Journal of Surgery
Flores Carvalho M, et al. J Surg 8: 1760
www.doi.org/10.29011/2575-9760.001760
www.gavinpublishers.com
Research Article

Machine Perfusion of the Pancreas: What Can We Learn from five Decades of Experimental Studies?

Mauricio Flores Carvalho[1], Fabio Staderini[2], Janina Eden[3], Nadia Navari[2], Mattia Dimitri[4], Andrea Corvi[4], Fabio Cianchi[2], Adriano Peris[5], Paolo Muiesan[6,7], Philipp Dutkowski[3], Fabio Marra[1,8], Daniele Dondossola[7], Andrea Schlegel[1,3,7,9*]

[1]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

[2]Department of Surgery and Translational Medicine, University of Florence, Firenze, Italy

[3]Swiss HPB and Transplant Center, Department of Visceral Surgery and Transplantation, University Hospital Zurich, 8091 Zurich, Switzerland

[4] Department of Industrial Engineering, University of Florence, Firenze, Italy

[5]Tuscany Regional Transplant Authority, Centro Regionale Allocazione Organi e Tessuti (CRAOT), Florence, Italy

[6]Hepatobiliary Unit, Careggi University Hospital, University of Florence, 50134 Florence, Italy

[7]Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Centre of Preclinical Research, 20122 Milan, Italy

[8]Center for Research, High Education and Transfer DENOThe, University of Florence, Florence, Italy

[9]Department of Pathophysiology and Transplantation , Università degli Studi Milan, 20122, Italy

[*]**Corresponding author:** Andrea Schlegel, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Centre of Preclinical Research, 20122 Milan, Italy

Citation: Flores Carvalho M, Staderini F, Eden J, Navari N, Dimitri M, et al. (2023) Machine Perfusion of the Pancreas: What Can We Learn from five Decades of Experimental Studies?. J Surg 8: 1760 DOI: 10.29011/2575-9760.001760

Received Date: 18 March, 2023; **Accepted Date:** 21 March, 2023; **Published Date:** 23 March, 2023

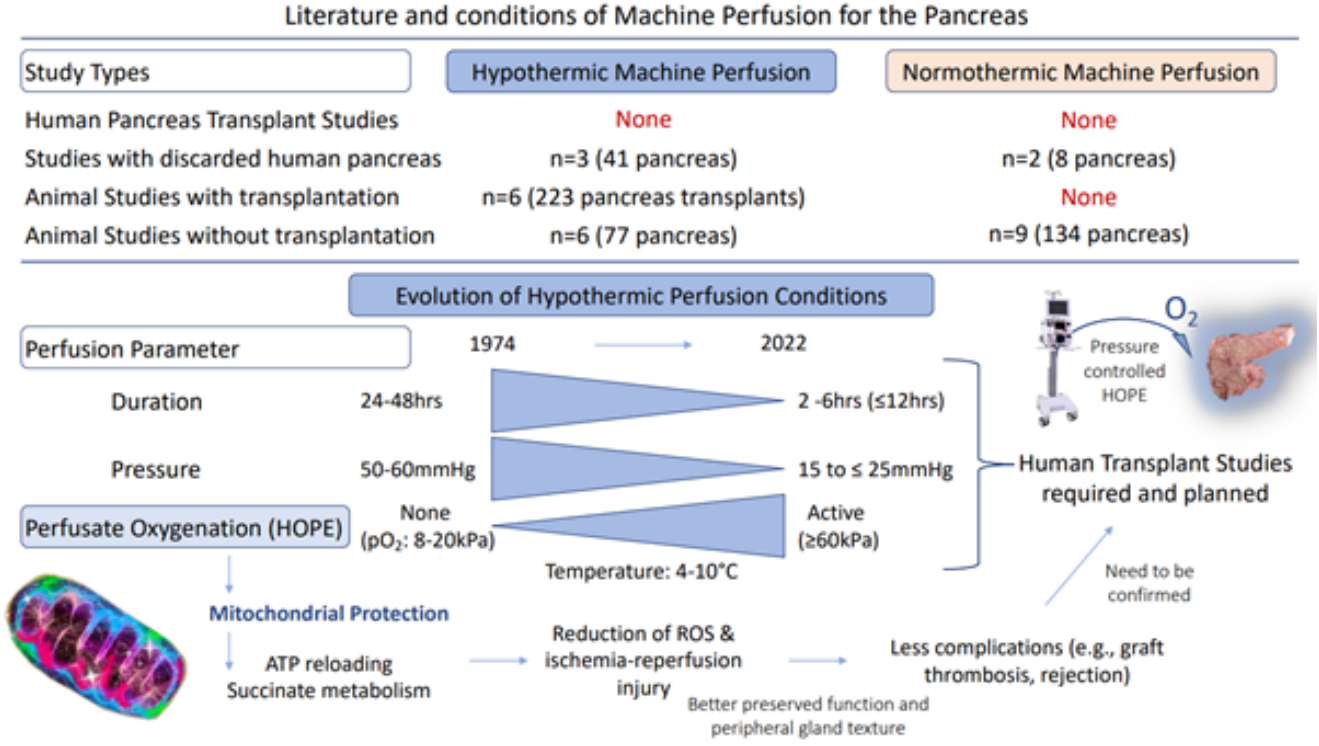
Abstract

Background: Pancreas transplantation is currently the best option for patients with severe complications of diabetes. This organ is however particularly vulnerable to Ischemia-Reperfusion-Injury (IRI) and the transplant procedure is associated with a high risk for recipient complications. It is therefore surprising, that testing and routine use of dynamic preservation strategies is lacking behind other solid organs.

Methods: This study includes first, a literature review on the evolution of cold and warm pancreas machine perfusion strategies. Second, pressure-controlled hypothermic oxygenated perfusion (HOPE, pO_2>60kPa) with fluoresceine is performed in porcine pancreases.

Results: No single human pancreas transplant study with machine perfusion is available. A few older animal studies exist with prolonged Hypothermic Machine Perfusion (HMP), with however high perfusion pressures and a lack of active perfusate oxygenation. Tissue oedema and inflammation were the direct consequences. Only recently, such HMP-conditions were adapted, providing an actively oxygenated perfusate at lower perfusion pressures and shorter durations. Such HOPE-treatment was found superior to cold storage and normothermic perfusion in early experimental studies. In our series, HOPE achieved a complete pancreas perfusion, as confirmed by fluorescence despite lower perfusion pressures.

Conclusion: HMP with active perfusate oxygenation may achieve similar protective effects in pancreases as seen with livers and kidneys. Lower perfusion pressures appear sufficient to distribute the required oxygen for mitochondrial reprogramming to reduce posttransplant IRI. Prospective clinical studies are planned to test the HOPE-technique in human pancreas transplantation.



Keywords: Hypothermic oxygenated perfusion; Oxygen; Pancreas; Perfusion parameters; Perfusion quality; Mitochondria

Introduction

Pancreas transplantation is the most effective treatment for patients with diabetes and related severe complications, including end-stage renal disease [1-4]. The pancreas is however particularly vulnerable to ischemia-reperfusion-injury (IRI) with microcirculatory failure and severe complications, including graft thrombosis and pancreatitis, responsible for 20-50% of graft losses [2,5,6]. Graft pancreatitis is directedly related to advanced IRI and often clinically silent. One third of such cases occurs as an early form within 3 months after transplantation and leads to graft loss in up to 90% [6]. The Standard Cold Storage (SCS) appears therefore insufficient to preserve such glands, particularly when procured from marginal donors or after circulatory death (DCD) with a high donor risk profile. Based on this and with the increasing donor age, the risk-appetite is rather low and leads to an overall high global pancreas discard rate of 30-50% with the exception of a few experienced centres [1,5]. Dynamic preservation techniques have gained renewed attention in all solid organs. Despite the first application of this technology in pancreases as early as in 1974, the routine clinical use of such concepts lacks behind most other solid organs [7-9]. The first perfusion concept includes the recirculation of artificial perfusion fluids under cold conditions. Although this Hypothermic Machine Perfusion (HMP) technique was tested in pancreas as early as in kidneys, it is not used in clinical pancreas transplantation yet. This is in contrast to the field of kidney transplantation, where HMP techniques have achieved commissioning for routine clinical use in several countries[10]. With an increasing need to improve available organs, the HMP equipment is currently developed further and the role of real-time viability tests is explored. While the HMP concept was introduced first in the United States (US), centres in Europe have started to use this technology to perfuse high-risk human livers in 2012 using highly oxygenated perfusates. The protective effect of this Hypothermic Oxygenated Perfusion (HOPE) on posttransplant outcomes was described in many retrospective clinical studies and randomized controlled trials (RCTs) in various solid organs [10-13]. Available studies showed a reduction of early allograft dysfunction (EAD) and recipient complications (e.g., biliary complications for livers and acute rejection for kidneys), better graft survival and lower retransplantation rates after liver and kidney transplantation [10-12,14,15]. The underlying mechanism of the HOPE-technique is based on mitochondrial protection with the reestablishment of an aerobe cellular respiration, previously interrupted during warm and cold ischemia. The direct consequence is the metabolism of accumulated toxic metabolites (e.g., succinate, NADH) and the reloading of Adenosine-Trisphosphate (ATP). This was demonstrated in hearts, lungs, livers and kidneys with a strict dependency on high perfusate oxygen levels of >60kPa [16-20]. In addition, a HOPE-duration of 2hrs seems important to effectively recharge cells with enough ATP [21,22].

In contrast, in most studies hypothermic pancreas perfusion was prolonged with >24hrs to replace cold storage with high perfusion pressures between 30-60mmHg [9,23-29]. Only more recently, such conditions were challenged and modified with lower pressures and shorter perfusion durations. Surprisingly, the most relevant molecule, oxygen, was kept rather low without active perfusate oxygenation in most studies [9,23,24,26-28]. Such controversial perfusion conditions are thought to be one main reason for the slow progress with dynamic perfusion technologies in pancreas transplantation. With the recent improvement of available perfusion devices, Normothermic Machine Perfusion (NMP) of the pancreas is increasingly explored. The advantage is the opportunity to test viability and to "imitate" the transplant setting as preclinical intervention [7,30,31]. Comparative clinical studies with transplantation after cold or warn machine perfusion are lacking. Only a few experimental studies exist, but not only for pancreas, also with other solid organs, such as livers or kidneys [32-34]. Only two studies are currently ongoing comparing different preservation methods in liver transplantation. For the pancreas, the next step is to better understand the underlying mechanisms of such techniques and the potential clinical benefit. The aim of this study was therefore to first, critically review and discuss the current literature on different perfusion concepts for the pancreas and secondly, to provide an initial experience of the HOPE-technique in pancreases with a perfusion device, routinely used for clinical liver and kidney perfusions before transplantation.

Materials and Methods

Literature Review

To identify the most beneficial settings for our perfusion series, a literature review was performed first, summarizing the evolution of the literature of pancreas machine perfusion between 1974 and 2023. The entire spectrum of machine perfusion concepts was considered.

Porcine Model and Experimental Groups

Next, porcine pancreases were procured en-bloc with liver and the bowel package from adult pigs (slaughterhouse). Healthy control pancreases were immediately flushed with UW-solution and sampled as control (DBD baseline control group, n=6). Organs in the injury group underwent 90min asystolic donor warm ischemia time (DWIT), followed by cold flush and 5hrs standard cold storage (SCS; n=6). In the perfusion group, such pancreases with DWIT underwent additional 2hrs of HOPE (n=6). To assess the pancreas perfusion quality, additional HOPE-experiments were done with the substitution of fluoresceine (0.25g; concentration 0.5g/5ml) to the perfusate (n=3).

Hypothermic Oxygenated Perfusion (HOPE) of The Pancreas

At the end of 90min DWIT all pancreas were flushed and stored in UW-solution for 5hrs. During later bench preparation another flush with 500ml UW-solution was performed and organs were separated from the liver, the bowel and the spleen with meticulous closure of small vascular branches to ensure appropriate perfusion. Both arterial vessels, supra-mesenteric and splenic artery were cannulated and used as perfusion route. Portal- and splenic veins were kept untouched for a passive outflow. The Vitasmart^{Ò} (Bridge to life Ltd; Medica) was used for the 2-hour HOPE procedures with UW-Machine Perfusion Solution (UW-MPS). This pressure-controlled device is CE-marked for clinical liver and kidney perfusions. The circuit with the standard disposable available for clinical applications was used and includes pressure, flow and temperature sensors and provides an active perfusate oxygenation (Oxygenator: Euroset^{Ò} EU5054). The device monitors pressure, flow, resistance and temperature in real-time.

Endpoints

Perfusate oxygen levels were measured through blood gas analysis. Further parameters of perfusion quality were measured by the device. HOPE-perfusates were obtained to quantify perfusate levels of Flavin-Mononucleotide-Levels (FMN) and NADH using spectroscopy. Such parameters are increasingly discussed as surrogate of mitochondrial function and injury [35-37]. Based on previous studies with other organs, perfusates were obtained within the first 30min of HOPE. Shortly, triplicates of 50ml perfusate were pipetted into standard 96 vial plates with a dilution of 1:4. Perfusate FMN levels were measured using the standard technology of spectroscopy with an excitation and emission wavelength of 485nm and 528nm, respectively. The perfusate NADH-levels were determined with an excitation and emission wavelength of 360nm and 460nm, respectively. Further details regarding the methodology can be found elsewhere [16,38,39]. At the end of cold storage or additional HOPE the pancreas weight was measured and tissues were obtained for histological assessment. Standard processing with formalin and embedding procedures were performed. Three staining procedures were done: Hematoxylin-Eosin (H&E), Toll-like-receptor-4 (TLR-4; macrophages, dendritic cells, Lifespan Bioscience: LS-B2070) and von Willebrand factor (vWF; endothelial cells, DAKO: A0082). Quantifications of TLR-4 - positive and vWF-positive cells were determined by manual counting in 20 random visual fields per experimental group. All histological analyses were performed in a blinded fashion with respect to the experimental groups. Vessels were excluded from the analysis.

Statistics, Quality Control and Ethical Approval

Completeness, plausibility, and validity of the data were independently verified (by MFC, RP and AS). Continuous variables are demonstrated as median and interquartile range (IQR). Statistical analysis was performed using the non-parametric Mann-Whitney-Wilcoxon U-test (GraphPad Prism, version 7.0, San Diego, CA, USA). P-values of <0.05 were considered significant. The experiments were carried out according to European Union (EU) directive guidelines (2010/63/EU) and Italian legislation (DLgs 26/2014) at the Centro Polyvalent Florence University (Cubo; Viale Gaetano Pieraccini, 6, 50139 Firenze FI, Italy).

Results

What Is Available In The Literature?

In 1974, Eloy et al presented the first study with Normothermic Machine Perfusion (NMP) of a canine pancreas, performed for 100min to test the secretory pancreas function [8]. This was paralleled by a first experimental comparison of 24hrs hypothermic machine perfusion (HMP) and SCS with subsequent transplantation of the canine pancreas [9]. Although the outcome of this pioneer study was not very promising, it triggered a series of experimental studies in the following years and instigated a controversial discussion regarding the best possible perfusion concepts. A total of 23 studies, 15 with HMP and 11 with NMP, were reported in the literature. The majority included pancreases from brain death donors (DBD; n=18) exposed to long CS or HMP. While no study exists today with transplantation of perfused human pancreas, five studies utilized discarded human pancreases [29,40-43]. Only Hamaoui et al have assessed IRI-features during NMP after previous HMP or standard CS in discarded human pancreases. In this study, HMP was performed with active oxygenation (HOPE) and resulted in a better organ functionality (Table 1)[41]. Dogs (n=8) and pigs (n=9) were the most frequent species in non-human studies. Since the seventies, the following perfusion systems were referenced in the literature as being used for pancreas HMP including the Gambro^{Ò}, the RM3 Machine Perfusion Unit, the Max-100 perfusion device (Waters Medical System^{Ò}), the Waves kidney perfusion device or LifePort^{Ò} kidney transporter [5,26,27,29,41].

Normothermic Pancreas Perfusion

The known advantage of Normothermic Machine Perfusion (NMP) as seen in other solid organs is the opportunity to assess viability. To perform the best possible NMP with near-physiologic conditions, an optimized equipment is needed. Organ positioning is one key feature to avoid pressure necroses particularly during prolonged perfusion as recently described with full and partial human livers [44,45]. The first experimental pancreas study with NMP was done as early as in 1974 [8]. Until today, two studies describe NMP in discarded human pancreas (Table 1) and nine experimental non-transplant studies were published with pancreases from pigs, dogs or rodents (Supplementary Table 2). Unfortunately, the studies with human organs lack a comparator or

baseline control group [42,43]. Barlow et al performed a short (1-2hrs) endischemic NMP of discarded DBD pancreases after 13hrs cold storage and demonstrated the link between a higher donor risk and perfusate amylase and lipase levels. This study also showed higher levels of insulin secretion from younger pancreas[42]. Three of five organs developed focal acinar necrosis and one showed extensive fat necrosis in histology. In contrast, Nassar et al performed NMP for 6hrs after shorter cold storage of only 4hrs with better results. Such human pancreas showed healthier acini at the end of 6hrs NMP with normal chromogranin staining (Table 1)[43]. Such findings parallel recent clinical studies with NMP in livers. Posttransplant results were superior with NMP when performed instead of cold storage compared to an endischemic approach [46]. NMP in the recipient center after transport led to a comparably high rate of non-anastomotic strictures in livers with higher risk, e.g., from DCD donors [47,48]. Animal studies further supported the recent findings with perfusion of human pancreases. Shorter endischemic NMP of 90min to 5hrs resulted in well-maintained tissue and limited inflammation[8,30,31,49-54]. Most studies included DBD pancreas and lack a high risk donor model. In-house devices and ECMO-based equipment were used by many. Various perfusate compositions were reported in the literature with autologous whole blood or washed erythrocytes as oxygen carriers in most recent studies [30,31,49]. Particularly in older studies the cold ischemia time before NMP is frequently not reported (Supplementary Table 2) [8,52-55].

What are Optimal Perfusion Conditions for Hypothermic Machine Perfusion?

Five transplant studies explored the effect of non-actively oxygenated, prolonged HMP (24-48hrs) with high perfusion pressures (30-60mmHg) [9,23,24,56]. Three studies showed an equal or even better recipient survival and pancreas function after 24-48hrs SCS compared to such high-pressure-HMP [9,23,24]. Already in 1975, Tersigni et al described improved posttransplant results with lower perfusion pressures of 10-25mmHg[25]. While a perfusion at too low pressures (e.g., 10mmHg) led to incomplete pancreas perfusion with limited protective effects, high pressures between 30 and 70 mmHg caused pancreatic edema [5,7,24,27]. Such findings were paralleled by Karcz et al, who showed good outcomes and a minimal weight gain with a pressure between 15-23mmHg [57]. Mild or moderate edema may be even beneficial for islet digestion and isolation [49]. Most authors would probably agree with a peak perfusion pressure of 15-25mmHg during HMP [1,5,7]. Next, in addition to organ temperature and tissue quality, perfusion flow is related to the set pressures. Toledo-Pereyra et al demonstrated superiority of a pulsatile flow for arterial systems, to maintain sheer-stress regulating the inflammatory response of endothelial cells; an enhanced expression of Kruppel-like-factor-2 is discussed as relevant for the microcirculation with potential antithrombotic properties [5,58]. Flow- and pressure-related effects are also linked to the perfusion duration. Most older studies aimed to replace SCS with HMP and the subsequently long HMP-duration contributed to pancreas edema with often no superiority compared to SCS, particularly with high pressures. Shorter HMP, e.g., 12-24hrs, was found superior compared to 48hrs HMP, where posttransplant survivals were seen inferior as demonstrated by Florack et al [5,23,58]. The addition of mannitol to the perfusate can help to minimize graft edema [1,41,57]. In 1992, Kenmochi et al explored the impact of a short 1hr endischemic HMP demonstrating more pancreas edema, inflammation and dysfunction with prolonged Donor Warm Ischemia Times (DWIT) of 30-60min [56]. Despite this important step towards an endischemic HMP-approach, the perfusion was probably too short, with high pressures of 50mmHg (Table 1)[56]. Such findings were paralleled by perfusion studies in other organs. Results from HOPE-treatment of livers demonstrated that too high pressures induce damage already during the first perfusion hour and diminish the protective effect achieved with high perfusate oxygen levels [22].

How Much Perfusate Oxygen is Beneficial During Hypothermic Perfusion?

In recent studies, perfusion pressure and duration were reduced to 25mmHg and 6hrs, respectively. The low perfusate oxygen levels may be an additional reason for the limited HMP-effect in earlier studies. Authors often claim to use an oxygenated perfusate with however no further details on oxygenator type or size and the lack of perfusate oxygen partial pressures (Table 1). Only two recent studies present perfusate oxygen levels [41,59]. Lemkuil et al from the UK have demonstrated the perfusate oxygen-dependent ATP-reloading of the pancreas, similar as seen with other solid organs. Authors describe less inflammation and better endo- and exocrine pancreas function during later evaluation on the normothermic perfusion device (Tables 2,3)[40]. Similar results are known from kidneys and livers; perfusate oxygen levels of 21% or a pO2 of <20kPa (8-18kPa) is not enough to trigger metabolic changes and rebuild ATP [60-64]. Subsequently, HMP without active oxygenation as done with the LifePort® for many years, has limited effects. A recent RCT demonstrated clear superiority of oxygenated HMP in kidney transplantation [6]. Similar findings are seen with livers; deoxygenated HOPE induced the same IRI as seen with unperfused, cold stored controls [22,65]. Such results are paralleled by the observed tissue protection through the simple addition of oxygen to cold stored organs with persufflation (bubbling) techniques. Higher tissue ATP levels were seen after oxygen persufflation through the graft vasculature. Similarly, as with above described oxygenated HMP such pancreases demonstrated lower levels of IRI and less tissue edema after normothermic reperfusion [66]. The high relevance of oxygen was also demonstrated with the development of preservation concepts where oxygen can be added to standard cold

storage [66]. Kuroda et al. from Japan presented in 1988 a new "Two-layer Method". Perfluorocarbon was added to the conventional cold storage flush solution [67]. These molecules have the unique ability to reversibly bind much more oxygen with a 20-fold higher oxygen concentration compared to human blood. As shown in experiments, Perfluorocarbon forms the lowest layer below the cold storage solution and the oxygen diffuses passively into the pancreas with subsequent ATP recovery. A few early experimental studies demonstrated a prolonged viability of organs. An experimental transplant study showed that slightly more recipients of two-layer method preserved pancreases achieved insulin independency compared to standard cold storage [68]. Although these studies confirm the high relevance of oxygen, the effective penetration of oxygen into deep tissue layers without perfusion remains unproven. Of interest appears also another perfusion parameter: the perfusion duration. Several authors advocate for a 2hrs cold perfusion to achieve the required metabolic switch of mitochondria [16,21,22]. Within the first minutes of HOPE, oxygen reactivates mitochondrial complex proteins with normally directed electron flow and metabolism of previously accumulated toxic metabolites, (e.g., succinate and NADH). Such metabolic changes lead to lower ROS-levels and are key protective effects, also described with HOPE in livers, hearts and kidneys [16,18,19,22,69]. The ATP-reloading during HOPE in pancreas as demonstrated by Leemkuil et al parallels such findings [40].

Year, author, country	Number and type of Pancreas	Experimental groups Perfusion mode	Cold ischemia time before perfusion	Perfusion Duration	Perfusate oxygenation	Perfusion settings	Perfusion device & solution	Results	Discussion
Experimental Studies with hypothermic perfusion of discarded human pancreas and with evaluation during normothermic reperfusion									
Hamaoui et al, J of Surg Research, 2018	Porcine DCD and declined Human (30-55min dWIT) with HMP vs. SCS, 3 study phases (n=12 overall)	Hypothermic oxygenated, endischemic (after SCS), with 2hrs assessment during NMP (phase 2&3)	3-7hrs, 24hrs (porcine); 26.8 and 56hrs (human)	5hrs	Active: Yes pO_2 : 0.95-2.8 kPa/min/mL/g	Temperature: 4°C Pressure: 26-37mmHg (phase 1: 30mmHg, phase 2&3: 20mmHg) Flow: 24.8-29.7mL/ min/100 g; PFI: 0.7-1.19 mL/min/100 g/ mmHg	Waters Medical RM3 Machine Perfusion Unit , modified UW solution	Better endocrine viability and pancreas functionality with HMP-O_2. Weight gain between 3.9-140% in different groups during HMP, low pressure led to very limited weight changes	No transplant, but evaluation during NMP, no information on oxygen levels in perfusate.
Experimental Studies with hypothermic perfusion of discarded human pancreas without evaluation during normothermic reperfusion									
Branchereau J et al, Cryobiology 2018,	Human, DBD, discarded, HMP (n=7) vs. SCS (n=2; 12 & 24hrs), split group: p-head HMP, body/tail SCS	Hypothermic, pulsatile instead of cold storage	Not available*	24hrs	Active: Yes (low) pO_2 : Not available	Temperature: 4°C Syst. Pressure: 25mmHg Flow: pulsatile, rate not available	Waves® Belzer (Perf Gen®), Belzer machine perfusion solution	Cold stored pancreases developed ischemic lesions, which were not seen with HMP, normal immunhistochemistry, RI: 0.22-0.25, Insulin, glucagon normal after HMP	No transplant or evaluation during NMP, no information of cold storage duration before HMP, no information on oxygen levels in perfusate.
Leemkuil et al, Transplantation direct, 2018,	Human, DBD or DCD (14-24min dWIT) with HOPE or SCS (5 each group, total 20)	Hypothermic oxygenated, endischemic (after SCS)	6hrs	6hrs	Active: Yes (flow: 100ml/ min) pO_2 : Not available	Temperature: 4-7°C Syst. Pressure: fixed at 25mmHg Flow: median 36ml/min (DCD), 38.5 and 52ml/min (DBD)	Modified device from Organ Assist®, UW machine perfusion solution	HMP-O_2 uploaded ATP (6.8 fold DCDs, 2.6 fold DBD), high islet viability after isolation from DCD pancreas after HMP-O_2, no signs of ROS-release or inflammation; Amylase, Lipase, LDH increased after HMP-O_2,	No transplant or evaluation during NMP, active oxygenation, no information on oxygen levels in perfusate.
Experimental Studies with nomothermic perfusion of discarded human pancreas without transplantation									
Barlow AD et al, AJT 2015, UK	Human, DBD, discarded, endischemic NMP (n=5)	NMP	13hrs	1-2hrs	Active: Yes, physiological, pO_2 : Not available	Temperature: 37°C Syst. Pressure: fixed at 50-55mmHg Flow: mean: 35 ± 2.8mL/ min/100g	ECMO (pediatric pump, cardiopulmonary bypass), blood based + gelofusine, with sodium, hepatine, mannitol, glucose	Higher perfusate amylase and lipase with higher donor risk, more insulin secretion in pancreas from younger donors, 3/5 with focal acinar cell necrosis, 1/5 with extensive parenchymal and fat necrosis	No transplantation, small case number, DBD model, no comparator group, short perfusion
Nassar A et al, Artificial Organs, 2018, USA	Human, DBD, discarded, endischemic NMP (n=3)	NMP	4hrs 6min	6hrs (n=2), 12hrs (n=1)	Active: Yes, physiological, pO_2 : Not available	Temperature: 37°C Syst. Pressure: fixed at 60mmHg Flow: mean: 55mL /min/100g	In-house device, RBC and plasma, 1:3 ratio	C-peptide levels increased up to 18ng/ml within 6hrs NMP, healthy looking acini at 6 (n=2) and 12hrs (n=1), chromogranin staining normal	No transplantation, small case number, DBD model, no comparator group

*: considering that human pancreas are flushed cold and procured there should be some time of SCS, also because the procurement was en-bloc with donor livers, requiring back table separation and vascular reconstruction for the two main arteries; Fixed pressures is a pressure which is kept at a certain level by the perfusion device and subsequently maintains a specific flow; organ cannot regulate how much flow it prefers, but is forced to accept a specific pressure and flow.

Table 1: Literature overview on hypothermic and normothermic machine perfusion of the human pancreas.

Year, author, country	Number and type of Pancreas	Experimental groups	Cold ischemia time preperfusion	Perfusion Duration	Perfusate oxygenation	Perfusion settings	Perfusion device & solution	Results	Discussion
Experimental studies with hypothermic perfusion of animal pancreas with Transplantation									
Brynger H, Eur Surg Res, 1975, Sweden	DBD, mongrel dog pancreas, recipient pancreatectomy 2days before transplantation, n=20 donors and recipients	24hrs HMP (n=7), vs. 24hrs SCS (n=9), direct transplantation (n=4)	HMP or SCS, no relevant SCS before HMP	24hrs	Active: yes, no oxygenator, oxygenation of organ chamber pO_2 : Not available	Temp.: 6-8°C Pressure: 50/36-44mmHg Flow: 95ml/min	Gambro perfusion machine with oxygenation, buffer-inverted sugar solution	Significant edema during HMP (weight gain 130-270%), HMP: 4/7 death due to bleeding (n=3/7), hypoglycemia (n=1), SCS: 4/9 death due to bleeding (n=3/9), volvolus (n=1/9),	Loss of recipients due to bleeding in the two main groups, no information on oxygen levels
Tersigni R et al, Ann Surg, 1975, USA	DBD mongrel dog pancreas, n=25, allotransplantation	Healthy controls (n=5), HMP 24hrs: at 5mmHg (n=5), at 10mmHg (n=5), at 10mmHg with MCPP (n=5), at 25mmHg (n=5)	24hrs HMP, no relevant SCS before HMP	24hrs	Active: yes (2l/ min) pO_2 : Not available	Temp.: 6°C Pressure: 5, 10 or 25mmHg Flow: pulsatile, 10-60ml/min based on pressure	Pulsatile, max 100, Waters, CPP solution (methylpred, KCl, mannitol, penicillin, $MgSO_4$), MCPP	Better recipient survival with 10mmHg perfusion pressure with MCPP solution instead of HMP with CPP or perfusion with higher pressure of 25mmHg, better enzyme secretion	Experimental group with 25mmHg and MCPP solution is missing, no information on oxygen levels
De Gruyl et al, Br J Surg, 1977, Netherlands	DBD dog pancreas, n=38, 19 transplants	healthy control (n=9), 24hrs HMP (n=5), 24hrs SCS (n=5)	HMP or SCS, no relevant SCS before HMP	24hrs	Active: yes pO_2 : Not available	Temp.: 6-10°C Pressure: 60mmHg Flow: not available	laboratory Belzer machine, CPP solution	Better survival with 24hrs HMP compared to SCS, lower insulin peak in HMP and SCS compared to healhy controls	High perfusion pressure, no information on oxygen levels
Florack G et al, J of Surg Res, 1983, USA	DBD, mongrel dog pancreas, pancreatectomy and segmental pancreas tail transplantation, n=98, 4 and 12 weeks follow up	HMP: with SGF-I and II (24hrs, n=12 each; 48hrs, n=8-10 each); SCS: Collins solution (24hrs, n=12; 48hrs, n=10),or SGF-1 solution, 24hrs (n=12), 48hrs (n=12), 72hrs (n=10), healthy controls, transplant (n=20)	HMP or SCS, no relevant SCS before HMP	24-48hrs	Active: yes pO_2 : Not available	Temp.: 4°C Pressure: 30mmHg Flow: pulsatile, 4.5 (initially), 6.5 (24hrs), 6.3 (48hrs) ml/min SGF-1, 5.3-8ml/min SGF-II	Pulsatile perfusion, SGF-I (dextrose), SGF-II (no dextrose, but Mannitol, Insulin, PSP dye)*	Lower peak amylasis with HMP, long-term pancreas function rate better with SCS compared to HMP (50% with 48hrs HMP with SGF-II vs. 75% with 48hrs SCS with SGF-I), severe edema after high pressure HMP	Assessment of different perfusion solutions, long HMP, no informaiton on oxygen levels
Kenmochi T. et al, Transplantation, 1992, Japan	DCD, dog pancreas, transplants n=13, total n=26	DCD grafts with different donro warm ischemia times: 15min (n=5), 30min (n=9) or 60min (n=7), DBD (no ischemia) controls (n=4)	Details not available, but direct perfusion	1hr	Active: yes pO_2 : Not available	Temp.: 6-10°C Pressure: 50mmHg Flow: 69.3ml / min/100g	ORPH3000C perfusion machine, CPP solution, fibrinogen-free plasma	Groups with 30 & 60min DWIT had edema, more weight gain in 60min DWIT group, decreasing amylasis with higher DWIT, graft prognosis did not correlate with DWIT	High perfusion pressure, no comparison with longer perfusion >1hr, no information on oxygen levels
Prudhomme et al, Transplant international, 2021, UK & France	Porcine pancreas, Feasibility ex-situ perfusion of pancreas, second part: allotransplantation: n=14	Feasibility, HMP vs SCS (n=3 each), transplantation with SCS or HMP of 2 or 6hrs (n=14), diabetic porcine recipient	HMP or SCS, no relevant SCS before HMP	24hrs, 2hrs, 6hrs	Active: no oxygenator, 21% O_2, flow: 1l/min pO_2 : >21kPa (150mmHg)	Temp.: 4-7°C Syst. Pressure: 15mmHg Flow: pulsatile, rate not available	Waves© machine, Perfusate: IGL-1	Safety and feasibility to transplant in a porcine diabetes model, no differences in recipient survival comparing HMP and SCS, mean survival: 14 days	Pancreas with low risk, perfusate not actively oxygenated

MCPP: (replace mannitol, and add albumin, dextrose); HMP-O2: hypothermic perfusion with active oxygenation; PFI: perfusion flow indices; RI: resistance index; SCS: standard cold storage.

Table 2: Literature overview on Hypothermic Perfusion of animal pancreas with subsequent transplantation.

Year, author, country	Number and type of Pancreas	Experimental groups Perfusion mode	Cold ischemia time before perfusion	Perfusion Duration	Perfusate oxygenation	Perfusion settings	Perfusion device & solution	Results	Discussion
Experimental studies with animal pancreas WITHOUT transplantation but with evaluation during normothermic reperfusion on a device									
Ogbemudia et al, Transplant international, 2021, UK & France	Porcine DCD pancreas, 15-30min DWIT	SCS (n=4), HMP-O_2 with UW (n=4) or with IGL-2 (n=5)	3hrs SCS (HMP-O_2 group), 9hrs SCS group	6hrs	Active: Yes (low), casette oxygenator, 21% O_2, flow: 1l/min pO_2: >21kPa (150mmHg)	Temp.: 4-7˚C Syst. Pressure: 15mmHg Flow: pulsatile, rate not available	Waves machine with UW MPS or IGL-2	Higher flow rates and lower resistance during NMP after HMP compared to SCS, tissue weight decreased in HMP group compared to SCS, edema/patchy ischemia during NMP of SCS pancreas, homogenous NMP after HMP	No transplant, but evaluation during NMP, improved perfusion conditions during HMP, active oxygen but only 21kpa
Experimental studies with hypothermic perfusion of animal pancreas WITHOUT Transplantation or evaluation during normothermic reperfusion on a device									
Karcz M et al, Exp Clin Transplant, 2010, UK	Porcine pancreas, DCD (25min total DWIT), n=15	Only one group with HMP	150min	315min	Active: Yes pO_2: Not available	Temp.: 4-10˚C Pressure: 5-13mmHg (first 60min), then 15-23mmHg Flow: >65ml/min/g	Waters Medical RM3 Machine Perfusion Unit, Belzer MPS	Pancreas weight gain 3.2-18.3g, Significant reduction of acinar cell and islet cell damage during HMP	No transplant or evaluation during NMP, only one experimental group, no information on oxygen levels
Taylor MJ et al, Cell transplant, 2010, USA	Porcine pancreas, DBD and DCD	Healthy control (n=7), 24hrs SCS with or without prior DWIT (n=9 each), HMP with KPS-1 or Unisol with or without prior 30min DWIT (n=7 each)	0hrs (HMP), max 2hrs control	24hrs	Active: No pO_2: Not available	Temp.: 5-7˚C Pressure: 10mmHg, Flow: n.a.	Lifeport kidney transporter, Unisol or KPS-1	Islet isolation for viability testing, higher viable islet yield after HMP, also after DWIT, moderate edema	No transplant study or evaluation during NMP, no information on oxygen levels
Weegman BP et al, Cell Transplantation, 2012	Porcine pancreas perfusion and islet isolation and transplantation (nude mice)	HMP (n=4) vs SCS (n=6)		24hrs	Active: No pO_2: Not available	Temp.: 4-8˚C Pressure: 10mmHg, Flow: n.a.	Lifeport kidney transporter, Unisol or KPS-1	Islets isolated and transplanted, 4/4 islet recipients after HMP had complete diabetes reversal compared to 5/6 after SCS	Small case load, no information on oxygen levels, no whole organ transplantation or evaluation during nmp
Prudhomme et al, Artificial organs 2020	Baboon pancreas, SCS control (n=2), HMP (n=5)	SCS vs HMP: 3 groups, perfusion pressure 15mmHg (n=3), 20mmHg (n=1), or 25mmHg (n=1).	minimal	24hrs	Active: Yes pO_2: Not available	Temp.: 4˚C Pressure: 15, 20, 25mmHg Flow: pulsatile, rate not available	Waves kidney perfusion device, IGL-1	Similar edema with different perfusion pressures, increasing injury with prolonged SCS or HMP of >12hrs.	No transplant or evaluation during NMP, small numbers in groups with different perfusion pressures

HMP-O_2: hypothermic perfusion with active oxygenation; PFI: perfusion flow indices; RI: resistance index; SCS: standard cold storage.

Table 3: Literature Review on Hypothermic Perfusion of the animal pancreas without subsequent transplantation.

Author, year, country	Number and type of Pancreas	Experimental groups Perfusion mode	Cold ischemia before perfusion	Perfusion Duration	Perfusate oxygenation	Perfusion settings	Perfusion device & solution	Results	Discussion
Eloy et al, Eur Surg Res, 1974, France	DBD dog pancreas, n=15	NMP only, test endo-+exocrine hormone response	Not available	100min	Active: Yes pO_2: Not available, O_2-consumption: 0.75mL/min/100g	Temperature: 39-40˚C Pressure: not available Flow: 38-46mL/min/100g	Roller pump, disc oxygenator, heat exchanger, momologous erythrocytes+Earles salt, glucose, albumine, dextrane	Glucose tolerance test: constant insuline secretion, increased when glucose reached 140mg/mL, excocrine response to secretin/cholecystokinin infusion	No transplantation, DBD model, no comparator group, short perfusion,
Loubatières-Mariani MM et al, Diabetologia 1980, France	DBD model of wistar rats pancreas, n=unspecified	Low or high glucose concentration to stimulate secretion at 28˚C or 37.5˚C, affect of Tolbutamide, Acethylcholine, Arginine assessed	Not available	90min	Active: Yes pO_2: not available	Temperature: 28 or 37.5˚C Syst. Pressure: 35cmH2O (37.5˚C group); 37.5cmH2O (28˚C) Flow: 2.4ml/min	Unspecified perfusion system, perfusion with glucose for 45min + Krebs Ringer bicarb buffer+albumin, glucose	Lower insulin output at lower temperatures; Glucagon secretion not different at 28 and 37.5˚C; Insulin response to Acethylcholine was less at 28˚C than at 37.5˚C; B cell response to tolbutamine was less at 28˚C; Glucagon secretion stimulated by Arginine	Short NMP, no histological assessment, no transplantation
Eckhauser F et al, J Surg Res, 1981, USA	DBD dog pancreas, n not available	NMP group (n not available)	Not available	4-5hrs	Active: Yes pO2: Not available	Temperature: 37˚C Pressure: 90-110mmHg initially, 30-50mmHg Flow: 15-24mL/min	Perfusion apparatus, pulsatile, water bath heating,in-line dialysis, autolog red cells+albumin, mannitol, heparin	Edema in all pancreas, vascular resistance and oxygen consumption suggested as viability criteria,	No transplantation, unknown case number, no comparison with other techniques or perfusion conditions, cold storage prior to NMP not available
Pegg DE et al, J Surg Res, 1982, UK	DBD model, WAG rats, n=45	Recirculation (n=5) for 2hrs; Single-pass method for 2hrs (n=40),	Not available	2hrs	Active: Yes pO_2: not available	Temperature: 38˚C Syst. Pressure: 60mmHg Flow: 2ml/min	Single pass method (recirculation of perfusate) or two way tap, Gelatine polypeptide Haemaccel+calcium,+/-glucose	Lower wet/dry weight ratio with single pass method compared to perfusate recirculation	No transplantation
O`Maller VP et al, J Surg Res 1986, USA	DBD model of dog pancreas, n=24	Healthy controls (n=6), pancreatitis group (n=6), fluosol-perfused controls (n=6), fluosol-perfused pancreas with pancreatitis (n=6)	Not available	4hrs	Active: Yes pO_2: Not available	Temperature: 37˚C Syst. Pressure: 61-95mmHg Flow: 20ml/min	In-house perfusion device, heated chamber, oxygenator, sensors; Fluosol (FC-43)+Glucose, albumin or blood based with autologous blood+heparin, albumin, glucose	Pancreatitis grafts have edema and with fluosol the edema is reduced, lower Amylase-release with fluosol compared to blood-based NMP, histology comparable	No transplantation, panceatitis model instead of no donor model comparable to clinical situation, cold storage not reported,
Wahlberg J et al, Transplant Int 1989, USA	DBD model of dog pancreas, n=30	Healthy control (n=6); 24 or 48hrs SCS + NMP with/without Allopurinol (n=6 each; 4x6);	24 and 48hrs	2hrs	Active: Yes pO_2:350-450 mmHg	Temperature: 37˚C Syst. Pressure: 61-95mmHg Flow: 20ml/min	In-house device, roller pump, heat exchanger, oxygenator; UW solution with dextran with/without allopurinol	No effect of allopurinol, more weight gain and amylase release during NMP in SCS pancreas	No transplantation, NMP with UW and short
Kuan KG et al, Artif. Organs, 2017 UK, Australia	DBD porcine pancreas, n=4	Isolated pancreas perfusion (n=2), pancreas and kidney (n=2)	34min	2-4hrs	Active: Yes pO_2: Not available	Temperature: 37˚C Pressure: 70-80mmHg Flow: 0.37L/min	Roller pump system, water bath heating, autolog whole blood-based	Moderate/severe edema after 90min NMP, acinar damage, inflammation and thrombosis after NMP	No transplantation, small case number, no comparison with other techniques
Kumar R et al, In J Surg, 2018, UK	Porcine pancreas (n=9)	Control pancreas (50mmHg pressure, 3h perfusion) vs 20mmHg & 4hrs perfusion;	127 and 136min	4hrs	Active: Yes mean arterial O2 pressures: 76.7kPa; venous: 6.2kPa	Temperature: 37˚C Syst. Pressure: 50mmHg à 141ml/min flow; 20mmHg à 40ml/min flow	In-house device, autologous blood, heparin, cefuroxime	Low pressure (20mmHg) pancreas NMP achieved better results with significnatly better cell death profiles (e.g. lower anti-caspase 3 positivity and ATP-synthetase activity compared to 50mmHg	No transplantation, minimal injury
Parmentier C et al, JOVE 2022, Canada	Porcine DBD pancreas, n=7	NMP, one group, n=7	2hrs	3hrs	Active: Yes pO2: Not available, Oxygen consumption 100-200ml/min/g	Temperature: 37˚C Syst. Pressure: fixed at 20-25mmHg Flow: mean: 100-120ml/min	neonatal cardiopulmonary bypass equipment, washed erythrocytes, steen solution, ringers, heparin, bicarb, aprotinin	NMP: pH: 7.35-7.4; Amylase increases to >20000U/L; Lipase to 3500 U/L; good histological results after 3hrs NMP	No transplantation, small case number, DBD model, no comparator group

*: considering that human pancreas are flushed cold and procured there should be some time of SCS, also because the procurement was en-bloc with donor livers, requiring back table separation; Fixed pressures is a pressure which is kept at a certain level by the perfusion device and subsequently maintains a specific flow; organ cannot regulate how much flow it prefers, but is forced to accept a specific pressure and flow; HMP-O2: hypothermic perfusion with active oxygenation; PFI: perfusion flow index; RI: resistance index; SCS: standard cold storage

Table 4: Overview on Normothermic Perfusion of animal pancreas.

Series of Experimental HOPE-Perfusions of The DCD Pancreas

DCD pancreas underwent 5hrs SCS and subsequent 2hrs HOPE treatment (n=6) with the following conditions: pressure controlled system with a perfusion pressure of 15-20mmHg; the perfusion flow was pulsatile and ranged between 80-110ml/min/g; the observed resistance index was 0.7-0.8; the measured perfusion temperature was 6-10°C; perfusate: 1.5l UW-Machine perfusion solution. The median pancreas weight was 324g (IQR: 52g) with a weight gain of 4.2-14.8% during perfusion. Perfusate oxygenation was kept above a pO_2 of 60kPa, confirmed with blood gas analysis; pO_2 pressures of 69-83kPa were achieved within the first 30min of HOPE and maintained thereafter. Three DCD pancreases underwent HOPE with fluorescein and demonstrated a homogenous fluorescein staining throughout the entire gland within the first 15min of perfusion. The histological assessment confirmed the fluorescein distribution (Figure 1).

Figure 1: Pancreas preparation and Hypothermic oxygenated pancreas perfusion

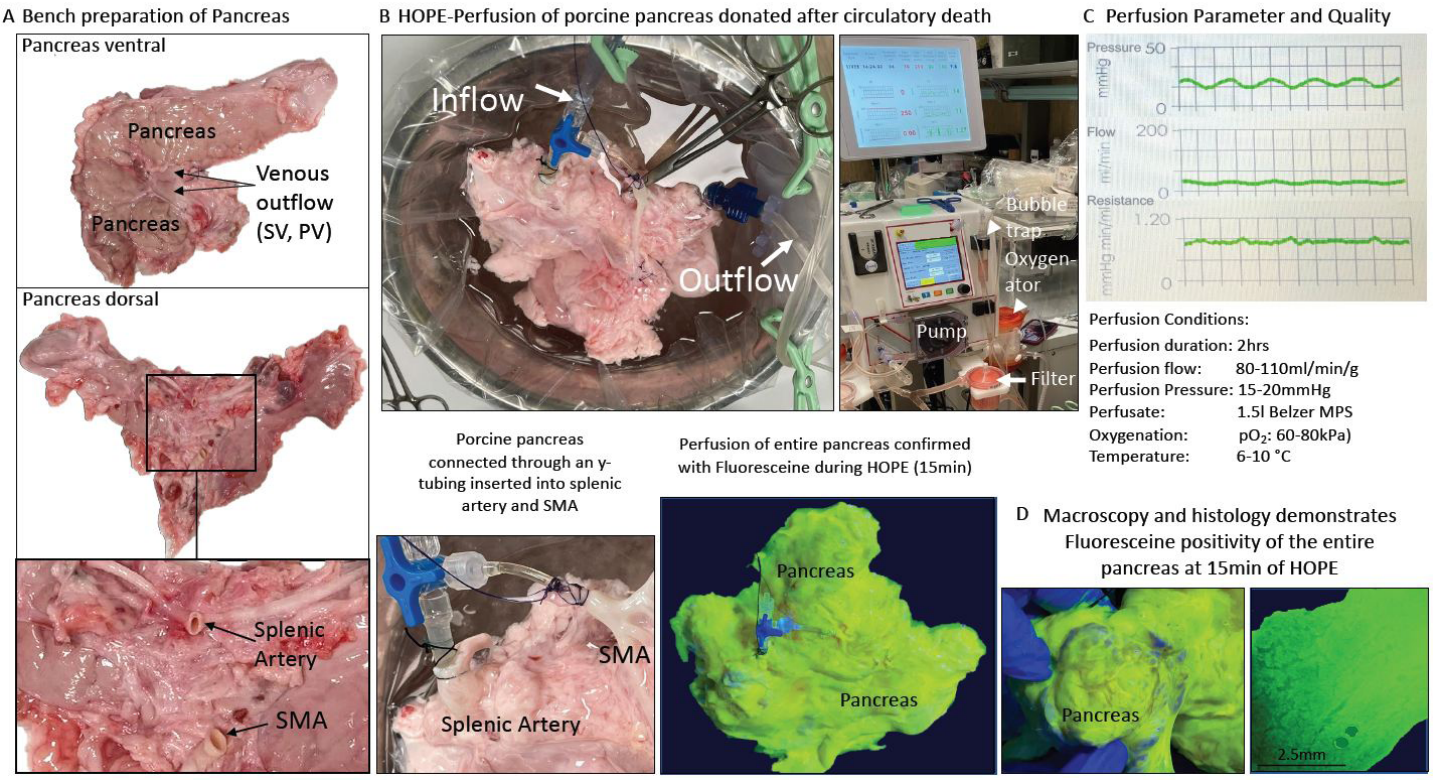


Figure 1: Pancreas preparation and Hypothermic oxygenated pancreas perfusion. Bench preparation of the porcine DCD pancreas **(A)** and cannulation of both arterial inflow vessels, e.g., SMA and SA to connect the pancreas with the device for hypothermic oxygenated perfusion (HOPE)**(B).** Pancreas perfusion parameters obtained during HOPE **(C).** Fluoresceine stained pancreas after 15min of HOPE, macroscopic and histological image after fluoresceine staining **(D).** HOPE: Hypothermic oxygenated perfusion; PV: portal vein; SA: splenic artery; SMA: supra-mesenteric artery; SV: splenic vein;

As described with different solid organs and first in hearts in 1969, mitochondrial complex-I releases flavin-mononucleotide (FMN) and NADH during re-oxygenation [16,22,39,69,70]. Such molecules have auto-fluorescent characteristics and are increasingly used for liver viability testing before transplantation. Expectedly, both molecules were released from pancreas mitochondria during HOPE with median perfusate concentrations of: FMN: 5461 A.U. (IQR: 4481-5926) and NADH: 7867 (IQR: 7066-8319) A.U.. Compared to HOPE in livers and kidneys, the amount of released FMN in pancreases appears 10- and 3-fold lower, respectively, also based on the smaller organ size, the different mitochondrial density and metabolic activity of the pancreas.

The histological assessment demonstrated no signs of ischemic necrosis or congestion, minimal macrophage activation and inflammation at the end of 2hrs HOPE compared to low risk, baseline control DBD organs (Figure 2). The known dilatation of the peripheral vasculature was also seen here after HOPE of the

pancreas (Figure 2A). This phenomenon was described earlier in other organs, e.g., livers or kidneys. With the need to completely preserve the entire periphery of this specific gland to reduce IRI-associated inflammation, this finding is of particular interest also in context of the here applied lower perfusion pressure of maximal 20mmHg during HOPE. No significant differences were observed regarding the number of proinflammatory tissue macrophages and endothelial cells throughout the study groups (Figure 2D).

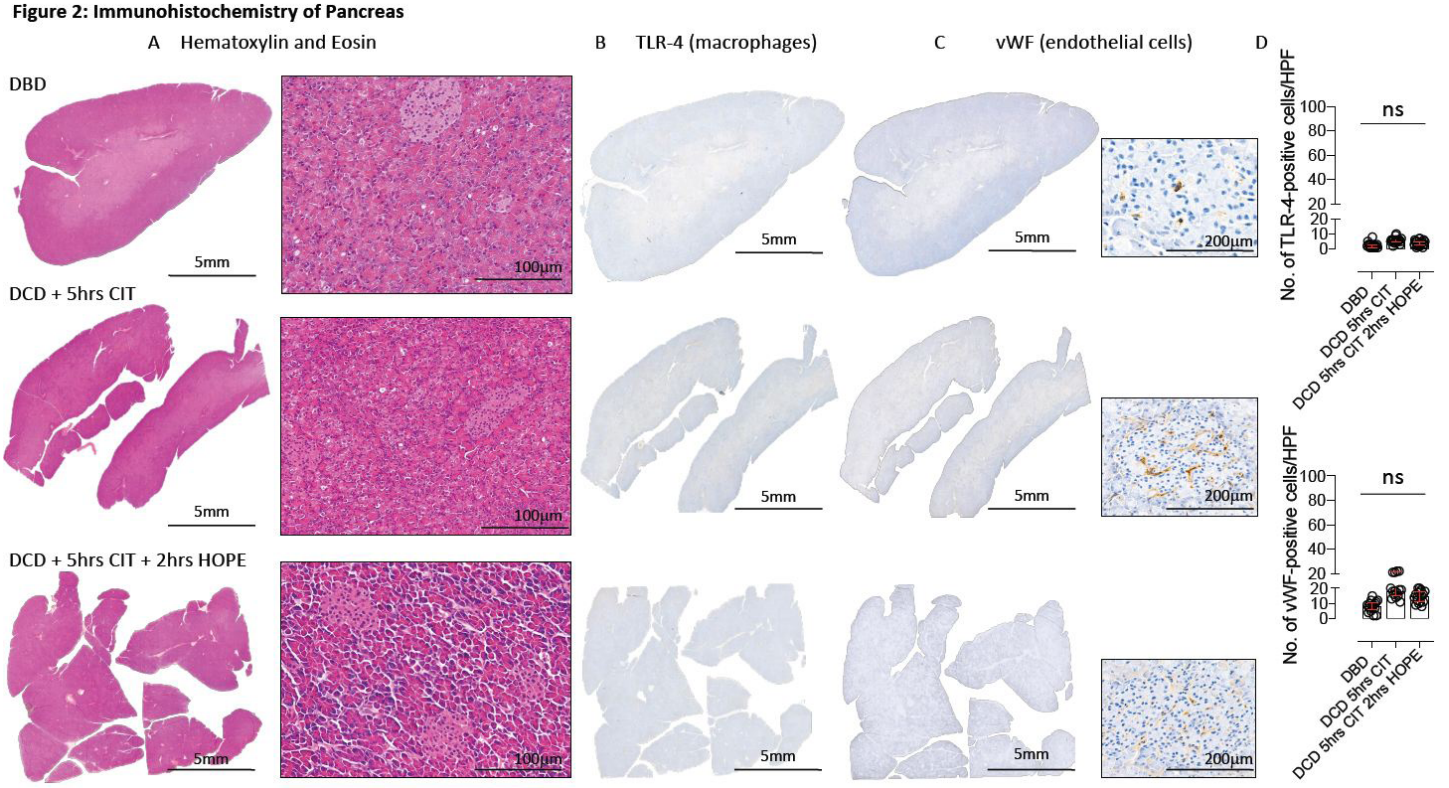


Figure 2: Immunohistochemistry of Pancreas. Images obtained after pancreas staining for Hematoxylin & Eosin **(A),** TLR-4 **(B)** and vWF-positive tissue **(C).** The number of positive cells were quantified per HPF **(D)**; CIT: cold ischemia time; DBD: Donation after brain death; DCD: donation after circulatory death; HOPE: Hypothermic oxygenated perfusion; TLR-4: toll-like receptor 4; vWF: von Willebrand factor;

Discussion

Despite the early testing in 1974, pancreas perfusion remains in its infancy and the routine clinical implementation lacks behind other abdominal organs [71]. Only a few research programs exist to overcome described hurdles, which include the quality of existing studies (e.g., small case load with various, suboptimal perfusion conditions; a few, old studies with pancreas transplantation), the lack of human transplant studies and the small number of active pancreas transplant programs [5,71]. With the low vascularization, the pancreas is particularly sensitive to barotrauma, caused by prolonged perfusion and high pressures. Knowing the various benefits of dynamic organ preservation, including re-oxygenation, toxin-elimination, viability assessment, therapeutic interventions and endothelial cell protection, the pancreas should benefit most [41,72,73].

This literature review and cases series of HOPE in pancreas confirms what is reported with other organs. HMP should be performed at limited systolic pressures of £ 25mmHg for at least 2hrs, but ideally not more than 6-12hrs [5,7,74]. Once the oxygen has converted mitochondrial metabolisms and rebuilt energy in the entire gland, the perfusion pressure could potentially be reduced in cases, where longer perfusion is needed to bridge logistical challenges and to avoid relevant edema and necrosis. Such findings were further paralleled by a greater islet viability isolated from discarded human pancreas after HOPE as shown by Doppenberg et al in 2021[74]. More studies are required to identify such timings and to explore the acceptable SCS-duration before and after HOPE-treatment. The increasing interest in Normothermic Machine Perfusion (NMP) for all solid organs refers to the opportunity to quantify released IRI-associated molecules for viability assessment, which is particularly interesting in steatotic

pancreas and organs from extended criteria donors. With regard to the preservation quality, comparative studies demonstrated however superiority of hypothermic perfusion techniques, thus none included a model of pancreas transplantation [5]. Hamaoui et al reported higher porcine and human pancreas damage with more tissue lesions after normothermic perfusion compared to HOPE [41]. Similar results were shown for livers. In addition, the injury observed during NMP was more pronounced with relevant SCS before perfusion. Recent clinical transplant studies showed this for livers, paralleled by two recent experimental studies with NMP in human pancreas [47,48,75,76]. While, Barlow et al demonstrated severe pancreas damage after endischemic NMP with previous 13hrs SCS, Nassar et al found healthy acini at the end of NMP performed after only 4-6hrs SCS, in a however small number of perfusions (n=3)[42,43]. As with HMP, perfusion pressures during NMP should not exceed 20mmHg[49]. A few groups with increasing expertise in pancreas perfusion are currently developing better circuits to further explore the role of NMP in pancreas. Barlow et al. demonstrated the feasibility of ex-situ pancreas NMP, where perfusate amylase levels correlated well with pancreas fat infiltration and exocrine function [5].

Most viability parameters assessed during NMP appear however quite peripheral to the known IRI-instigator, mitochondria. Pancreas injury and secretory function is frequently assessed during NMP through perfusate amylase and lipase levels; lactates, lactate dehydrogenase, insulin, glucagon, glucose, and C-peptide are further markers of injury and B-cell function [5]. The histology and the perfusion resistance indices may serve as additional parameters to evaluate the pancreas.

In contrast to such markers of downstream injury and function, molecules released from complex-I, such as FMN, were identified by many during the reperfusion of kidneys, livers, hearts and even brain under various conditions, and seem also evident in pancreas as shown here [38,39,70,77]. Perfusate FMN-levels are currently used to accept high-risk DCD livers for transplantation [10,50,52].

The experimental part of this study has some limitations. We are aware that this study does not present functional, molecular or dynamic parameters, which will require larger, prospective trials comparing most recent perfusion concepts in the same donor and recipient risk categories, as currently under preparation.

To increase the utilization of DCD pancreas, where in addition to exocrine dysfunction a low islet yield and beta cell function is known, organs would benefit from HOPE treatment and assessment, explored in upcoming studies [78-80].

The best perfusion conditions suggested for the pancreas based on current literature include: a pulsatile, hypothermic perfusion at 6-10°C with pressure between 15-25mmHg and related flows for a duration of 2-6hrs (max. 12hrs), using a highly oxygenated (pO_2 >60kPa) perfusion solution (e.g., UW-MPS). Devices in current clinical use for livers and kidneys before transplantation can provide safe pancreas perfusion, given they are pressure controlled and administer the required perfusate oxygen needed to recondition mitochondria.

Author Contributions

Data curation, Fabio Staderini, Janina Eden, Nadia Navari and Andrea Schlegel; Formal analysis, Mauricio Flores Carvalho; Funding acquisition, Paolo Muiesan and Andrea Schlegel; Methodology, Mauricio Flores Carvalho and Andrea Schlegel; Resources, Mattia Dimitri, Fabio Cianchi, Adriano Peris, Paolo Muiesan, Philipp Dutkowski, Fabio Marra and Andrea Schlegel; Supervision, Andrea Schlegel; Writing - original draft, Mauricio Flores Carvalho and Andrea Schlegel; Writing - review & editing, Andrea Corvi, Philipp Dutkowski and Fabio Marra.

Funding

Funding was provided by the Swiss National Science Foundation grant no: 32003B-140776/1, 3200B-153012/1, 320030-189055/1, and 31IC30-166909 to P.D. and A.S. This study was supported by University Careggi grant no 32003B-140776/1 dedicated to P.M. This work was further supported by the OTT grant No.: DRGT641/2019 (cod.prog. 19CT03). The authors confirm that all funders played no role in study design, data collection, data analysis, interpretation, or writing of the report.

References

1. Hamaoui K, Papalois V (2019) Machine Perfusion and the Pancreas: Will It Increase the Donor Pool? Curr Diab Rep 19.
2. Gruessner A.C, Gruessner R.W.G (2016) Long-Term Outcome after Pancreas Transplantation: A Registry Analysis. Curr Opin Organ Transplant 21: 377-385.
3. Boggi U, Vistoli F, Marchetti P, Kandaswamy R, Berney T, Andres A, Arbogast H.P, Badet L, Baronti W, Bartlett S.T (2021) First World Consensus Conference on Pancreas Transplantation: Part I-Methods and Results of Literature Search. Am J Transplant 21: 1-16.
4. Boggi U, Vistoli F, Andres A, Arbogast H.P, Badet L, Baronti W, Bartlett S.T, Benedetti E, Branchereau J, Burke G.W (2021) First World Consensus Conference on Pancreas Transplantation: Part II – Recommendations. American Journal of Transplantation 21: 17-p59.
5. Prudhomme T, Kervella D, le Bas-Bernardet S, Cantarovich D, Karam G, Blancho G, Branchereau J (2020) Ex Situ Perfusion of Pancreas for Whole-Organ Transplantation: Is It Safe and Feasible? A Systematic Review. J Diabetes Sci Technol 14: 120-134.
6. Nadalin S, Girotti P, Königsrainer A (2013) Risk Factors for and Management of Graft Pancreatitis. Curr Opin Organ Transplant 18: 89-96.
7. Branchereau J, Hunter J, Friend P, Ploeg R (2020) Pancreas Preservation: Clinical Practice and Future Developments. Curr Opin Organ Transplant 25: 329-335.

8. Eloy M.R, Kachelhoffer J, Pousse A, Dauchel J, Grenier J.F (1974) Ex Vivo Vascular Perfusion of the Isolated Canine Pancreas. Experimental Procedure Haemodynamic Data and Experimental Applications. Eur Surg Res 6 : 341-353.

9. Brynger H (1975) Twenty-Four-Hour Preservation of the Duct-Ligated Canine Pancreatic Allograft. Eur Surg Res 7 : 341–354.

10. Jochmans I, Brat A, Davies L, Hofker H.S, van de Leemkolk F.E.M, Leuvenink H.G.D, Knight S.R, Pirenne J, Ploeg R.J, Abramowicz D (2020) xygenated versus Standard Cold Perfusion Preservation in Kidney Transplantation (COMPARE): A Randomised Double-Blind Paired Phase 3 Trial. The Lancet 396 :1653-1662.

11. Schlegel A, Mueller M, Muller X, Eden J, Panconesi R, von Felten S, Steigmiller K, Sousa Da Silva R.X, de Rougemont O, Mabrut J.-Y (2023) A Multicenter Randomized-Controlled Trial of Hypothermic Oxygenated Perfusion (HOPE) for Human Liver Grafts before Transplantation. J Hepatol.

12. van Rijn R, Schurink I, de Vries Y, van den Berg A, Cortes Cerisuelo M, Darwish M, Erdmann J, Gilbo N, de Haas R, Heaton N (2021) Hypothermic Machine Perfusion in Liver Transplantation — A Randomized Trial. New England Journal of Medicine .

13. Patrono D, Cussa D, Sciannameo V, Montanari E, Panconesi R, Berchialla P, Lepore M, Gambella A, Rizza G, Catalano G (2022) Outcome of Liver Transplantation with Grafts from Brain-Dead Donors Treated with Dual Hypothermic Oxygenated Machine Perfusion with Particular Reference to Elderly Donors. Am J Transplant 22: 1382-1395.

14. Ravaioli M, Germinario G, Dajti G, Sessa M, Vasuri F, Siniscalchi A, Morelli M.C, Serenari M, del Gaudio M, Zanfi C (2022) Hypothermic Oxygenated Perfusion in Extended Criteria Donor Liver Transplantation-A Randomized Clinical Trial. Am J Transplant .

15. Czigany Z, Pratschke J, Froněk J, Guba M, Schöning W, Raptis D, Andrassy J, Kramer M, Strnad P, Tolba R (2021) Hypothermic Oxygenated Machine Perfusion (HOPE) Reduces Early Allograft Injury and Improves Post-Transplant Outcomes in Extended Criteria Donation (ECD) Liver Transplantation from Donation After Brain Death (DBD): Results from a Multicenter Randomized Con. Ann Surg .

16. Schlegel A, Muller X, Mueller M, Stepanova A, Kron P, de Rougemont O, Muiesan P, Clavien P.A, Galkin A, Meierhofer D (2020) Hypothermic Oxygenated Perfusion Protects from Mitochondrial Injury before Liver Transplantation. EBioMedicine 60.

17. Kron P, Schlegel A, Mancina L, Clavien P.A, Dutkowski P (2018) Hypothermic Oxygenated Perfusion (HOPE) for Fatty Liver Grafts in Rats and Humans. J Hepatol 68: 82-91.

18. Wyss R, Méndez Carmona N, Arnold M, Segiser A, Mueller M, Dutkowski P, Carrel T, Longnus S (2020) Hypothermic Oxygenated Perfusion (HOPE) Provides Cardioprotection via Succinate Oxidation Prior to Normothermic Perfusion in a Rat Model of Donation after Circulatory Death (DCD). American Journal of Transplantation.

19. Darius T, Vergauwen M, Smith T, Gerin I, Joris V, Mueller M, Aydin S, Muller X, Schlegel A, Nath J (2020) Brief O 2 Uploading during Continuous Hypothermic Machine Perfusion Is Simple yet Effective Oxygenation Method to Improve Initial Kidney Function in a Porcine Autotransplant Model . American Journal of Transplantation.

20. Nakajima D, Chen F, Okita K, Motoyama H, Hijiya K, Ohsumi A, Sakamoto J, Yamada T, Sato M, Aoyama A (2012) Reconditioning Lungs Donated after Cardiac Death Using Short-Term Hypothermic Machine Perfusion. Transplantation 94: 999-1004.

21. Koetting M, Lüer B, Efferz P, Paul A, Minor T (2011) Optimal Time for Hypothermic Reconditioning of Liver Grafts by Venous Systemic Oxygen Persufflation in a Large Animal Model. Transplantation.

22. Schlegel A, Rougemont O. de; Graf R, Clavien P.A, Dutkowski P (2013) Protective Mechanisms of End-Ischemic Cold Machine Perfusion in DCD Liver Grafts. J Hepatol 58: 278-286.

23. Florack G, Sutherland D.E.R, Heil J, Squifflet J.P, Najarian J.S (1983) Preservation of Canine Segmental Pancreatic Autografts: Cold Storage versus Pulsatile Machine Perfusion. J Surg Res 34: 493-504.

24. de Gruyl J, Westbroek D.L, MacDicken I, Ridderhof E, Verschoor L, van Strik R (1977) Cryoprecipitated Plasma Perfusion Preservation and Cold Storage Preservation of Duct-Ligated Pancreatic Allografts. Br J Surg 64: 490-493.

25. Tersigni R, Toledo Pereyra L.H, Pinkham J, Najarian J.S (1975) Pancreaticoduodenal Preservation by Hypothermic Pulsatile Perfusion for Twenty-Four Hours. Ann Surg 182: 743-748.

26. Taylor M.J, Baicu S, Greene E, Vazquez A, Brassil J (2010) Islet Isolation from Juvenile Porcine Pancreas after 24-h Hypothermic Machine Perfusion Preservation. Cell Transplant 19: 613-628.

27. Weegman B.P, Taylor M.J, Baicu S.C, Scott W.E, Mueller K.R, Kitzmann J.D, Rizzari M.D, Papas K.K (2012) Hypothermic Perfusion Preservation of Pancreas for Islet Grafts: Validation Using a Split Lobe Porcine Model. Cell Med 2: 105-110.

28. Prudhomme T, Renaudin K, lo Faro M.L, Cantarovich D, Kervella D, Minault D, Hervouet J, le Bas-Bernardet S, Karam G, Blancho G (2020) Ex Situ Hypothermic Perfusion of Nonhuman Primate Pancreas: A Feasibility Study. Artif Organs 44: 736-743.

29. Branchereau J, Renaudin K, Kervella D, Bernadet S, Karam G, Blancho G, Cantarovich D (2018) Hypothermic Pulsatile Perfusion of Human Pancreas: Preliminary Technical Feasibility Study Based on Histology. Cryobiology 85: 56-62.

30. Kuan K.G, Wee M.N, Chung W.Y, Kumar R, Mees S.T, Dennison A, Maddern G, Trochsler M (2017) A Study of Normothermic Hemoperfusion of the Porcine Pancreas and Kidney. Artif Organs 41: 490-495.

31. Parmentier C, Ray S, Mazilescu L, Kawamura M, Noguchi Y, Nogueira E, Ganesh S, Arulratnam B, Kalimuthu S, Selzner M (2022) Normothermic Ex Vivo Pancreas Perfusion for the Preservation of Pancreas Allografts before Transplantation. J Vis Exp.

32. Schlegel A, Kron P, Graf R, Dutkowski P, Clavien P.A. Warm vs (2014) Cold Perfusion Techniques to Rescue Rodent Liver Grafts. J Hepatol 61: 1267-1275.

33. Boteon Y.L, Laing R.W, Schlegel A, Wallace L, Smith A, Attard J, Bhogal R.H, Neil D.A, Hübscher S, Perera M.T.P (2018) Combined Hypothermic and Normothermic Machine Perfusion Improves Functional Recovery of Extended Criteria Donor Livers. Liver Transplantation.

34. Kron P, Schlegel A, de Rougemont O, Oberkofler C.E, Clavien P.-A, Dutkowski P (2016) Short Cool and Well Oxygenated - HOPE for Kidney Transplantation in a Rodent Model. Ann Surg.

35. Rouslin W, Ranganathan S (1983) Impaired Function of Mitochondrial Electron Transfer Complex I in Canine Myocardial Ischemia: Loss of Flavin Mononucleotide. J Mol Cell Cardiol.

36. Stepanova A, Sosunov S, Niatsetskaya Z, Konrad C, Starkov A, Manfredi G, Wittig I, Ten V, Galkin A (2019) Redox-Dependent Loss of Flavin by Mitochondrial Complex I in Brain Ischemia/Reperfusion Injury. Antioxid Redox Signal 20: 608-622.

37. Panconesi R, Flores Carvalho M, Mueller M, Meierhofer D, Dutkowski P, Muiesan P, Schlegel A (2021) Viability Assessment in Liver Transplantation—What Is the Impact of Dynamic Organ Preservation? Biomedicines.

38. Muller X, Schlegel A, Kron P, Eshmuminov D, Würdinger M, Meierhofer D, Clavien P, Dutkowski P (2019) Novel Real Time Prediction of Liver Graft Function during Hypothermic Oxygenated Machine Perfusion Prior to Liver Transplantation. Ann Surg 270: 783-790.

39. Wang L, Thompson E, Bates L, Pither T.L, Hosgood S.A, Nicholson M.L, Watson C.J.E, Wilson C, Fisher A.J, Ali S (2020) Flavin Mononucleotide as a Biomarker of Organ Quality - A Pilot Study. Transplant Direct.

40. Leemkuil M, Lier G, Engelse M.A, Ploeg R.J, de Koning E.J.P, T'Hart N.A, Krikke C, Leuvenink H.G.D (2018) Hypothermic Oxygenated Machine Perfusion of the Human Donor Pancreas. Transplant Direct.

41. Hamaoui K, Gowers S, Sandhu B, Vallant N, Cook T, Boutelle M, Casanova D, Papalois V (2018) Development of Pancreatic Machine Perfusion: Translational Steps from Porcine to Human Models. Journal of Surgical Research 223: 263-274.

42. Barlow A.D, Hamed M.O, Mallon D.H, Brais R.J, Gribble F.M, Scott M.A, Howat W.J, Bradley J.A, Bolton E.M, Pettigrew G.J (2015) Use of Ex Vivo Normothermic Perfusion for Quality Assessment of Discarded Human Donor Pancreases. Am J Transplant 15: 2475.

43. Nassar A, Liu Q, Walsh M, Quintini C (2018) Normothermic Ex Vivo Perfusion of Discarded Human Pancreas. Artif Organs 42: 334-335.

44. Eshmuminov D, Becker D, Bautista Borrego L, Hefti M, Schuler M.J, Hagedorn C, Muller X, Mueller M, Onder C, Graf R (2020) An Integrated Perfusion Machine Preserves Injured Human Livers for 1 Week. Nat Biotechnol.

45. Mueller M, Hefti M, Eshmuminov D, Schuler M.J, da Silva R.X.S, Petrowsky H, de Oliveira M.L, Oberkofler C.E, Hagedorn C, Mancina L (2021) Long-Term Normothermic Machine Preservation of Partial Livers: First Experience With 21 Human Hemi-Livers. Ann Surg 274: 836-842.

46. Nasralla D, Coussios C.C, Mergental H, Akhtar M.Z, Butler A.J, Ceresa C.D.L, Chiocchia V, Dutton S.J, García-Valdecasas J.C, Heaton N (2018) A Randomized Trial of Normothermic Preservation in Liver Transplantation. Nature.

47. Mergental H, Laing R.W, Kirkham A.J, Perera M.T.P.R, Boteon Y.L, Attard J, Barton D, Curbishley S, Wilkhu M, Neil D.A.H (2020) Transplantation of Discarded Livers Following Viability Testing with Normothermic Machine Perfusion. Nat Commun 11.

48. Gaurav R, Butler A.J, Kosmoliaptsis V, Mumford L, Fear C, Swift L, Fedotovs A, Upponi S, Khwaja S, Richards J (2022) Liver Transplantation Outcomes From Controlled Circulatory Death Donors: SCS vs in Situ NRP vs Ex Situ NMP. Ann Surg 275.

49. Kumar R, Chung W.Y, Runau F, Isherwood J.D, Kuan K.G, West K, Garcea G, Dennison A.R (2018) Ex Vivo Normothermic Porcine Pancreas: A Physiological Model for Preservation and Transplant Study. Int J Surg 54: 206-215.

50. Mazilescu L.I, Parmentier C, Kalimuthu S.N, Ganesh S, Kawamura M, Goto T, Noguchi Y, Selzner M, Reichman T.W (2022) Normothermic Ex Situ Pancreas Perfusion for the Preservation of Porcine Pancreas Grafts. Am J Transplant.

51. Wahlberg J, Southard J.H, Belzer F.O (1989) Preservation-Induced Pancreatitis in an Isolated Perfused Pancreas Model in the Dog. Transpl Int 2: 165-167.

52. Pegg D.E, Klempnauer J, Diaper M.P, Taylor M.J (1982) Assessment of Hypothermic Preservation of the Pancreas in the Rat by a Normothermic Perfusion Assay. J Surg Res 33: 194-200.

53. Eckhauser F, Knol J.A, Porter-Fink V, Lockery D, Edgcomb L, Strodel W.E, Webb D, Simmons J (1981) Ex Vivo Normothermic Hemoperfusion of the Canine Pancreas: Applications and Limitations of a Modified Experimental Preparation. J Surg Res 31: 22-37.

54. Loubatières-Mariani M.M, Chapal J, Puech R, Lignon F, Valette G (1980) Different Effects of Hypothermia on Insulin and Glucagon Secretion from the Isolated Perfused Rat Pancreas. Diabetologia 18: 329–333.

55. O'Malley V.P, Keyes D.M, Postier R.G (1986) The Fluosol-Perfused Isolated Canine Pancreas: A Model for the Study of Blood Component Effects in Acute Pancreatitis. J Surg Res 40: 210-215.

56. Kenmochi T, Asano T, Nakagouri T, Enomoto K, Isono K, Horie H (1992) Prediction of Viability of Ischemically Damaged Canine Pancreatic Grafts by Tissue Flow Rate with Machine Perfusion. Transplantation 53: 745-750.

57. Karcz M, Cook H.T, Sibbons P, Gray C, Dorling A, Papalois V (2010) An Ex-Vivo Model for Hypothermic Pulsatile Perfusion of Porcine Pancreata: Hemodynamic and Morphologic Characteristics. Exp Clin Transplant 8: 55-60.

58. Toledo Pereyra L.H, Valgee K.D, Castellanos J, Chee M (1980) Hypothermic Pulsatile Perfusion: Its Use in the Preservation of Pancreases for 24 to 48 Hours before Islet Cell Transplantation. Arch Surg 115: 95-98.

59. Ogbemudia A.E, Hakim G, Dengu F, El-Gilani F, Dumbill R, Mulvey J, Sayal K, Prudhomme T, Mesnard B, Rozenberg K (2021) Development of Ex Situ Normothermic Reperfusion as an Innovative Method to Assess Pancreases after Preservation. Transpl Int 34: 1630-1642.

60. Pravisani R, Baccarani U, Molinari E, Cherchi V, Bacchetti S, Terrosu G, Avital I, Ekser B, Adani G.L (2022) PO2 21% Oxygenated Hypothermic Machine Perfusion in Kidney Transplantation: Any Clinical Benefit? Int J Artif Organs 45: 666-671.

61. Lazeyras F, Buhler L, Vallee J.P, Hergt M, Nastasi A, Ruttimann R, Morel P, Buchs J.B (2012) Detection of ATP by "in Line"31P Magnetic Resonance Spectroscopy during Oxygenated Hypothermic Pulsatile Perfusion of Pigs' Kidneys. Magnetic Resonance Materials in Physics Biology and Medicine 25: 391-399.

62. Venema L.H, Brat A, Moers C, Hart N.A, Ploeg R.J, Hannaert P, Minor T, Leuvenink A.H.G.D (2019) Effects of Oxygen during Long-Term Hypothermic Machine Perfusion in a Porcine Model of Kidney Donation after Circulatory Death. Transplantation.

63. Lindell S.L, Muir H, Brassil J, Mangino M.J (2013) Hypothermic Machine Perfusion Preservation of the DCD Kidney: Machine Effects. J Transplant 2013: 1-7.

64. Lindell S.L, Klahn S.L, Piazza T.M, Mangino M.J, Torrealba J.R, Southard J.H, Carey H. v (2005) Natural Resistance to Liver Cold Ischemia-Reperfusion Injury Associated with the Hibernation Phenotype. Am J Physiol Gastrointest Liver Physiol 288: G473-80.

65. Schlegel A, Kron P, Graf R, Clavien P.-A, Dutkowski P (2014) Hypothermic Oxygenated Perfusion (HOPE) Downregulates the Immune Response in a Rat Model of Liver Transplantation. Ann Surg 260: 937-938.

66. Dholakia S, Royston E, Sharples E.J, Sankaran V, Ploeg R.J, Friend P.J (2018) Preserving and Perfusing the Allograft Pancreas: Past Present and Future. Transplant Rev 32: 127-131.

67. Kuroda Y, Fujino Y, Kawamura T, Suzuki Y, Fujiwara H, Saitoh Y (1990) Mechanism of Oxygenation of Pancreas during Preservation by a Two-Layer (Euro-Collins' Solution/Perfluorochemical) Cold-Storage Method. Transplantation 49: 694-696.

68. Matsumoto S, Kandaswamy R, Sutherland D.E.R, Hassoun A.A, Hiraoka K, Sageshima J, Shibata S, Tanioka Y, Kuroda Y (2000) Clinical Application of the Two-Layer (University of Wisconsin Solution/ Perfluorochemical plus O2) Method of Pancreas Preservation before Transplantation. Transplantation 70: 771-774.

69. van Golen R.F, van Gulik T.M, Heger M (2012) Mechanistic Overview of Reactive Species-Induced Degradation of the Endothelial Glycocalyx during Hepatic Ischemia/Reperfusion Injury. Free Radic Biol Med 52: 1382-1402.

70. Chance B, Thorell B (1959) Localization and Assay of Respiratory Enzymes in Single Living Cells: Fluorescence Measurements of Mitochondrial Pyridine Nucleotide in Aerobiosis and Anaerobiosis. Nature.

71. Liu Q, Simioni A, del Angel Diaz L, Quintini C (2020) Pancreas Perfusion Preservation: State of the Art with Future Directions. Artif Organs 44: 445-448.

72. Bellini M.I, Yiu J, Nozdrin M, Papalois V (2019) The Effect of Preservation Temperature on Liver Kidney and Pancreas Tissue ATP in Animal and Preclinical Human Models. J Clin Med 8.

73. Jomaa A, Gurusamy K, Siriwardana P.N, Claworthy I, Collier S, de Muylder P, Fuller B, Davidson B (2013) Does Hypothermic Machine Perfusion of Human Donor Livers Affect Risks of Sinusoidal Endothelial Injury and Microbial Infection? A Feasibility Study Assessing Flow Parameters Sterility and Sinusoidal Endothelial Ultrastructure. In Proceedings of the Transplantation Proceedings; 45: 1677-1681.

74. Doppenberg J.B, Leemkuil M, Engelse M.A, Krikke C, de Koning E.J.P, Leuvenink H.G.D (2021) Hypothermic Oxygenated Machine Perfusion of the Human Pancreas for Clinical Islet Isolation: A Prospective Feasibility Study. Transpl Int 34: 1397-1407.

75. Watson C, Kosmoliaptsis V, Pley C, Randle L, Fear C, Crick K, Gimson A, Alli M, Upponi S, Brais R (2018) Observations on the Ex Situ Perfusion of Livers for Transplantation. American Journal of Transplantation .

76. Mohkam K, Nasralla D, Mergental H, Muller X, Butler A, Jassem W, Imber C, Monbaliu D, Perera M.T.P, Laing R.W (2022) In Situ Normothermic Regional Perfusion versus Ex Situ Normothermic Machine Perfusion in Liver Transplantation from Donation after Circulatory Death. Liver Transpl.

77. Stepanova A, Kahl A, Konrad C, Ten V, Starkov A.S, Galkin A (2017) Reverse Electron Transfer Results in a Loss of Flavin from Mitochondrial Complex I: Potential Mechanism for Brain Ischemia Reperfusion Injury. Journal of Cerebral Blood Flow and Metabolism.

78. Prudhomme T, Kervella D, Ogbemudia A.E, Gauttier V, le Bas□ Bernardet S, Minault D, Hervouet J, Cantarovich D, Karam G, Renaudin K (2020) Successful Pancreas Allotransplantations after Hypothermic Machine Perfusion in a Novel Diabetic Porcine Model: A Controlled Study. Transplant International.

79. Tanioka Y, Hering B.J, Sutherland D.E.R, Kronson J.W, Kuroda Y, Gilmore T.R, Aasheim T.C, Rusten M.C, Leone J.P (1997) Effect of Pancreatic Warm Ischemia on Islet Yield and Viability in Dogs. Transplantation 64: 1637-1641.

80. Corlett M.P, Scharp D.W (1988) The Effect of Pancreatic Warm Ischemia on Islet Isolation in Rats and Dogs. J Surg Res 45: 531-536.