# Expanding Donor Heart Utilization Through Machine Perfusion Technologies

Jeanette E. Villanueva[1,2] · Yashutosh Joshi[1,2,3] · Sam Emmanuel[1,2,3] · Ling Gao[1] · Peter S. Macdonald[1,2,3]

## Abstract

**Purpose of Review** Recent advances in donor heart preservation have allowed the utilization of hearts that would typically be discarded due to prolonged ischemic times or donation via the circulatory death pathway. This review will discuss recent advances in donor heart preservation including optimization of machine perfusion technologies and future strategies of potential benefit for the donor heart and transplant outcomes.

**Recent Findings** Improvements in organ preservation strategies have enabled retrieval of donor hearts that were not ideal for static cold storage. Machine perfusion (normothermic and hypothermic) and normothermic regional perfusion have ultimately expanded the donor pool for adult heart transplantation. Xenotransplantation has also incorporated machine perfusion for porcine donor heart preservation.

**Summary** Traditional static cold storage is feasible for non-complex donors and transplants. Machine perfusion has enabled increased donor heart utilization however optimal preservation strategies are dependent on the donor criteria, predicted ischemic times and surgical complexity.

## Abbreviations

| | |
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| DBD | Donation after brain death |
| DCD | Donation after circulatory death |
| HMP | Hypothermic machine perfusion |
| hTBM | Human thrombomodulin |
| NIHP | Non-ischemic hypothermic perfusion |
| NMP | Normothermic machine perfusion |
| NRP | Normothermic regional perfusion |
| OCS | Organ Care System |
| PGD | Primary graft dysfunction |
| SCS | Static cold storage |
| TA-NRP | Thoraco-abdominal normothermic regional perfusion |
| WLS | Withdrawal of life support |

✉ Peter S. Macdonald
peter.macdonald@svha.org.au

1 Cardiac Physiology & Transplantation, Victor Chang Cardiac Research Institute, Darlinghurst, NSW, Australia

2 Faculty of Medicine, St Vincent's Clinical School, UNSW Sydney, Randwick, Australia

3 Heart & Lung Transplant Unit, St Vincent's Hospital, 390 Victoria Street Darlinghurst New South Wales, Darlinghurst, Australia

## Introduction

Heart transplantation is the most effective treatment for end-stage heart failure; however, despite increased deceased organ donation rates in developed countries [1], less than 10% of patients with end-stage heart disease receive a donor heart. The limited donor heart supply impacts waitlist mortality since many who might benefit from heart transplantation are never referred and ~20% of those listed for transplant die waiting. A large proportion of hearts procured from donation after brain death (DBD) donors are deemed unsuitable for transplantation due to advanced donor age, pre-existing heart disease, injury that occurs to the heart following brain death, or from prolonged cold ischemia during storage and/or transport. Until recently, hearts from donation after circulatory death (DCD) donors were considered unsuitable for heart transplantation due to the injury induced by the obligatory warm ischemia that occurs following withdrawal of life support (WLS) and declaration of circulatory

death. Traditionally, static cold storage (SCS) has been the default preservation method for donor hearts; however, the past decade has seen significant advances in donor heart preservation. It is now clear that cellular and tissue injury sustained by the donor heart due to ischemia and subsequent reperfusion can be mitigated using various preservation techniques including both normothermic and hypothermic machine perfusion as well as normothermic regional perfusion (NRP). Whilst these methods can help improve donor heart preservation and broaden the donor pool, the recent report of the first pig-to-human heart xenotransplantation using a genetically modified pig is a potential breakthrough as it is an avenue that can directly address the increasing gap between the supply and demand of human donor hearts (Fig. 1).

## Normothermic Machine Perfusion

Clinical preservation of donor hearts using NMP was made possible with the Organ Care System (OCS) developed by TransMedics. The OCS method requires 1.2–1.5L of donor blood to be collected prior to heart retrieval, after which the heart is flushed with cold preservation solution, explanted, and prepared on a back-table for instrumentation onto the OCS via a perfusion cannula connected to the donor aorta.

The donor heart is then reperfused and reanimated on the OCS. Normothermic machine perfusion (NMP) was used to assess donor heart function prior to transplantation in brain dead donors as part of the PROCEED-II trial [2]. In that prospective randomized trial, NMP using the OCS system was compared with SCS. Donor and recipient selection criteria were restricted to standard-criteria DBD donors and uncomplicated recipients. Clinical outcomes were similar between groups. Given this neutral outcome and the high cost and complexity of the OCS device, there has been little uptake of this technology for straight-forward heart transplants. Rather, attention has shifted to the application of NMP to preserving donor hearts where there is an increased risk of severe primary graft dysfunction (PGD) due either to adverse donor characteristics (e.g., increased donor age, donor left ventricular dysfunction), or anticipated long ischemic times or adverse recipient characteristics (e.g., complex adult congenital heart disease, ventricular assist device explants) [3, 4]. The multicenter prospective EXPAND (Expanded Criteria Donor Hearts for Transplantation) clinical trial assessed the use of donor hearts that at the time of procurement did not meet standard criteria: ≥ 4-h ischemic time, plus either left ventricular hypertrophy, ejection fraction 40–50% or donor age > 55 years old [5]. Overall, these extended criteria hearts had a utilization rate of 81% and overall low rates of PGD. In terms of patients who
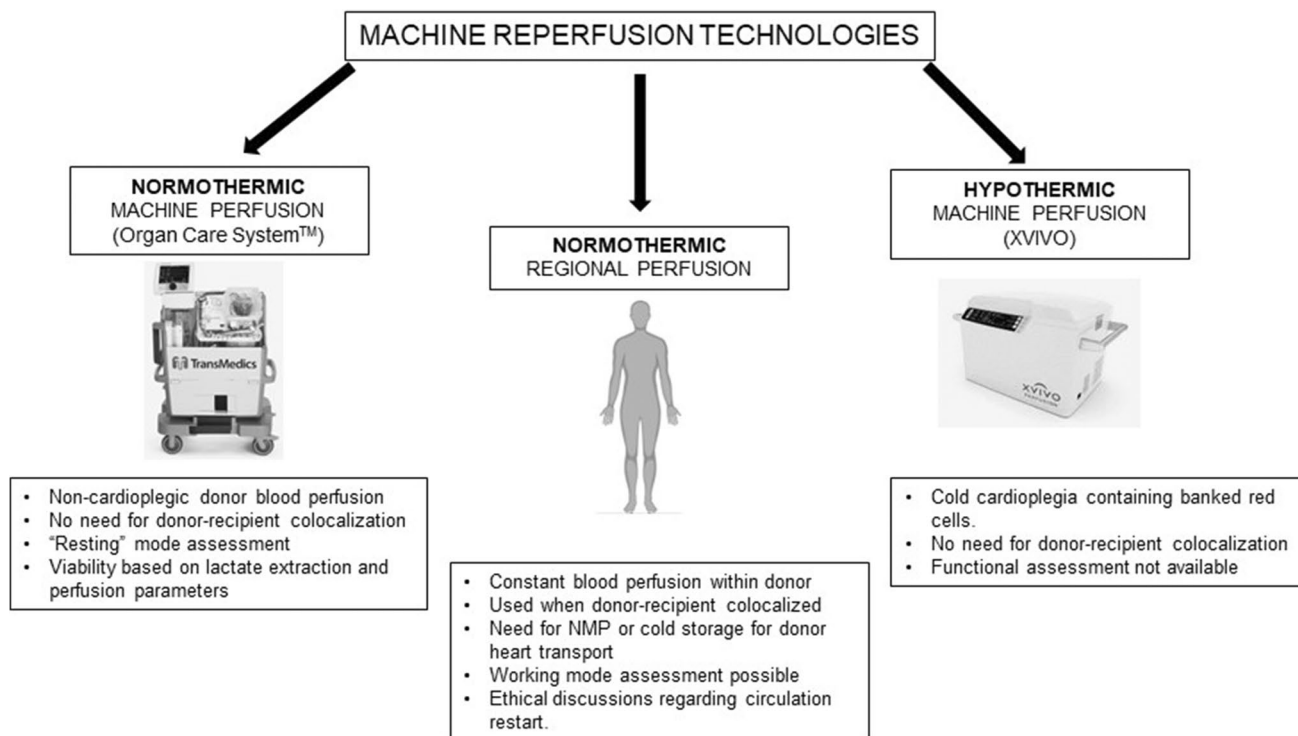


**Fig. 1** Overall comparison of current machine perfusion technologies including normothermic machine perfusion with the Organ Care System™, normothermic regional perfusion, and hypothermic machine perfusion with the XVIVO Perfusion system

have been supported on a ventricular assist device, compared to SCS hearts, the use of NMP for donor hearts has reduced the need for post-transplant mechanical support [6], reduced cardiopulmonary bypass times, and reduced the incidence of early PGD [6, 7]. Both SCS and NMP groups showed similar post-transplant survival at 1 month [6] and 1 year with a lower incidence in acute rejection [7].

Another emerging application for the use of NMP is the recovery of hearts from DCD donors. Following pre-clinical studies [8, 9], it was first implemented for clinical use in DCD heart transplantation in Australia [10••, 11]. The use of NMP with the OCS in DCD heart transplantation has since been established for clinical use in the UK and Belgium although in some instances heart procurement involves NRP discussed later in this review. DCD heart retrievals using the OCS are also in clinical use in the USA [12], with outcomes of the first randomized trial recently reported [13, 14]. In the US trial, the outcomes of DCD with OCS ($n=80$) were compared to DBD-SCS ($n=86$). Recipient survival rate at 24 months was significantly higher in the DCD-OCS group compared to the DBD-SCS group: 93% versus 83% ($p=0.0362$). Although higher rates of PGD were observed in the DCD-OCS group (16% versus 5% in DBD-SCS group), the PGD rates in the DCD-OCS group were lower than previously reported PGD rates using similar procurement protocols [10••, 11]. The PGD rates were similar to those from Papworth [15]; however, it is important to note that NMP has been used in conjunction with NRP (discussed later in this review), where blood circulation is restarted within the donor after declaration of circulatory death, in some transplant centers.

The use of the OCS for NMP of donor hearts is primarily used for adult donors due to compatibility of the OCS device for a donor weight greater than 45 kg and small pediatric donors fall well below this weight requirement. Given the success of the OCS in adult heart procurement and preservation, pediatric patients would also benefit since recipients with congenital heart disease can have a higher degree of surgical complexity and the OCS can help minimize overall ischemic times. There are limited reports on the use of the OCS in pediatric cases. A recent retrospective study [16•] compared pediatric transplant outcomes in eight recipients that received a donor heart (donor weight ≥ 45 kg) maintained on the OCS with thirteen recipients that received a donor heart maintained with SCS. There were no differences observed in PGD between both OCS and SCS groups, no difference in length of hospital stay, and 1 year survival rates were comparable between OCS (88%) and SCS (85%). Due to the incompatibility of smaller donor hearts on the OCS, these hearts were allocated to the SCS group which impacted the randomization of the study and resulted in a higher donor-to-weight ratio in OCS patients (1.84 versus 1.2 $p=0.03$). Nevertheless, this study provides evidence that

the OCS could potentially be used for pediatric heart transplants where there are prolonged ischemic times or surgical complexities.

The study by Fleck et al. [16•] was comprised of DBD hearts; therefore, the outcomes of clinical pediatric DCD heart transplants with NMP are unknown. Successful heart transplantation from pediatric DCD donors using SCS was first reported in 2008 [17]. In that report, donors and recipients were co-located in adjacent operating theaters and a range of controversial measures including ante-mortem cannulation and very short "stand-off" times were utilized to minimize the warm ischemic time that occurred during WLS. In contrast to this early success, a subsequent ISHLT Registry analysis of 21 pediatric DCD heart transplants performed across 5 centers between 2005 and 2014 reported that 1-year survival of DCD heart transplant recipients was significantly lower than for recipients of hearts from DBD donors (57% versus 93%) with 24% of DCD heart recipients dying from primary graft failure [18]. This experience highlights the need for better preservation strategies if DCD donors are to be successfully utilized for pediatric heart transplantation.

A recent study using a porcine pediatric DCD model placed hearts on an ex vivo perfusion circuit and assessed donor heart recovery comparing two strategies for perfusion: pressure-targeted and flow-targeted [19]. The current OCS does not allow for working mode assessment for the heart and uses a flow-targeted perfusion approach. For each perfusion strategy, hearts were subjected to 15-min warm ischemic time after circulatory death was established, cardioplegia administered (supplemented with erythropoietin and glyceryl trinitrate), heart instrumented onto perfusion circuit and then perfused for 2 h in resting (Langendorff) mode followed by 1.5 h in working mode. No differences in hemodynamics were observed between pressure-targeted and flow-targeted groups during resting mode perfusion. In contrast, when hearts were perfused in working mode, those perfused with a flow-targeted approach showed significantly increased cardiac output, stroke volume, and ejection fraction and reduced coronary vascular resistance [19]. This study provides evidence that the current OCS strategy (flow-targeted perfusion) can potentially maintain pediatric DCD hearts in an experimental setting. Overall, some limiting factors remain in the application of the OCS for pediatric use including the limited amount of donor blood available to maintain the perfusion circuit and the limitation of donor heart size for instrumentation onto the OCS.

One of the major advantages of maintaining the donor heart with NMP is that continual blood perfusion maintains oxygenation and allows for transportation to recipient hospitals over greater distances. To date, the longest time a DBD donor heart has been maintained on an OCS is 15 h with the recipient surviving 3 years at the time of reporting despite

having a ventricular assist device prior to transplant [20]. At our center, the longest we have maintained a DCD heart on an OCS is approximately 6 h and 50 min, with the patient having survived over 6 years at the time of writing. One potential limitation to the use of the OCS NMP device is the need to collect approximately 1.2–1.5L of donor blood which is the major constituent of the OCS perfusate. While collection of blood from DBD donors is usually straight-forward, collection of blood after cessation of the circulation in the DCD donor is less certain particularly in the absence of ante-mortem heparin. Furthermore in a direct procurement sequence, the delivery of cardioplegia prior to explantation of the heart must occur after donor blood is collected and therefore the cannulation of the right atrium and subsequent collection of donor blood adds to the warm ischemic time [10••]. The option of using banked blood to meet volume requirements has been investigated in both pre-clinical and clinical studies, however the addition of banked blood to the perfusate has been associated with coronary vasoconstriction and failure of the graft during NMP [21].

## Supplementation of Blood Collection Bag and Perfusate

Across various transplant centers, there is generally no consensus as to the composition of the perfusate of the OCS or whether any supplements are added to the blood collection bag. The addition of heparin to the blood collection bag seems to be consistent across all NMP studies — an advantage in jurisdictions that do not allow the use of ante-mortem interventions of the donor. Tirofiban, a glycoprotein IIb/IIIa inhibitor that acts as an anti-platelet agent and prevents platelet activation and aggregation, is now routinely added to the blood collection bag during OCS retrievals in Australia as a means of ameliorating module filter clotting issues experienced by our retrieval team [22]. The addition of levosimendan to the perfusate has been shown to increase calcium sensitivity and subsequent myocardial contractility [20]. The ideal composition of the perfusate for NMP is yet to be established and is an important area for future research.

## Viability Assessment of the Donor Heart During NMP

One of the disadvantages of NMP using the OCS is the inability to measure donor heart function in working mode. The TransMedics OCS perfuses the heart in Langendorff mode (resting mode) and as such the viability of the donor heart is determined based on arterial and venous lactate measurements. Donor hearts are deemed viable based on visual assessment of contractility (primarily right ventricular contractility as the left ventricle is vented), coronary flow measurements, and whether there is adequate lactate extraction

(arterial lactate > venous lactate), as well as an overall down-trending lactate profile, with the goal of achieving overall lactate measurements under 5 mmol/L initially proposed. However, as clinical experience of the OCS grew, a strict adherence of waiting for lactate to fall below 5 mmol/L was replaced in favour of an overall downward trend [10••]. There is debate as to whether lactate measurements are an accurate indicator of donor heart performance after transplantation which has led to research for alternative markers that can be used as accurate predictors of graft performance.

## NMP and Multi-organ Retrieval

In cases where NMP is used for DCD heart donors, retrieval of lungs and abdominal organs (e.g., kidney, liver) does not appear to be negatively impacted. One of the concerns is that the additional time required to collect donor blood for the OCS prior to organ procurement will cause delays in additional organ retrieval however this delay can be easily mitigated. A recent study by Feizpour et al. [23] used NMP where both the heart and liver were retrieved from the same DCD donor. In this case study, 1.5L donor blood was collected and then the heart was cold-flushed with Del Nido cardioplegia, the IVC vented, the liver flushed with University of Wisconsin solution and then both heart and liver were cold dissected simultaneously by the retrieval surgeons. The time taken to retrieve both organs was 9 min. In our transplant center, we have high utilization of lungs from the same DCD donors.

## Thoraco-abdominal Normothermic Regional Perfusion

The use of normothermic regional perfusion (NRP) to preserve donor abdominal organs has been approved for use in France, Spain, Italy, and the UK for almost a decade. In these instances, the donor heart was either discarded or retrieved via a direct procurement protocol. In 2016, thoraco-abdominal NRP (TA-NRP) was introduced as an alternative to NMP for monitoring of donor heart function prior to transplantation. To date only the UK, Belgium, Spain, and the USA have used NRP for donor heart preservation. In TA-NRP, once death has been declared and the stand-off period has been observed, the aortic arch branches are clamped to inhibit cerebral blood flow prior to commencement of NRP. In order to start NRP, cannulae are placed in the ascending aorta and right atrium which are then connected to a circuit containing a centrifugal pump and oxygenator [24]. There are several benefits afforded using TA-NRP to preserve donor organs. TA-NRP allows for direct functional assessment of the DCD donor heart using a Swan-Ganz catheterization and echocardiography

[24] rather than relying on lactate extraction. The continual supply of warm blood perfusion reduces the ischemic impact on abdominal organs, reduces organ discard rate and offers a cost-effective solution of donor heart preservation compared to standard OCS retrievals which is over double the cost of SCS [4]. TA-NRP has shown to be particularly beneficial for assessing DCD donor heart function prior to transplantation and is quite feasible when the donor and recipient are co-localized within the hospital [25, 26] and has been successful for pediatric DCD heart transplants [27, 28].

Following TA-NRP and cardioplegia administration, the donor heart is maintained either in SCS or placed onto the OCS prior to transplantation. One of the caveats to placing the heart on the OCS after TA-NRP is the need for donor blood to prime the OCS; however, this can be overcome with the use of donor matched washed packed blood cells [29]. SCS is still used in some NRP-based organ retrievals. Recent cases have maintained NRP for periods of under 25 min after which the donor heart is perfused with cold cardioplegia and then cold stored [30, 31••, 32, 33, 34, 35]. Although the cold ischemic times for these hearts ranged from under 15 min where the recipient was co-localized with the donor [32] to 177 min where the recipient was ~900 km away [33]. Post-transplant outcomes were favorable with very few recipients requiring post-transplant mechanical support: three recipients required an intra-aortic balloon pump [33], one required ECMO due to primary lung graft dysfunction [34]; however, all recipients had excellent left ventricular function upon discharge.

Despite the benefits of TA-NRP in increasing the utilization of both thoracic and abdominal organs, the concept of TA-NRP remains contentious in many countries due to ethical concerns that surround restarting of circulation within the donor after death has been declared.

## Hypothermic Machine Perfusion

Hypothermic machine perfusion (HMP) has been investigated as another alternative to mitigate the problems associated with SCS. Steen et al. [36] studied the efficacy of non-ischemic hypothermic perfusion (NIHP) by using porcine donor hearts following a brain-death model. These hearts were preserved using NIHP for a duration of 24 h prior to transplantation. The NIHP system used was developed in-house, resembling a cardiopulmonary bypass machine which included an automatic pressure and flow-controlled perfusion system, gas exchange system, leucocyte filter, arterial filter, and a heater-cooler unit. All NIHP-preserved porcine hearts were successfully transplanted and stable throughout the 24-h post-transplant observation period. This study also utilized 3 pigs as a control group whereby hearts were preserved in SCS for 24 h. While the NIHP group were weaned with ease from cardiopulmonary bypass, the SCS control group were difficult to wean and failed shortly thereafter.

To investigate the pathophysiological impact of NIHP, Qin et al. [37] demonstrated that in a porcine model, coronary artery endothelium maintained normal function in terms of vascular contractility and relaxation after 8 h of NIHP. Furthermore, they showed that myocardial contractility in right ventricular trabeculae after 24 h of NIHP demonstrated a normal response. In addition to this work, Michel and Madsen [38] reported that a NIHP porcine model resulted in hearts with less depletion of ATP stores and improved preservation of mitochondrial structure. Critchley et al. [39] echoed the physiological benefits of NIHP — in this study, six Swedish pigs underwent heart retrieval. Three organs were preserved with SCS for 2 h and the remainder were preserved for 8 h with NIHP. The NIHP group demonstrated less ischemic-reperfusion injury as well less cell death and better maintenance of tissue viability. Additionally, the NIHP group demonstrated loss of viable white cells compared to the SCS group, which may present a unique immunodepletion phenomenon resulting in less organ rejection. Langin et al. [40] went on to describe how this unique immunodepletion phenomenon can benefit xenotransplantation. In this study, the authors divided pigs with the genetically modified multi-modified α1,3-galactosyltransferase-knockout pig hearts into 3 groups undergoing xenotransplantation to baboon. Group I consisted of five pigs with hearts preserved in SCS, group II consisted of four pigs with hearts preserved in NIHP, while group III consisted of five pigs with hearts preserved in NIHP as well as adjustment in post-operative care such as tighter blood pressure management, quicker tapering of steroids as well as anti-rejection immunosuppression consisting of sirolimus to combat cardiac overgrowth. In group I (SCS). only one animal survived past 3 days, compared to the group II (NIHP) whereby all animals survived for 3 or more days. However, with the aid of NIHP and the aforementioned post-operative care modifications, all participants in group III survived to 6 months.

NIHP use in human cardiac transplantation was first implemented in 2020 by Nilsson et al. [41••]. In this study, six patients were assigned to NIHP technology, and twenty-five patients were assigned to SCS. The median preservation time for NIHP was 223 min compared to 194 min for SCS. All six patients who underwent NIHP achieved event-free survival (freedom from severe PGD, no ECMO post-operatively and free of severe acute-cellular rejection) compared to 18 (72%) of the patients in the SCS group. Currently, further studies are underway to investigate the benefits of NIHP in a human cardiac transplant model — these include a European trial as well as an Australia-New Zealand trial utilizing the XVIVO Perfusion heart module for NIHP preservation [42].

## Xenotransplantation

The first genetically modified pig-to-human heart xenotransplant took place in early January 2022 and was deemed a success with the 57-year-old recipient surviving 2 months post-op [43, 44]. Although it is unclear whether there was any evidence of antibody-mediated rejection or if the recipient's death was due to other comorbidities, this breakthrough presents an alternative avenue for the procurement of donor hearts to help alleviate demand. Heart xenotransplantation has been a subject of preclinical studies for decades. Initial studies by Mohiuddin et al. [45] included three genetic modifications in porcine donors: galactosyltranferase knockout and insertions of human CD46 and human thrombomodulin (hTBM). These initial heart xenotransplants in non-human primates displayed a median survival of 298 days with an immunosuppression regime of MMF and anti-CD40 antibodies [45]. Similar studies using genetically modified heart xenografts demonstrated that the use of cold perfusion to preserve the donor heart reduced the incidence of perioperative cardiac xenograft dysfunction compared to SCS [40, 46, 47••]. Subsequent xenotransplant studies by Mohiuddin et al. [48] expanded the genetic modification of porcine donor hearts by using a maximum of nine modifications: insertions that regulate complement activation (hCD46, hDAF), coagulation (hTBM, hEPCR) and inflammation (hCD47, hHO-1), and deletions to minimize porcine cell antigenicity (GGTA1, B4GalNT2, CMAH) and cell growth (growth hormone receptor). In addition, cold perfusion was used for donor heart preservation in all but two donor porcine hearts.

The protocol used for the first pig-to-human transplant was a combination of the above studies — a total of ten genetic modifications were used for the heart xenograft: six insertions (hCD46, hDAF, hTBM, hEPCR, hCD47, hHO-1) and four deletions (GGTA1, B4GalNT2, CMAH, GHR). Although the specific details of the case study are yet to be published, the donor xenograft was preserved with cold perfusion using the XVIVO Perfusion system, the recipient was placed on an immunosuppressive regime that included anti-CD40 monoclonal antibodies and an undisclosed experimental medication [43]. Although more details are needed as to whether the cardiac xenograft showed signs of graft rejection, this early clinical experimental phase provided a breakthrough that could help alleviate the heart transplant waitlists.

## Conclusions

SCS is widely used in heart transplant centers globally to preserve and transport donor hearts in instances of standard DBD donors, short ischemic times, and non-complex surgeries. However, the use of either NMP, NRP, or HMP has started to gain traction worldwide and has expanded the donor heart pool to include marginal BD donors and DCD donors and has enabled heart transplantation where there are large geographical distances between donor and recipient hospitals. These methods of donor heart preservation have been mainly used for adult heart transplantation. For NMP, the configurations of commercial perfusion devices that only accommodate heart sizes from donors ≥ 45 kg places limitations for pediatric donors. However, recent evidence shows that these technologies have been successful in cases of pediatric heart transplantation and can further expand the donor pool for pediatric heart transplant. Recent clinical use of a genetically modified porcine heart donor has allowed for further clinical investigation into alternative donor sources. Over time, the increased uptake of machine perfusion and NRP amongst transplant centers will increase donor heart utilization, alleviating wait-list times and patient wait-list mortality.

## Declarations

**Conflict of Interest** The authors declare no competing interests.

**Human and Animal Rights** All reported studies with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

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