discarded (NHSBT, National Health Service Blood and Transplant annual activity report). Although there are multiple reasons for organ discard, it can be appreciated that by introducing enhanced preservation methods such as machine perfusion that may allow viability testing and prolongation of ischaemia times, discard rates would improve by reducing uncertainty about allografts' functional potential. Our group has already demonstrated with discarded human pancreases that HMP is feasible and safe [17] and replicated similar conclusions with healthy nonhuman primate pancreases [18]. In this study, we demonstrated the feasibility of successful pancreas allotransplantation after varying preservation periods on HMP in a novel diabetic porcine model. Additionally, we compared transplantation outcomes of HMP to classical SCS. Reassuringly, we did not observe any differences in recipient survival between both HMP and SCS groups which further moves the needle towards adoption in transplantation hopefully in the form of first in human clinical trials.

The pancreases in this study were procured from healthy young pigs, and we used a procurement model not completely similar to a DBD model as we did not replicate brain stem death in the donor pigs, and therefore, it will be without the unfavourable systemic cytokine storm associated with brain death.

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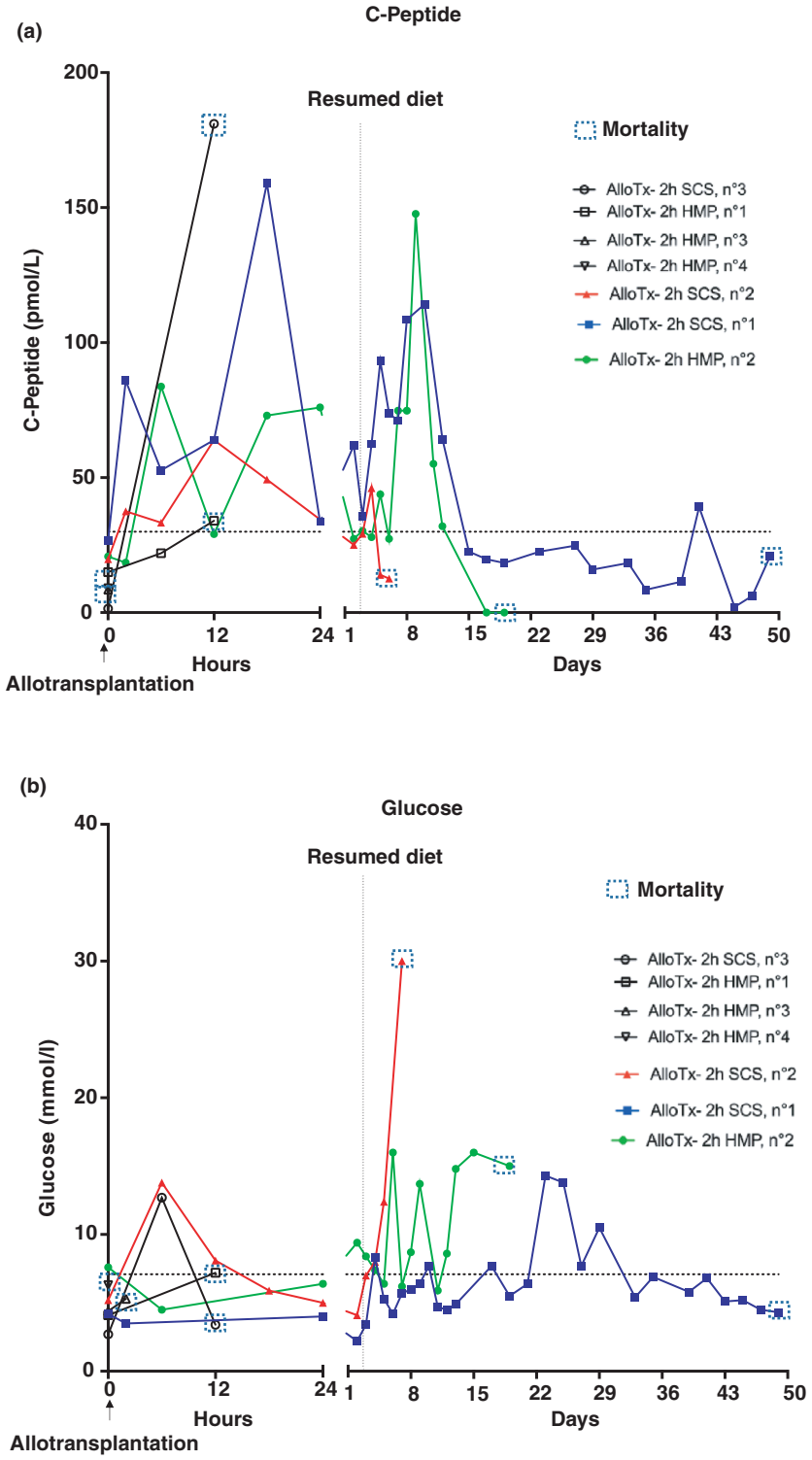

Figure 5 Postpancreas allotransplantation C-peptide and glucose levels after 2 and 6 h preservation by static cold storage (SCS) and hypothermic machine perfusion (HMP).

Amylase, lipase and LDH levels were found to be nonsignificantly higher in SCS preservation at 24 h suggesting HMP could be protective to the exocrine component of the pancreas. However, at T0h, these markers were higher in the HMP group, suggesting the deleterious effect of manipulating the pancreas to

connect it to the perfusion machine. This deleterious effect is then compensated by the HMP. Although the exocrine component of the pancreas is not required for beta-cell replacement, it is the contributing component associated with graft thrombosis and pancreatitis.

There are several mobile perfusion machines, specifically designed for kidney preservation that are currently commercially available for use, in this study we used the Waves© machine for the HMP of the pancreases. Our choice of this device was because it provides both hypothermic and pulsatile perfusion within a sufficiently large, sterile cartridge that conveniently accommodates a whole pancreas graft for perfusion.

The preference for pulsatile perfusion compared to nonpulsatile perfusion is based on the observation that former more closely mimics physiological blood flow. Pulsatile perfusion which consists of "systolic" and "diastolic" pressures may confer a protective benefit to the endothelium by activating endothelial protective genes such as Kruppel-like factor 2 [28]. This gene is overexpressed by the endothelium during pulsatile perfusion and is thought to have an antithrombotic action to support the microcirculation [29].

Fukae *et al.* [30] associates pulsatile perfusion, compared to nonpulsatile with the inhibition of proinflammatory responses via the reduction of sympathetic nerve activity. Also, they associated pulsatile perfusion with the reduction of vascular resistance which may improve the microcirculation and therefore organ function in a canine renal model.

There were limitations to this research which we aim to address in our future work. We worked with healthy, uninjured porcine, nonclassical DBD/DCD pancreases with short cold ischaemia times before preservation and subsequent transplantation. Although this is not an exact model to the "real world" heterogeneous pool of donor pancreases, we achieved our goal of establishing the safety and feasibility of HMP followed by transplantation in a porcine diabetic model. We chose not to perform pancreatic biopsies in the allografts for transplantation to minimize potential damage.

Moreover, we performed pancreas allotransplantations after short period of hypothermic preservation (2 and 6 h). A longer period of hypothermic preservation (12 h) would better reflect clinical practice [5]. However, for organizational reasons, we were not able to extend the hypothermic preservation method because the development of the porcine diabetic model was very time-consuming.

We had 8/14 recipient deaths in the early postoperative period (within 24 h) due to significant intraabdominal haemorrhages and we classify these as technical failures. We suspect this is due to a higher surgical morbidity of having two major procedures, namely total pancreatectomy and allotransplantation in the same operation. In future work, we plan to have a recovery period of a week after total pancreatectomy before pancreas allotransplantation and in the recovery period support the animals with exogenous insulin. We have successfully trialled this protocol in one pilot case. Additionally, we will start an autologous blood bank (blood donation taken at time of initial pancreatectomy) to allow for blood transfusion if required.

We have already begun work with a DCD porcine model that includes warm ischaemia time (WIT) to replicate extended criteria pancreases and we will be comparing the effect of HMP of pancreases with oxygenation versus without, prior to transplantation. Our hypothesis is that the addition of oxygen to HMP reduces free radical production to attenuate the harmful effects of anaerobic metabolism.

We also plan to work on the possibility to reduce IRI, with all its associated complications (vascular thrombosis, pancreatitis...) by using normothermic machine perfusion (NMP) of the pancreas. Barlow *et al.* [31] demonstrated the feasibility of *ex situ* pancreas NMP in which they could correlate amylase levels with fat infiltration of the organ and exocrine function.

Conclusion

This study reports the first successful series of pancreas allotransplantation in a novel diabetic porcine model after HMP preservation for up to 6 h with no evidence of graft thrombosis.

Authorship

TP: participated in research design, writing the paper, data collection and analysis. DK: participated in research design, writing the paper, data collection and analysis. AEO: participated in writing and editing the paper. VG: participated in data collection and analysis. SLB-B: participated in data collection and analysis. DM: participated in data collection and analysis. JH: participated in data collection and analysis. DC: participated in data collection and analysis. GK: participated in data collection and analysis. KR: participated in research design, data collection and

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analysis. GB: participated in research design, writing the paper, data collection and analysis. JB: participated in research design, writing the paper, data collection and analysis.

Funding

T. Prudhomme has received a grant from the French Association of Urologist. J. Branchereau has received research support from Institut Georges Lopez.

Conflict of interest

The authors of the manuscript have conflicts of interest to disclose. J. Branchereau has received research support from Institut Georges Lopez.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Pancreas macroscopic oedema assessment at 12 and 24 h of preservation.

Figure S2. Phase 1 hypothermic machine perfusion (HMP) parameters for three pancreases over 24 h.

Figure S3. Immunohistochemical analyses of static cold storage (SCS) versus hypothermic machine perfusion (HMP) groups.

Figure S4. HMP Pancreas macroscopic oedema assessment at 12 and 24 h of preservation.

Figure S5. Relationship between macroscopic appearance and histologic grading after HMP.

Table S1. Histological analysis of HMP pancreases.

REFERENCES

1. Kandaswamy R, Stock PG, Gustafson SK, *et al.* OPTN/SRTR 2017 annual data report: pancreas. *Am J Transplant.* 2019; **19**(Suppl 2): 124.
2. Redfield RR, Rickels MR, Naji A, Odorico JS. Pancreas transplantation in the modern era. *Gastroenterol Clin North Am* 2016; **45**: 145.
3. Neidlinger NA, Odorico JS, Sollinger HW, Fernandez LA. Can 'extreme' pancreas donors expand the donor pool? *Curr Opin Organ Transplant* 2008; **13**: 67.
4. Mittal S, Gilbert J, Friend PJ. Donors after circulatory death pancreas transplantation. *Curr Opin Organ Transplant* 2017; **22**: 372.
5. Muthusamy AS, Mumford L, Hudson A, Fuggle SV, Friend PJ. Pancreas transplantation from donors after circulatory death from the United Kingdom. *Am J Transplant* 2013; **13**: 824.
6. Benz S, Bergt S, Obermaier R, *et al.* Impairment of microcirculation in the early reperfusion period predicts the degree of graft pancreatitis in clinical pancreas transplantation. *Transplantation* 2001; **71**: 759.
7. Carlsson PO, Palm F, Andersson A, Liss P. Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. *Diabetes* 2001; **50**: 489.
8. Sade RM. Transplantation at 100 years: Alexis carrel, pioneer surgeon. *Ann Thorac Surg* 2005; **80**: 2415.
9. Belzer FO, Ashby BS, Dunphy JE. 24-hour and 72-hour preservation of canine kidneys. *Lancet* 1967; **2**: 536.
10. Moers C, Smits JM, Maathuis MH, *et al.* Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med* 2009; **360**: 7.
11. Florack G, Sutherland DE, Heil J, Squifflet JP, Najarian JS. Preservation of canine segmental pancreatic autografts: cold storage versus pulsatile machine perfusion. *J Surg Res* 1983; **34**: 493.
12. Squifflet JP, Gruessner RW, Sutherland DE. The history of pancreas transplantation: past, present and future. *Acta Chir Belg* 2008; **108**: 367.
13. Kojima Y, Nakagawara G, Takeyama S, Imahori T, Miyazaki I. Perfusion preservation of cadaver rat pancreas: I. Morphological observation and biological function of the islets. *Jpn J Surg* 1984; **14**: 42.
14. Leeser DB, Bingaman AW, Poliakova L, *et al.* Pulsatile pump perfusion of pancreata before human islet cell isolation. *Transplant Proc* 2004; **36**: 1050.
15. Taylor MJ, Baicu S, Leman B, Greene E, Vazquez A, Brassil J. Twenty-four hour hypothermic machine perfusion preservation of porcine pancreas facilitates processing for islet isolation. *Transplant Proc* 2008; **40**: 480.
16. Leemkuil M, Lier G, Engelse MA, *et al.* Hypothermic oxygenated machine perfusion of the human donor pancreas. *Transplant Direct* 2018; **4**: e388.
17. Branchereau J, Renaudin K, Kervella D, *et al.* Hypothermic pulsatile perfusion of human pancreas: preliminary technical feasibility study based on histology. *Cryobiology* 2018; **85**: 56.
18. Prudhomme T, Renaudin K, Lo Faro L, *et al.* Ex-situ hypothermic perfusion of non-human primate pancreas: a feasibility study. *Artif Organs* 2020; **44**: 736.
19. Kuan KG, Wee MN, Chung WY, *et al.* A study of normothermic hemoperfusion of the porcine pancreas and kidney. *Artif Organs* 2017; **41**: 490.
20. Kelly WD, Lillehei RC, Merkel FK, Idezuki Y, Goetz FC. Allotransplantation of the pancreas and duodenum along with the kidney in diabetic nephropathy. *Surgery* 1967; **61**: 827.
21. Ploeg RJ, Goossens D, Sollinger HW, Southard JH, Belzer FO. The Belzer-UW solution for effective long-term preservation in canine pancreas transplantation. *Transplant Proc* 1989; **21**: 1378.
22. Belzer FO, D'Alessandro AM, Hoffmann RM, *et al.* The use of UW solution in clinical transplantation. A 4-year experience. *Ann Surg* 1992; **215**: 579; discussion 84–5.
23. Carden DL, Granger DN. Pathophysiology of ischaemia-reperfusion injury. *J Pathol* 2000; **190**: 255.

24. Lakey JR, Tsujimura T, Shapiro AM, Kuroda Y. Preservation of the human pancreas before islet isolation using a two-layer (UW solution-perfluorochemical) cold storage method. *Transplantation* 2002; **74**: 1809.
25. Corlett MP, Scharp DW. The effect of pancreatic warm ischemia on islet isolation in rats and dogs. *J Surg Res* 1988; **45**: 531.
26. Tanioka Y, Hering BJ, Sutherland DE, *et al*. Effect of pancreatic warm ischemia on islet yield and viability in dogs. *Transplantation* 1997; **64**: 1637.
27. Zhao H, Alam A, Soo AP, George AJT, Ma D. Ischemia-reperfusion injury reduces long term renal graft survival: mechanism and beyond. *EBioMedicine* 2018; **28**: 31.
28. van Thienen JV, Fledderus JO, Dekker RJ, *et al*. Shear stress sustains atheroprotective endothelial KLF2 expression more potently than statins through mRNA stabilization. *Cardiovasc Res.* 2006; **72**: 231.
29. Adams GN, Stavrou EX, Fang C, *et al*. Prolylcarboxypeptidase promotes angiogenesis and vascular repair. *Blood* 2013; **122**: 1522.
30. Fukae K, Tominaga R, Tokunaga S, Kawachi Y, Imaizumi T, Yasui H. The effects of pulsatile and nonpulsatile systemic perfusion on renal sympathetic nerve activity in anesthetized dogs. *J Thorac Cardiovasc Surg* 1996; **111**: 478.
31. Barlow AD, Hamed MO, Mallon DH, *et al*. Use of ex vivo normothermic perfusion for quality assessment of discarded human donor pancreases. *Am J Transplant* 2015;**15**: 2475.