# Twenty-four-hour normothermic perfusion of isolated ex vivo hearts using plasma exchange



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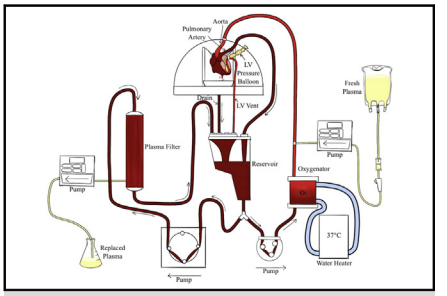
## ABSTRACT

**Objective:** Cross-circulation of plasma from a paracorporeal animal allows successful ex vivo heart perfusion (EVHP) for 3 days. Little is known about the feasibility of prolonged EVHP without a paracorporeal animal. These experiments evaluated plasma exchange (PX) that infuses fresh plasma, whereas an equal amount is removed to replace paracorporeal cross-circulation.

**Methods:** Ten hearts were procured from 8 to 10 kg piglets and maintained with EVHP. The EVHP circuit was primed with platelet- and leukocyte-reduced blood. Plasma obtained from stored porcine blood (4°C for ≤7 days) was infused and removed with a plasma separator at 1 mL/h/g cardiac tissue (n = 5) in the PX group. Controls (n = 5) used the same EVHP without PX. Antegrade aortic perfusion was adjusted to reach physiologic coronary flow of 0.7 to 1.2 mL/min/g, normothermia (37°C), and hemoglobin ≥8 g/dL. Viability was assessed by hemodynamic metrics, metabolic assays, and histopathology.

**Results:** All PX hearts remained viable for 24 hours compared with only 1 control ($P = .015$). Coronary resistance was higher in the PX versus controls (1.06 ± 0.06 mm Hg/mL/min; 0.58 ± 0.02 mm Hg/mL/min [$P < .05$]). Lactate levels were lower in PX (2.8-4.2 mmol/L) versus controls (3.6-7.6 mmol/L) ($P < .05$). PX demonstrated a trend toward preservation of left ventricle systolic pressure (63.0 ± 10.9 mm Hg) versus controls (37 ± 22.0 mm Hg) ($P > .05$). In mixed effect models, oxygen consumption was higher with PX ($P < .05$). Histopathologic evaluation confirmed extensive myocardial degeneration and worse interstitial edema in controls.

**Conclusions:** These results demonstrate that EVHP can be successfully maintained for at least 24 hours using continuous PX. This eliminates the need for a paracorporeal animal and provides an important step toward clinical application. (J Thorac Cardiovasc Surg 2022;164:128-38)




**Ex vivo heart perfusion circuit with plasma exchange.**

### CENTRAL MESSAGE

Normothermic perfusion of ex vivo hearts, without paracorporeal support, can be extended for at least 24 hours with plasma exchange into a platelet- and leukocyte-reduced blood perfusate.

### PERSPECTIVE

We have established a clinically relevant animal model of continuous ex vivo cardiac perfusion using plasma exchange that substantially extends organ protection. Prolonged ex vivo perfusion is desirable for pretransplant organ evaluation and therapeutic interventions. This should expand the donor pool, reduce geographic restrictions, and allow for an elective approach to transplantation.

See Commentaries on pages 139 and 140.

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**Abbreviations and Acronyms**

AO = aorta
EVHP = ex vivo heart perfusion
Hb = hemoglobin
HR = heart rate
LV = left ventricle
LVSP = left ventricular systolic pressure
NEVP = normothermic ex vivo perfusion
OCS = Organ Care System
PA = pulmonary artery
PCA = paracorporeal animal
PX = plasma exchange
RV = right ventricle

Current cardiac transplantation preservation techniques rely on nonphysiologic cold immersion, which is effective for up to 6 hours.[1] Static cold storage longer than 6 hours invariably subjects organs to detrimental ischemia and significantly compromises organ performance. Beyond this time frame, organs undergo endothelial damage and loss of vasomotor tone with edema leading to delayed function or organ failure.[2-4] In the case of renal grafts where the effects of prolonged cold ischemia (>6 hours) has been extensively studied, the loss of endothelial integrity in the grafts were identified via immunohistochemistry.[5] In a rodent model, it has been shown that prolonged cold storage induced coronary endothelial dysfunction in a time-dependent relationship.[2] In clinical practice, it has been established that cold ischemia time beyond 6.25 hours is associated with poor long-term cardiac graft survival, decreased patient survival, and higher 30-day and overall mortality.[6]

Because of this time restriction, marginal organs are not considered for interventions that could optimize function. Normothermic ex vivo perfusion (NEVP) is a method to expand the donor pool by reducing cold ischemia time, enabling graft repair or reconditioning, and providing real-time graft evaluation during perfusion. Furthermore, extending ex vivo perfusion from hours to days will remove geographic restrictions for organ allocation and transform transplantation into elective procedures.

Normothermic perfusion has already been shown to facilitate support of hearts ex vivo. In clinical medicine, NEVP has been shown to be noninferior to current cold static storage in the Ex-Vivo Perfusion of Donor Hearts for Human Heart Transplantation (PROCEED II) trial using the Organ Care System (OCS), with normothermic perfusion lasting on average 210 minutes.[7] Experimentally, it has been established that continuously supplying fresh blood at normothermia from a live animal via cross-circulation maintains normal coronary vasomotor tone and cardiac graft function up to 24 hours. Further advances from our laboratory have shown in an ovine model that plasma cross-circulation at the rate of 1 L/h from a live, awake, paracorporeal animal (PCA) extended ex vivo heart perfusion (EVHP) to 3 days with hemodynamic parameters of good cardiac function, histopathologic confirmation of healthy myocardium, and preservation of hormonal responsiveness.[8] However, the requirement for a PCA limits the potential clinical application of this technology. Building on our prior work, we hypothesized that continuous plasma exchange (PX) from freshly pooled porcine blood will constitute an adequate perfusate for EVHP and thus eliminate the need for a PCA.

In this study, we established a clinically relevant animal model for prolonged EVHP at normothermia using PX in the perfusate. This work is important because prolonged preservation to 1 or more days will facilitate the optimization (medically or surgically) of marginal hearts before transplantation. It also illustrates the possibility of preserving hearts without support from a live animal. The findings presented herein could lead to long-term preservation of a variety of organs in dedicated organ banks.

## METHODS

### Animals

Ten healthy piglets were utilized in this pilot study (n = 5 in the experimental and control arms of the project). Five animals in each study group provide at least 90% power to detect moderately large differences between group measurements for our primary outcome organ failure. Animals had a median weight of 9 kg (range, 8-10 kg). The piglet model was chosen given the size of the donor hearts and the smaller volume of plasma required in our weight-based exchange protocol. All animals received humane care in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and protocols were approved by the University of Michigan Institutional Animal Care and Use Committee.

### Surgical Procedure

Animals underwent general anesthesia via induction with 7 mg/kg tiletamine-zolazepam combined with 3 mg/kg xylazine before skin incision, were prepped and draped in standard sterile manner, and intravenous antibiotics were administered (25 mg/kg nafcillin and 2.25 mg/kg gentamicin). Vascular access was obtained via the femoral vessels and 1 mg/kg lidocaine was administered intravenously before a midline sternotomy. The pericardial fat pad was removed for accurate weight measurements. The great vessels were isolated and loosely encircled with ligatures. The animals received a dose of 400 IU/kg unfractionated heparin (Sagent Pharma, Schaumburg, Ill). Care was taken to preserve the pericardium around the heart to prevent desiccation during ex vivo perfusion. The azygous and hemiazygous veins were ligated. Following documentation of adequate systemic anticoagulation, the distal innominate artery and other branch vessels from the transverse arch were ligated, the superior and inferior vena cava were ligated, the middescending thoracic aorta (AO) was crossclamped, and the left side of the heart was decompressed by transecting the inferior pulmonary veins. Cold del Nido cardioplegia (CAPS Inc, Detroit, Mich) (50 mL/kg) was infused antegrade via the proximal innominate artery. Concurrently, topical cooling with sterile iced saline was applied. Following cardioplegia administration, the hearts were excised with the pericardium intact, weighed, and placed in an ice bath. Cold ischemia time did not exceed 40 minutes.

### Back Table Preparation

All cannulas used were products of Terumo (Ann, Arbor, Mich). A 10Fr venous drainage cannula (Medtronic Inc, Dublin, Ireland) and

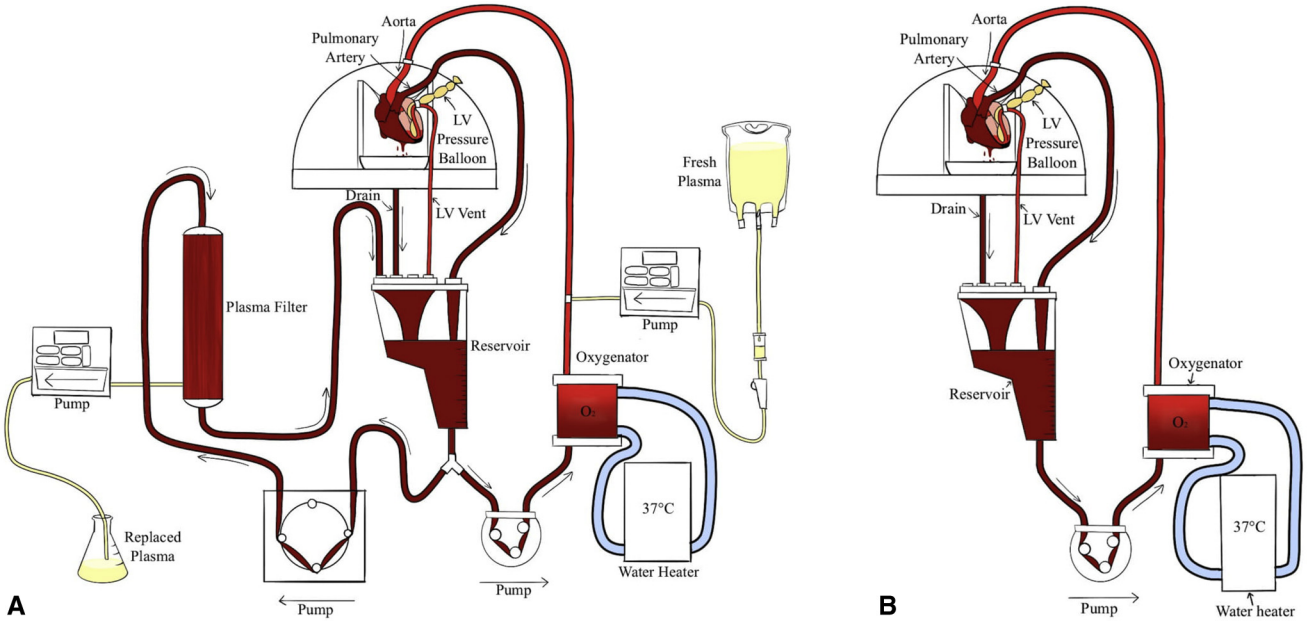



**FIGURE 1.** Ex vivo heart support system. Inflow of blood was achieved via antegrade aortic cannula and drainage via pulmonary artery catheter. Regurgitant blood through the aortic valve was drained via a left ventricle (*LV*) venting catheter. A, The heart was maintained under a humidified dome. Plasma was infused into the aortic catheter in the experimental hearts and filtered out at a similar rate from the reservoir drainage line. The previous plasma cross circulation experiments included removal of plasma from the perfusion circuit and return to the paracorporeal animal, creating continuous plasma exchange.[8] In the current experiments, continuous plasma exchange was done by continuous infusion of bank plasma, and continuous removal of the same amount of plasma by a plasma separator as illustrated in panel A. The plasma removed was measured and discarded. B, Control hearts were perfused with platelet- and leuko-reduced blood only.

a high-compliance balloon connected to a pressure-transducing apparatus for left ventricle (LV) isovolumetric pressure monitoring were inserted into the LV via a left atriotomy. The mitral valve orifice was approximated with a 5-0 prolene suture and secured to the LV balloon to prevent balloon migration. A 24Fr DLP venous drainage cannula (Medtronic Inc, Dublin, Ireland) was placed in the right ventricle (RV) via the pulmonary artery (PA). A ¼ × ¼-inch with Luer Lock cannula (Medtronic Inc, Minneapolis, Minn) was secured in the AO root for antegrade coronary perfusion. All remaining branches were ligated and the heart and cannulas were de-aired and connected to the perfusion apparatus.

## Ex Vivo Heart Perfusate and Circuit

The perfusion circuit, as illustrated in Figure 1, *A* and *B*, consisted of commercially available components including a reservoir (Terumo) filled with donor blood collected into citrate phosphate dextrose adenine bag. Citrate phosphate dextrose adenine allows storage of blood for 35 days at 1°C to 6°C[9]; however, oxidative damage can occur as early as 7 to 14 days.[9,10] Therefore, blood storage at 4°C was limited to 7 days. The priming volume was approximately 300 mL platelet- and leukocyte-reduced blood with a hemoglobin (Hb) concentration goal >8 g/dL for both PX and control hearts. Platelet and leukocyte reduction was achieved via centrifugation of citrated whole blood at 3600 rpm at 25°C for 20 minutes.

Perfusion was accomplished using a FX05 Baby Capiox Oxygenator (Terumo) and roller pump (Stockert, Munich, Germany). AO flows were slowly increased and adjusted to maintain PA flow (surrogate for coronary flow) at 0.7 to 1.2 mL/min/g tissue, concordant with physiologic coronary flow.[11] PA and LV drainage were collected and returned to the reservoir of the perfusion circuit. The LV balloon was inflated to maintain end-diastolic pressure within 10 to 12 mm Hg. Temperature was maintained at 37°C using a water heater. The sweep gas (50% oxygen, 45% sodium, and 5% carbon dioxide at 0.1-0.2 L/min) was adjusted to maintain carbon dioxide tension 40 ± 5 mm Hg. If fibrillation occurred, the heart was defibrillated with 5 to 20 J using internal defibrillation paddles. After 60 minutes of normothermic perfusion, the perfusate was exchanged using 300 mL platelet- and leukocyte-reduced blood with Hb level >8 g/dL to eliminate residual cardioplegia and toxins that may have accumulated from reperfusion.

In experimental hearts, a continuous infusion of plasma was thus started at the rate of 1 mL/h/g heart tissue for 24 hours or until heart failure. We called this process PX. Based on unpublished data from our group, the lowest rate of infusion of plasma cross-circulation required from a PCA is 1 mL/h/g cardiac tissue. Bank plasma was obtained by centrifugation from pooled porcine blood and collected into sterile bags. Plasma bags were anticoagulated with 10,000 IU heparin per 100 mL plasma. To avoid dilution of hemoglobin and alteration in the coagulation profile of the perfusate, plasma was infused into the AO inflow at the set rate and an equal amount was removed from the reservoir using a plasma separator (Plasmaflo OP-05W[A], Asahi Kasei Medical MT Corp, Oita, Japan).

Cardiac failure was defined as asystole or any of the following for 2 consecutive hours: LV systolic pressure consistently <30 mm Hg, intractable arrhythmia, or lactate level >7 mmol/L. At the end of each experiment, an epinephrine challenge (0.1 mg) was delivered to the hearts to evaluate hormonal responsiveness as evidenced by increase in heart rate and LV pressure. Postmortem, hearts were decannulated, drained, weighed, photographed, and sent to pathology in 10% buffered formalin.

## Histologic Analysis

Following perfusion, random sections from each cardiac chamber were sampled, weighed, and stored in a desiccator for 7 days for wet/dry weight ratios. The remainder of the organs were sent to pathology for routine hematoxylin and eosin staining. A scoring system ranging from 0 to 4 based

on myofiber degeneration, myocardial hemorrhage, interstitial edema, and endothelial changes was developed in conjunction with veterinary pathologists.[8,12,13] The interpreting pathologists were blinded to experimental groups. Average scores using nonquantitative/ordinal data for each injury type were reported for each cardiac chamber.

### Data Collection and Analysis

Over the course of each experimental run, hemodynamic parameters, including heart rate (HR), AO and PA flows, AO root pressure (coronary perfusion pressure), LV pressure, temperature, and electrocardiogram data were monitored continuously and single measurements were recorded every 30 minutes for each parameter. AO flow, AO arterial blood gas, PA arterial blood gas, electrolytes, and lactate were measured hourly with electrolyte repletion done if necessary, to maintain normal values. Mean AO root pressure and PA flow were used to calculate coronary vascular resistance as follows:

$$\textbf{Coronary resistance}\left(\textbf{mmHg}.\,\textbf{min}.\,\textbf{mL}^{-1}\right) = \frac{\textbf{Mean Aortic Pressure}\,(\textbf{mmHg})}{\textbf{PA Flow}\left(\frac{\textbf{mL}}{\textbf{min}}\right)}$$

Oxygen content and glucose concentration were measured in the infusion (ie, AO) and drainage (ie, PA) blood and oxygen consumption was calculated hourly.

Statistical analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC) and Excel 2019 (Microsoft Corp, Redmon, Wash). All data are expressed as mean ± standard deviation. Mixed effect models were used to analyze longitudinal data, which applied an autoregressive covariance structure to account for correlation between measurements. The interaction term between time and treatment groups was tested in the models. The significance of the interaction suggested that the group effect varied across time. Group effect at each time was estimated and tested in the models. The differences at individual time points were based on the models using SAS PROC MIXED. SAS PROC MIXED allows such comparisons when time is modeled as a categorical variable in longitudinal analyses. Wilcoxon rank-sum tests were used to test the difference in wet/dry ratios between treatment groups or between LV and RV. Box-and-whisker plots were constructed for illustration. A graphical description of the experimental overview for control and PX groups is provided in Figure 2.

## RESULTS

PX hearts (n = 5) functioned well for 24 hours, maintaining normal contractility and electrical activity before elective termination. Control hearts failed at 15, 16, 17, 17, and 24 hours. One control lasted 24 hours but termination criteria for that experiment was based solely on lactate levels because the LV pressure balloon malfunctioned. The response to hormonal stimulation by epinephrine bolus was present in all 5 PX hearts with increased strength and rate of contractility compared with only 2 controls.

### Ex Vivo Perfusion Circuit Stability

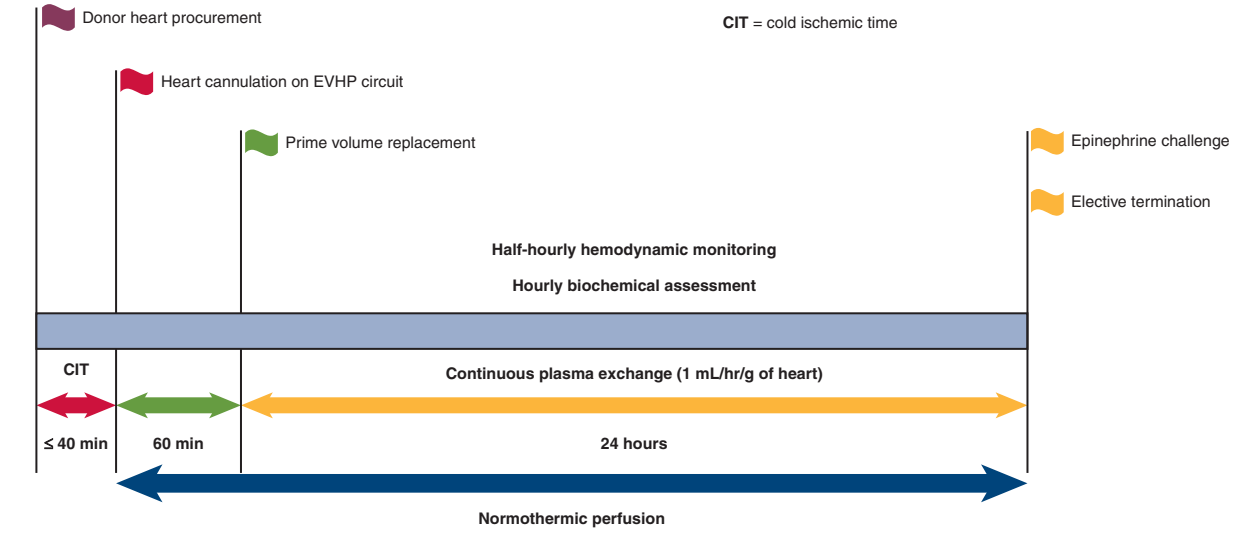
Perfusion conditions in both PX and control groups were tightly regulated within physiologic parameters and stable throughout the duration of perfusion. Flows were maintained on average at 89.6 ± 4.6 mL/min in the PX group and 86.7 ± 6.0 mL/min in the control group (Figure 3, *A*). Resultant coronary (ie, PA) flows were within target range in PX (0.8-0.9 mL/min/g) and control hearts (0.7-1.2 mL/min/g) as seen in Figure 3, *B*. The circuit was kept at a mean temperature of 36.7°C ± 0.2°C throughout the duration of perfusion in PX group versus 37.9°C ± 0.3°C in control hearts (Figure 3, *C*). The pH of the perfusate was maintained within physiologic range: 7.34 ± 0.03 for PX hearts and 7.37 ± 0.04 for controls (Figure 3, *D*). In both the PX and control groups, Hb level was also kept at goal: 8.2 ± 0.5 g/dL versus 8.7 ± 0.7 g/dL, respectively (Figure 3, *E*). Both PX and control hearts required packed red blood cell transfusion (4-5 times over the duration of perfusion). Control hearts received on average 110 mL packed red blood cells versus 160 mL for the PX hearts, likely due to additional hemolysis in the plasma separator. Average potassium levels were also within physiologic levels in both groups: 4.77 ± 0.1 mEq/L and 5.2 ± 0.2 mEq/L in PX and control hearts, respectively (Figure 3, *F*). There were no episodes of hypoglycemia and glucose levels in PX and control hearts averaged 272.07 ± 51.7 mg/dL and 132 ± 52.0 mg/dL, respectively (Figure 3, *G*).

### Ex Vivo Cardiac Performance

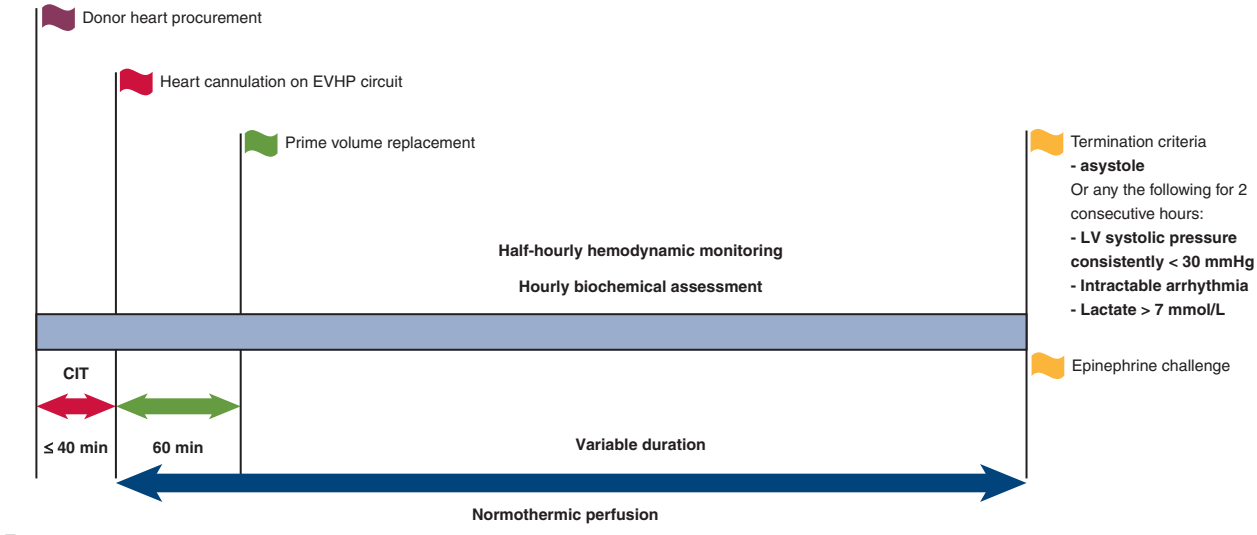
HR was a stable sinus rhythm between 90 and 110 bpm in both PX and control groups (Figure 4, *A*). The difference in HR between groups was not statistically significant ($P > .05$) with the exceptions highlighted in Figure 4, *A*. The resultant AO root pressure was on average higher in PX hearts (48.8 ± 1.3 mm Hg) versus control hearts (32.4 ± 0.7 mm Hg), a difference that was statistically significant from hour 4 to hour 24 (*P* value as shown in Figure 4, *B*).

With diastolic pressure maintained at approximately 12 mm Hg, LV systolic function was monitored with a pressure-transducing balloon, which showed that the average LV systolic pressure (LVSP) was higher in the PX compared with control hearts. In Figure 4, *C*, percent change from baseline value shows that in both groups, on average, there was an initial decrease to 60% of baseline in LV systolic function between hour 1 and hour 5 that could be the result of residual cardioplegia or cold ischemia. In the treatment group, LVSP recovered to 80% of baseline, whereas control hearts remain at 60% and trended down to 50% of baseline after hour 17. At the end of perfusion, LVSP was on average 63.0 ± 10.9 mm Hg and 37 ± 22.0 mm Hg for PX and control hearts, respectively. Across time, these differences were not statistically significant ($P > .1$). No data on LVSP were available after hour 17 for the control heart that progressed to 24 hours due to a failure of the balloon.

Coronary resistance was on average higher in the PX versus control hearts (1.06 ± 0.06 mm Hg/mL/min vs 0.58 ± 0.02 mm Hg/mL/min, respectively). That difference




A




B

**FIGURE 2.** Experimental timeline for plasma exchange (A) and control (B) hearts perfusion. Hearts were procured and after a brief period of cold ischemia (≤40 minutes) to allow cannulation, were perfused on the normothermic circuit. The priming perfusate was replaced after 60 minutes on the circuit to eliminate residual cardioplegia and reperfusion toxins. Perfusion was electively terminated at 24 hours or earlier (if asystole or any of the following end criteria were met for 2 consecutive hours: Left ventricle (*LV*) systolic pressure consistently <30 mm Hg, intractable arrhythmia, or lactate >7 mmol/L). *EVHP*, Ex vivo heart perfusion; *CIT*, cold ischemic time.

was only statistically significant from hour 5.5 until hour 12 with $P < .05$ (Figure 4, *D*).

### Metabolism

Lactate levels over the duration of perfusion were lower in PX ranging between 2.8 and 4.2 mmol/L versus control hearts where level ranged between 3.6 and 7.6 mmol/L. These differences were statistically significant as noted in Figure 5, *A*.

Calculated oxygen metabolism (corrected to weight) increased over time and indicated higher average oxygen utilization in PX hearts (2.89 ± 0.1 mL/min/100 g vs 1.8 ± 0.1 mL/min/100 g), with statistical significance as shown in Figure 5, *B*.

### Pathology

Photos of organs following perfusion show the gross appearance of the pericardial surface of these hearts with diffuse areas of necrosis more predominant in the control hearts (Figure 6).

Wet/dry ratios were obtained on gross specimens at the time of necropsy to evaluate organ edema. Data are

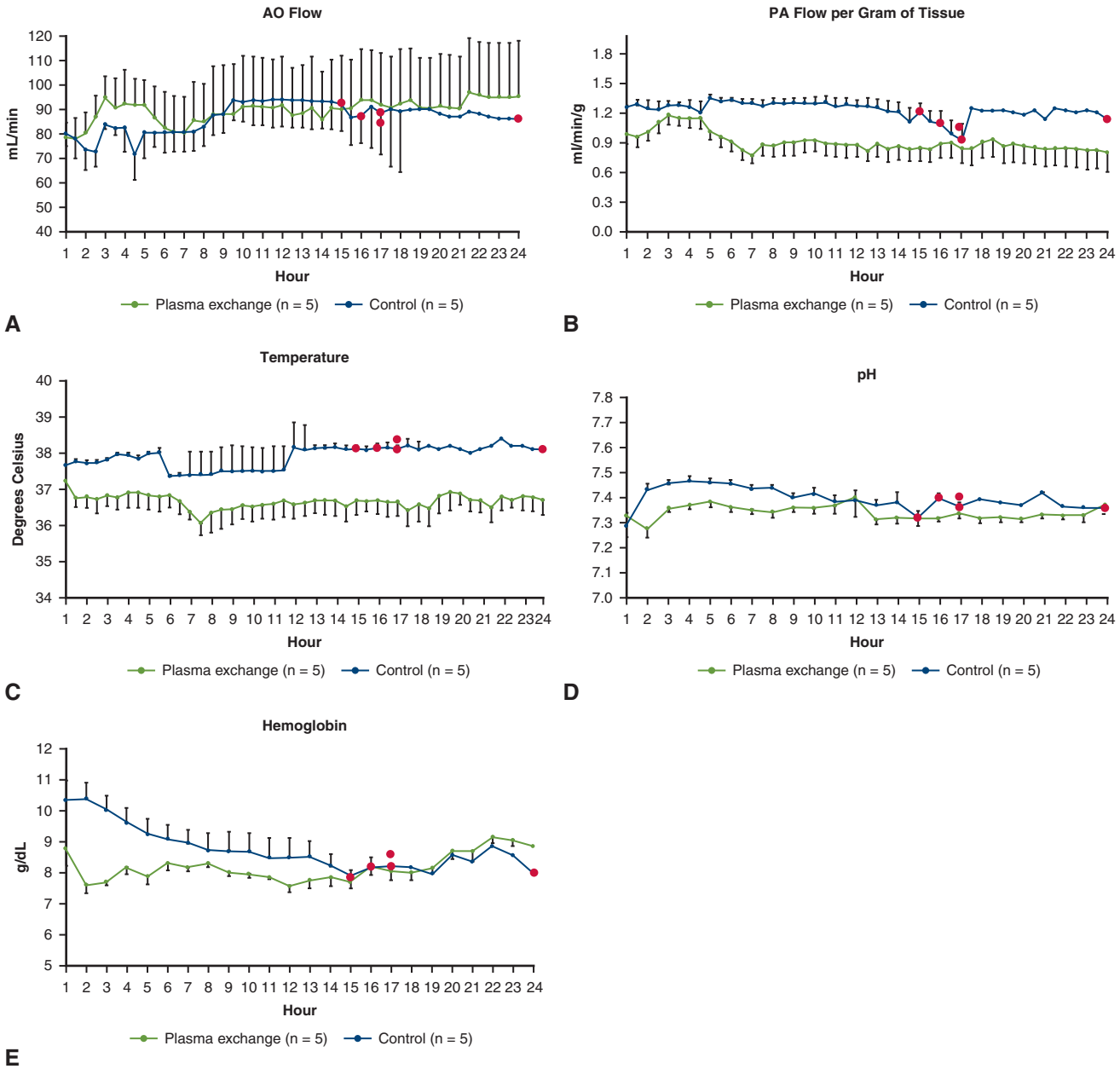



**FIGURE 3.** Perfusion parameters during prolonged ex vivo support remained stable and within target physiologic range in both plasma exchange and control experiments. A, Aortic (*AO*) flow. B, Pulmonary artery (*PA*) flow. C, Temperature. D, pH. E, Hemoglobin. F, Potassium. G, Glucose. Values are presented as mean ± standard error. *Red dots* indicate failing control hearts. Although AO flow, pH, and initial potassium levels were nearly identical in both groups, plasma exchange was associated with minor differences in PA flow, temperature, hemoglobin, and glucose concentrations as documented in the results.

presented in a box-and-whisker plot as seen in Figure 7. The difference between LV or RV in control versus PX was not statistically significant ($P = .09$ and $P = .14$). Similarly, there was no significant difference between PX LV versus RV ($P = .29$) or between control LV versus RV ($P = .83$).

An injury score for each cardiac chamber was assigned for each control and PX heart. Averages of those scores are outlined in Table 1 with the most notable difference being that PX hearts exhibited less myocardial degeneration and interstitial edema than controls. Myocardial hemorrhage and endothelial injury were more pronounced in the right atrium of controls.

## DISCUSSION

This study is a proof of concept that, in a porcine model, normothermic extracorporeal heart perfusion using PX is

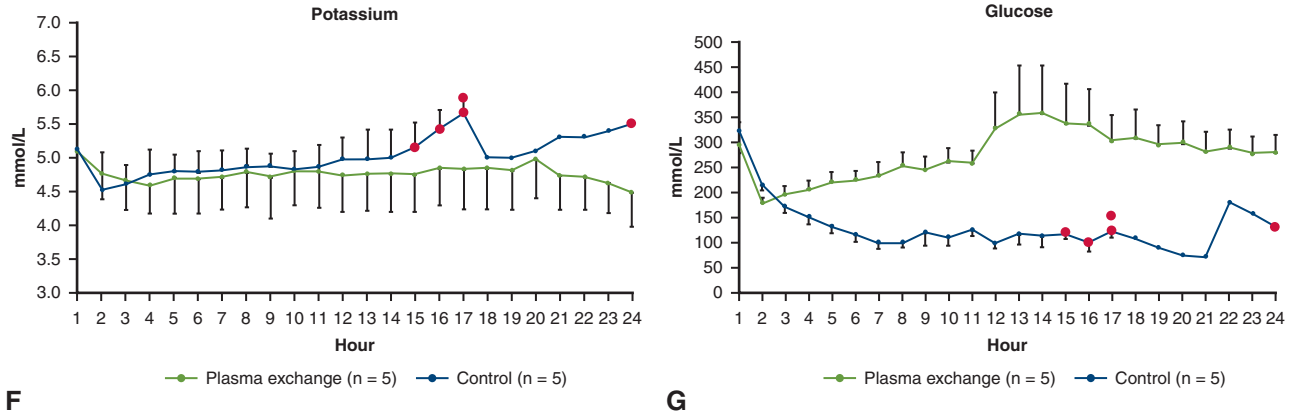



**FIGURE 3.** *(Continued).*

capable of maintaining ex vivo cardiac function for at least 24 hours. The primary findings of interest in this study are 4-fold: PX hearts all functioned well for 24 hours, compared with controls, fresh pooled PX can effectively replace plasma cross-circulation from a paracorporeal animal, lower lactate levels indicate that PX hearts have better myocardial perfusion, and PX preserves myocardial systolic function as demonstrated by higher LV systolic pressures and oxygen consumption.

AO flow was kept constant in both PX and control groups to achieve a physiologic coronary (ie, PA) flow; however, mean AO root pressures were lower in the

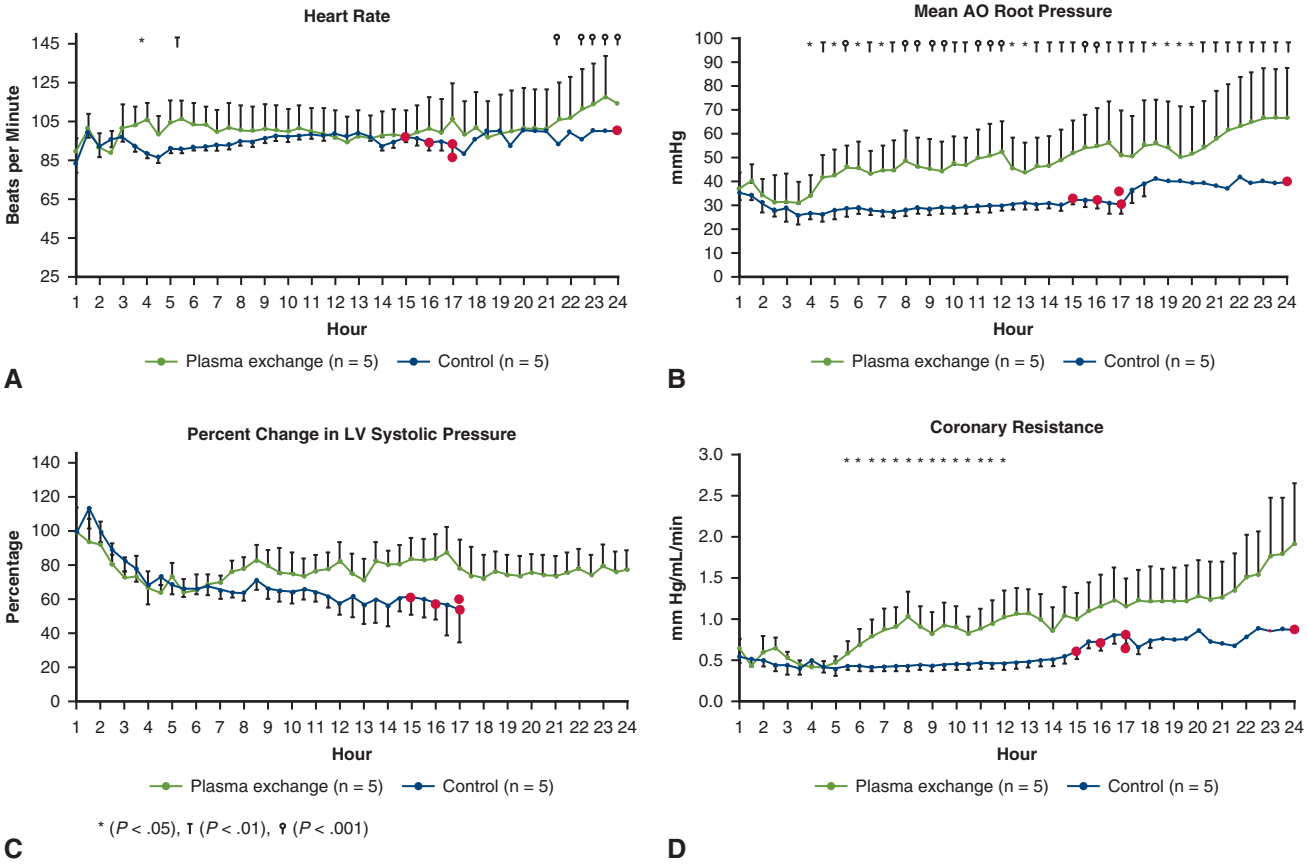



**FIGURE 4.** Evaluation of cardiac function overtime during ex vivo perfusion. A, Heart rate. B, Mean aortic root pressure. C, Percent change in left ventricle (*LV*) systolic pressure. Only 4 control hearts are represented due to a failed LV balloon in 1 of the control experiments. D, Coronary resistance. Values are presented as mean ± standard error. *Large red dots* indicate failing control hearts. *AO*, Aortic.

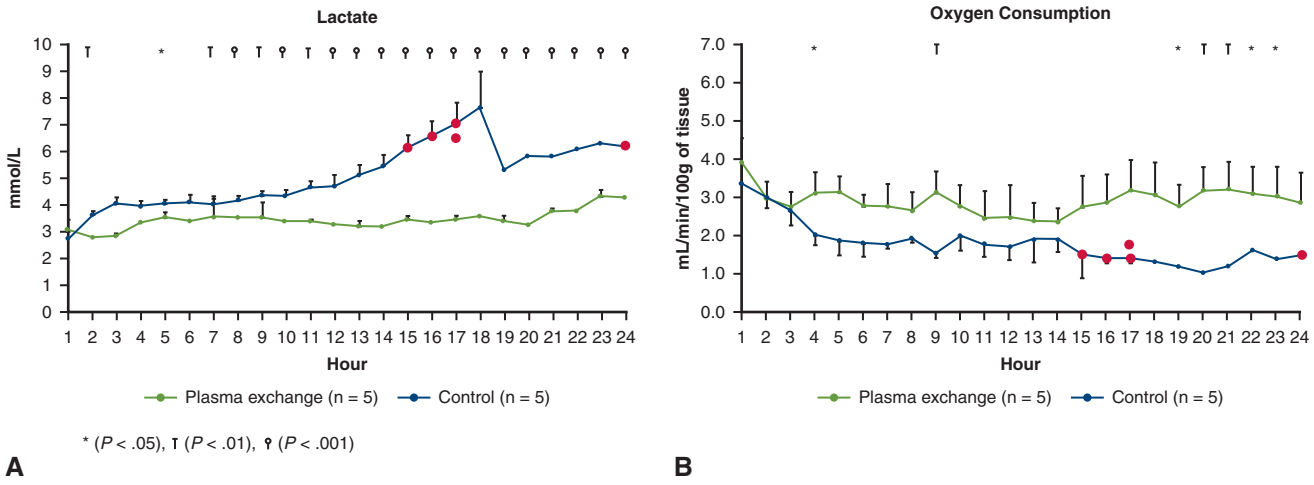



**FIGURE 5.** Metabolic functions of ex vivo hearts with comparison of (A) lactate levels and (B) oxygen consumption per 100 g cardiac tissue. *Large red dots* indicate failing control hearts.

control hearts, suggesting that distal coronary vasomotor tone was diminished in control hearts as hypothesized. Higher calculated coronary resistance in plasma group also supports this conclusion. The endothelium plays a critical role in the control of vasomotor tone in coronary vessels.[14] Although cardioplegia impairs endothelium-mediated relaxation of coronary vessels,[15,16] both groups received identical cardioplegia dosing with the difference in coronary resistance unlikely to be a consequence of cardioplegia. Higher vasomotor tone in PX hearts was interpreted as evidence of preserved endothelial integrity. These findings corroborate the results of prior studies with plasma cross-circulation that factors in plasma maintain coronary vasomotor tone and better endothelial cell preservation, which could mitigate the accelerated coronary artery disease in cardiac allograft, previously linked to endothelial cell injury.[17] The determinants of coronary resistance that were variable in the current experiments included characteristics of blood (viscosity and quality of flow) and extrinsic forces on the vasculature (compression and endothelial injury). Given the addition of a colloid in the form of plasma, blood viscosity was altered and could explain the higher coronary resistance in the PX group. Nevertheless, hematocrit is the major determinant of blood viscosity and was similar in both PX and control groups.

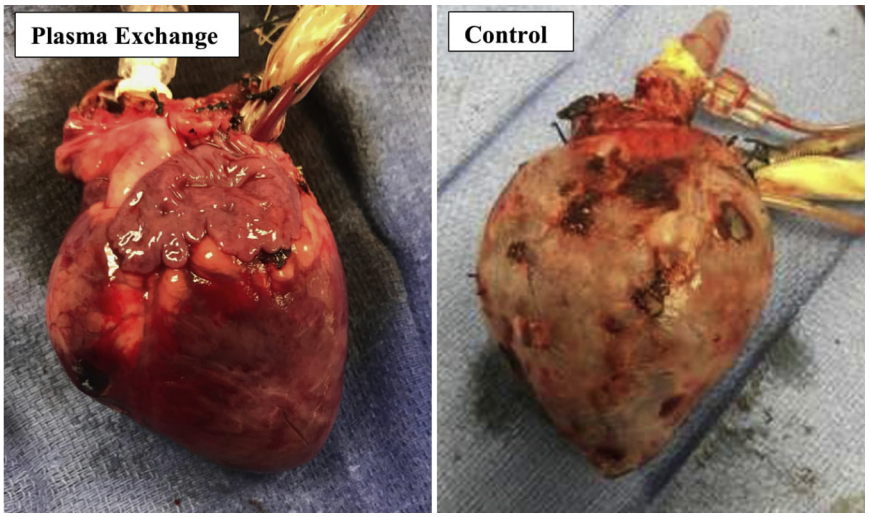



**FIGURE 6.** Gross appearance of plasma exchange (PX) and control hearts at necropsy. The PX heart was edematous and, because it had been perfused for 24 hours until elective termination and necropsy, it appeared engorged when compared with a typical donor heart preserved for <6 hours with nonphysiologic cold cardioplegia and immersion. Nonetheless, the physiologic parameters of the PX heart indicated that it should be suitable for transplantation. The control heart was not suitable for transplantation.

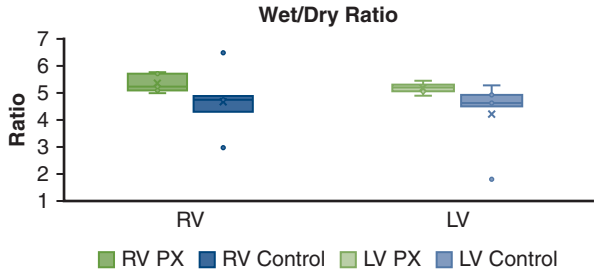



**FIGURE 7.** Comparisons of right ventricle (*RV*) (*full box*) and left ventricle (*LV*) (*hatched box*) wet/dry ratios in plasma exchange (*PX*) versus control ex vivo heart perfusion. Data are shown as box-and-whisker plots. The *upper* and *lower borders* of the *boxes* represent the upper and lower quartiles, respectively. The *middle horizontal line* represents the median. The *upper* and *lower whiskers* represent the maximum and minimum values of nonoutliers, respectively. *Extra dots* represent outliers and the cross is the mean value. There was no significant difference in wet/dry ratios between PX and control hearts.

Several reports have investigated novel perfusates in NEVP and to this date, only the Transmedics OCS (Andover, Mass) is clinically available, which uses a proprietary priming solution supplemented with insulin, antibiotics, methylprednisolone, sodium bicarbonate, multivitamins, and fresh blood. Despite improved results when compared with cold static storage, hearts perfused in the OCS still experience a time-dependent progressive functional decline and preservation with the OCS still has not exceeded 12 hours.[18,19] Continuous plasma supplementation in normothermic perfusate whether from cross-circulation or PX has consistently extended normothermic EVHP to 24 hours at least while allowing real-time evaluation of graft function.[8]

PX hearts had improved end-organ perfusion as evidenced by lower lactate levels and better oxygen consumption when compared with control hearts despite similar oxygen-carrying capacity in the perfusate. As a result, myocardial function was overall better in the PX group that generated higher LV systolic pressure. Studies using the OCS for heart preservation established that lactate levels >5 mmol/L were predictive of poorer posttransplant outcomes.[7,20] Worsening lactatemia was noted in the controls. In future experiments, perfusate filtration in the form of hemofiltration or dialysis could be added to remove toxins and further improve the quality of the perfusate.

Although there was no notable episode of hypoglycemia, glycemic levels were higher in the PX group because of ongoing glucose loading during plasma supplementation. With the small organ mass, insulin infusion had little effect in controlling hyperglycemia. Nevertheless, no detrimental effect of this hyperglycemia on perfusion was appreciated.

Increased functional capacity of the PX hearts was associated with lower injury grade. A validated myocardial injury scoring system was used to correlate hemodynamic performance with pathological data. Overall composite injury score was better for PX hearts versus controls with respect to myocardial degeneration and interstitial edema. In other categories, including myocardial hemorrhage and endothelial changes, little difference in scores was observed. It is worth noting that the lack of specific findings in sampled sections does not indicate the absence of lesions in other areas of the hearts. Additional testing with dyes such as tetrazolium may aid in additional quantitative assessment of tissue viability.

There was no significant difference in wet/dry ratios between PX and control hearts, which is discordant with previous publications where successfully perfused hearts exhibited less edema and weight gain.[8,13] Although organ edema was not different, it must be noted that 5 out of 5 control organs failed before 24 hours and thus, this comparison is looking at organs with different ex vivo life span. It is not unreasonable to think that had the control hearts been electively perfused for 24 hours (regardless of hemodynamic end criteria), they would have experienced worse capillary leakage and edema.

The current study supports a previously published report that plasma cross-circulation can maintain ex vivo cardiac function in an ovine model for at least 3 days (Figure 8).[8] These results also demonstrate the reproducibility of successful EVHP for at least 24 hours using supplemental plasma across species (ovine and porcine), a major step for preclinical human application. Before this study, only 3 other publications have documented successful prolonged (>24 hours) ex vivo cardiac perfusion and all of those studies relied on cross-circulation from a paracorporeal animal.[8,21,22] By eliminating the requirement for a paracorporeal animal, this work demonstrates that PX should allow the clinical application of EVHP for prolonged organ preservation.

**TABLE 1. Histopathologic evaluation of myocardial lesions**

| | Myocardial degeneration | | Myocardial hemorrhage | | Interstitial edema | | Endothelial changes | |
|---|---|---|---|---|---|---|---|---|
| Location | Control | Plasma | Control | Plasma | Control | Plasma | Control | Plasma |
| RA | 3.0 | 1.8 | 2.2 | 1.2 | 2.4 | 1.8 | 1.6 | 1.2 |
| RV | 3.0 | 2.8 | 2.2 | 2.2 | 2.2 | 2.6 | 1.6 | 1.4 |
| LA | 2.6 | 2.2 | 1.8 | 1.8 | 1.2 | 1.2 | 1.2 | 1.2 |
| LV | 3.0 | 2.0 | 1.6 | 1.4 | 2.2 | 1.2 | 1.6 | 1.4 |

*RA*, Right atrium; *RV*, right ventricle; *LA*, left atrium; *LV*, left ventricle.

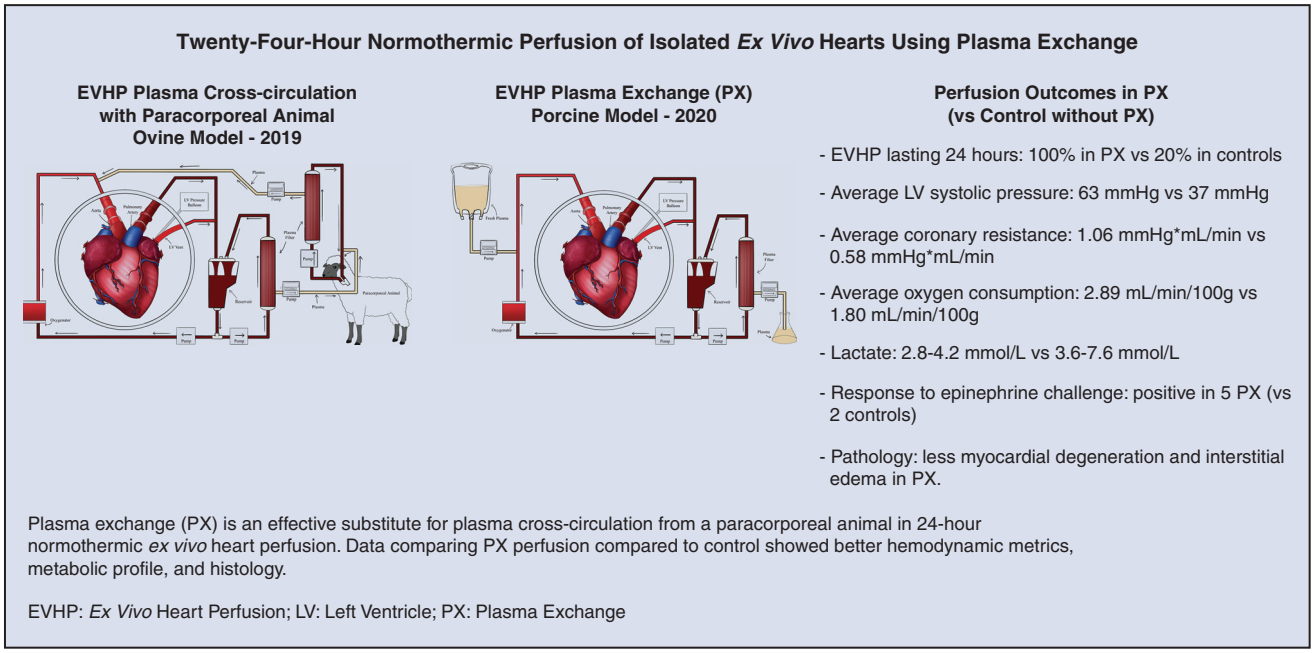



**FIGURE 8.** Twenty-four-hour normothermic perfusion of isolated ex vivo hearts using plasma exchange (PX). *EVHP*, Ex vivo heart perfusion; *LV*, left ventricle.

### Limitations of the Study

There are several limitations to the present study. This is a pilot study involving a small sample size from which to derive a complex longitudinal model. Although some of the differences in organ function and metabolic parameters during ex vivo perfusion between control and PX hearts were statistically significant, it remains to be shown whether they translate into clinical significance. The best test for organ viability would be the transplantation of PX hearts and measurement of clinical parameters related to cardiac function in the recipient animals. These transplants will be completed in upcoming experiments.

This study involved hearts procured from young and healthy swine donors. Although preserving hearts from older donors would be more clinically relevant, data presented here still highlight that PX is an effective substitute for plasma cross-circulation to prolong EVHP. Additional studies using PX could investigate injured organ recovery ex vivo and therapeutic interventions such as immunomodulation.[23]

Heart failure during EVHP is attributed to a primary change in endothelial integrity and function.[8,12,13] We observed higher resistance (vasomotor tone) with PX but no difference in wet/dry ratios between both groups. Nevertheless, hematoxylin and eosin staining demonstrated that globally there was minimal disruption to the endothelial lining in the PX group.

## CONCLUSIONS

In this study, we demonstrated the longest successful duration of EVHP without support from a paracorporeal animal. This study illustrates the feasibility of ex vivo heart support for clinical application and underscores the practicality of a perfusion system for real-time assessment of donor organs and interventions for marginal organs. As the field of heart transplantation progresses into the non-heart-beating donor arena, more objective systems to assess organs become critically important. The conclusions of this work and prior published experiments continue to support the importance of PX to achieve at least 24 hours of normothermic perfusion. It remains to be shown what molecules contained in plasma are vital to successful ex vivo organ perfusion.

### Conflict of Interest Statement

The authors reported no conflicts of interest.

The *Journal* policy requires editors and reviewers to disclose conflicts of interest and to decline handling or reviewing manuscripts for which they may have a conflict of interest. The editors and reviewers of this article have no conflicts of interest.

All statistical analyses were performed by Xiaoting Wu, PhD, biostatistician for the Department of Cardiac Surgery, University of Michigan. Circuit illustrations were designed by Bokai Zhang, University of Michigan.

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