Detailed Methods

Male Yorkshire pigs (40 ± 4 kg, Caughell Farms, ON, CA) were used to perform 17 orthotopic heart transplants. The experimental protocol was approved by our institutional animal care committee and followed the ARRIVE guidelines. [16] Animals were treated following the “Guide for the Care and Use of Laboratory Animals”.

Donor Procedure

Anesthesia, monitoring and baseline measurements

Premedication was undertaken with an intramuscular injection of Midazolam (0.3 mg/kg) and Ketamine (20 mg/kg). Anesthesia was induced and maintained using inhalational isoflurane through an oral endotracheal tube (end tidal concentration: 1-3%). An arterial line was inserted through the right common carotid artery, a central venous line was introduced into the left jugular vein, and a pulmonary artery catheter was inserted via the right jugular vein and directed into position beyond the pulmonary artery bifurcation. After performing a median sternotomy, the heart and great vessels were exposed. An umbilical tape was placed around the inferior vena cava, and a pressure-volume conductance catheter (Millar Instruments Inc, Houston, TX, USA) was inserted into the left ventricle through a small apical ventriculotomy. Systemic anticoagulation was achieved with an intravenous injection of 30,000 U heparin. Following a baseline assessment, donor animals were randomly assigned to undergo beating-heart donation (BHD; n=9) or DCD (n=8) (**Figure 1**).

Beating-heart donation (BHD group)

Following baseline evaluation, a purse-string suture was placed in the ascending aorta and inferior vena cava (IVC) to permit placement of a cardioplegia cannula and an 18F venous cannula via right atrium, respectively. We collected approximately 1.5 L of whole blood into an autotransfusion system (Frensenius Kabi C.A.T.S., Terumo, USA) to isolate the red blood cells (RBC). Simultaneously, the aorta was cross-clamped, and hearts arrested with 1L of histidine-ketoglutarate-tryptophan (HTK) solution at 4ºC. The heart was excised and placed in ice-cold HTK for 1 hour while being cannullated for ESHP.

Donation after circulatory death (DCD group)

Before cessation of mechanical ventilation, Propofol (1 mg/kg/min) and Remifentanyl (1 µg/kg/min) infusions were commenced, ensuring adequate anesthesia. Mechanical ventilation was discontinued

in the pre-heparinized animal resulting in progressive hypoxia and circulatory arrest. Asystole was determined as the loss of pulsatility on the arterial waveform. After the onset of asystole, a 15-minute warm ischemic period was observed. Functional warm ischemic time was defined as the time since systolic blood pressure fell below 50 mmHg until the organ was flushed with cold HTK. Whole blood was then collected, and hearts flushed and retrieved as described in the BHD group.

Ex Situ Heart Perfusion

A custom ESHP system was developed for these experiments as previously described. [13] In brief, the system consisted of a venous reservoir (Affinity Fusion®, Medtronic, Minneapolis, MN), a combined oxygenator/heat exchanger (Affinity Fusion®, Medtronic, Minneapolis, MN), a heater (Sarns Dual Heater Cooler Model 11160) and two centrifugal pumps (560A, Medtronic, Minneapolis, MN) mounted on to a Medtronic Performer CPB machine (Medtronic, Minneapolis, MN). The system was primed with 500 mL of STEEN solution™ (XVIVO Perfusion, USA), 500 mL of SOM-TRN-001 (Somahlution, USA), Mannitol (1 mg/kg), Methylprednisolone (500 mg), Heparin (10,000 u), and Cefazolin (1 g). A sufficient volume of RBC was added to achieve a hematocrit of 15%. Calcium chloride 10%, sodium bicarbonate 8.4%, and dextrose 50% was added to correct calcium (1.1-1.3 mmol/L), glucose (5-10 mmol/L), and bicarbonate (24-30 mmol/L) concentration, respectively.

Hearts were mounted on to the ESHP system following 1 hour of cold-storage. Once deairing was complete, retrograde aortic root perfusion was started at a pressure of 40 mmHg and hearts were rewarmed over 30 minutes to 37º C. Continuous infusions of Dobutamine (0.05 mcg/min), Nitroglycerin (1mcg/kg/min), and Insulin (5 units/h) were maintained throughout perfusion. Fraction of inspired oxygen (FiO2) and gas flow through the oxygenator were titrated to maintain a pH between 7.35 and 7.45, a pO_2 between 100 and 300 mmHg, and a pCO_2 between 35 and 45 mmHg. All hearts were perfused in Langendorff-mode, during which hourly perfusate samples were obtained for metabolic assessment. At 3 hours, hearts were transitioned into working-mode by loading the left and right atriums (LA and RA, respectively) at a flow corresponding to a cardiac index of 1.8 L/min/m^2 based on the donor animal's weight. Aortic diastolic pressure was maintained between 25-30 mmHg during functional assessment.

Recipient Procedure

We performed orthotopic cardiac transplantations as previously described by our group.[17] In brief, sedation and anesthesia were performed as in the donor protocol. After median sternotomy, we exposed the heart and great vessels and inserted an arterial line and pulmonary artery catheter as described in the donor protocol. The superior and inferior vena cava were encircled with umbilical

tapes. Systemic anticoagulation was achieved with an intravenous injection of 30,000 U heparin. Ascending aortic and bicaval cannulations were used to place the recipient on cardiopulmonary bypass (CPB). Flow rates were adjusted to maintain a mean arterial pressure above 50 mmHg. Norepinhephrine (0.05-0.15 mcg/kg/min) was administered during CPB if needed to aid in pressure regulation. Normothermia was maintained throughout CPB.

After aortic cross-clamping, the recipient's heart was excised, the left hemiazygous vein was suture-ligated at its insertion into the coronary sinus, and the anastomotic margins were inspected and trimmed in preparation for transplantation. At 4 hours of ESHP, the donor heart was flushed with and initial dose of cold blood cardioplegia and removed from the perfusion system. For implant, we used a standard biatrial anastomotic technique in sequence: left atrium, right atrium, pulmonary artery and ascending aorta. Cardioplegic protection consisted of 300ml of a 2:1 mixture of blood/crystalloid and was delivered at 10°C after the completion of each anastomosis. Before removal of the aortic cross-clamp, an additional 300 mL of warm blood cardioplegia and 500mg of methylprednisolone were administered.

Once the aortic cross-clamp was removed, hearts were reperfused for 60 minutes. Ventricular arrhythmia was treated with internal defibrillation (20J). If required, ventricular epicardial pacing was used to maintain a heart rate of 100 beats per minute. After 60 minutes of reperfusion, 1g calcium chloride was administered, and they were weaned from CPB. Weaning was deemed successful if the animals maintained a systolic arterial pressure of 60 mmHg for 30 minutes after the discontinuation of CPB. A vasoactive infusion of Dobutamine (5 mcg/kg/min) and Norepinephrine (0.1 mcg/kg/min) was used to assist in weaning from CPB. Hemodynamic and biventricular functional assessments were performed at 3 hours post-reperfusion, following which, the experiment was terminated.

Biochemical Parameters

Blood gases, electrolytes and oncotic pressure. Arterial and venous samples were collected at baseline, following DCD induction, hourly during ESHP, and hourly following transplantation. Electrolyte, lactate and hemoglobin concentrations, pH, pO_2, pCO2, and oxygen saturation (SpO_2) were measured using a blood gas analyzer (RAPIDPoint® 500 Blood Gas Systems, Siemens). All metabolic parameters were assessed with hearts perfused in Langendorff-mode.

Myocardial Viability Assessment. Measurement of metabolic parameters during ESHP has been used to gauge myocardial viability prior to transplantation.[18] Myocardial lactate etraction, coronary

vascular resistance, myocardial oxygen consumption, and oxygen extraction rate were derived as detailed below.

Myocardial lactate metabolism was determined as follows:

$$\text{Myocardial Lactate Metabolism } \left[\frac{\text{mmol}}{\text{L}}\right] = \text{Pulmonary Artery Lactate} - \text{Aortic Lactate } [\frac{\text{mmol}}{\text{L}}]$$

A positive result means production, whereas a negative result means extraction.

Coronary vascular resistance (CVR) was calculated as follows:

$$\text{CVR } \left[\frac{\text{mmHg x min}}{mL\ x\ 100g}\right] = 100 \text{ x} \frac{(\text{Mean Aortic Pressure} - \text{Mean Right Atrial Pressure [mmHg]})/\ \text{CBF } [\frac{\text{mL}}{\text{min}}]}{\text{Heart weight [g]}}$$

Where CBF is the coronary blood flow, assumed to be equivalent to the aortic blood flow.

Indexed myocardial Oxygen consumption (MVO_2) was as follows:

$$\text{MVO}_2 \left[\frac{\text{mLO}_2}{\text{min}\ x\ 100g}\right] = 100 \text{ x } \frac{\text{CBF } [\frac{\text{mL}}{\text{min}}]\ \times (\text{C}_\text{a}\text{O}_2 - \text{C}_\text{V}\text{O}_2\ [\frac{\text{mLO}_2}{\text{dL}}])}{\text{Heart weight [g]}}$$

Where C_aO_2 is the arterial oxygen concentration (aorta) and C_vO_2 is the venous oxygen concentration (pulmonary artery).

CaO_2 and CvO_2 can be calculated as follows:

$$\text{C}_{\text{a/v}}\text{O}_2\ [\frac{\text{mL}}{\text{dL}}] = \frac{\left(1.34\ \times \text{Hb concentration } \left[\frac{\text{g}}{\text{dL}}\right] \times \frac{\text{SpO}_2[\%]}{100}\right) + \left(0.0031\ \times\ p_{\text{a/v}}\text{O}_2\ [\text{mmHg}]\right)}{10}$$

Oxygen extraction rate was calculated as follows:

$$\text{O}_2 \text{ extraction } (\%) = 1 - (\frac{\text{C}_\text{v}\text{O}_2}{\text{C}_\text{a}\text{O}_2})\ \times 100$$

Contractility Assessment

In vivo. Global cardiac function was evaluated by cardiac output measurements using the pulmonary artery catheter and the traditional thermodilution technique. Cardiac index (CI) was then calculated as

the ratio between cardiac output and body surface area. Right ventricular function was assessed with the same catheter using Right Ventricular Stroke Work Index (RVSWI). Left ventricular (LV) function was assessed using a conductance catheter (Ventri-Cath-507S, Millar Inc, Houston, TX). Volume-dependent measurements were collected under steady conditions, while volume-independent measurements were collected during intermittent IVC occlusions. Developed pressure (dP), maximum rate of developed pressure (dP/dt_{max}), stroke work (SW), preload recruitable stroke work (PRSW), dP/dt_{max} vs. end-diastolic volume (EDV), end-systolic (E_{es}) and maximum (E_{max}) elastance were used to assess systolic function. Diastolic function was assessed using minimum rate of ventricular pressure change (dP/dt_{min}), isovolumetric relaxation constant (Tau), and end-diastolic pressure-volume relationship. Data was collected and analyzed using IOX v1.8.9.13 software (EMKA Technologies Inc., Falls Church, VA). All assessments were performed at baseline and at 3 hours of reperfusion post-transplant. Percent recovery was then calculated relative to baseline values.

Ex situ. Biventricular functional parameters (described above) were measured using a conductance catheter (Ventri-Cath-507S, Millar Inc, Houston, TX). Volume-dependent measurements were collected under steady loading conditions (CI = 1.8 L/min/m^2), while volume-independent measurements were collected by decreasing the RA and LA loading. Mean (MAP) and systolic aortic pressure (SBP), mean right ventricular pressure (RVP), systolic pulmonary artery pressure (SPAP), left (LAP) and right (RAP) atrial pressures and stroke volume (SV = cardiac output / heart rate) were recorded to derive the following non-invasive (NI) measurements of PRSW and E_{max} as previously described. [12,19] In brief, PRSW is the slope of the linear regression between LV or RV SW and atrial pressures, and E_{max} is the slope of the linear regression between SBP or SPAP and atrial pressures. Where, LV SW = MAP (mmHg) x SV (mL) and RV SW = RVP (mmHg) x SV (mL).

Echocardiography. Surface echocardiography was performed using a standard 3D transesophageal echocardiographic probe (Z6Ms, Siemens, ACUSON SC2000). The scan protocol includes 3 views: left ventricular short axis, apical four and two chamber. Systolic function is evaluated by ejection fraction (EF), fractional area change (FAC), global longitudinal strain (GLS), and global circumferential strain (GCS).

Statistical Analysis

Statistical analysis was performed using the SPSS 24 (IBM, USA) and Prism 8 (GraphPad, USA). Data is reported as median values (interquartile range). Comparisons between BHD and DCD hearts were performed using Mann-Whitney, Kruskal-Wallis or Friedmann tests, were appropriate. Spearman correlation analyses were used to determine the correlation between ESHP parameters and post-transplant function. Correlations were classified as weak ($r < 0.5$), moderate ($r = 0.5$-0.7),

or strong ($r > 0.7$) as previously described. {Mukaka:2012wo} Significance was set at $\alpha = 0.05$ for all statistical tests.

Supplemental Tables

For all supplemental tables the following color code was followed:

Table S1. Spearman correlation between post-transplant functional parameters and cardiac index.

Post-Transplant Combined	CI	% CI		CI	% CI
Developed Pressure	.658**	.631**	% Developed Pressure	.563**	.570**
Maximum dP/dt	.755**	.805**	% Maximum dP/dt	.723**	.802**
Minimum dP/dt	-.585**	-.534**	% Minimum dP/dt	0.407	0.315
Tau	-0.183	-0.238	% Tau	-0.385	-.436*
Stroke work	.896**	.875**	% Stroke work	.732**	.725**
Ees	0.375	0.261	% Ees	0.489	0.375
EDPVR	-0.239	-0.282	% EDPVR	0.114	0.032
PRSW	.857**	.821**	% PRSW	.768**	.761**
Maximum dP/dt vs EdV	0.375	0.261	% Maximum dP/dt vs EdV	.930**	.902**
Emax	0.468	0.354	% Emax	.543*	0.443
RVSWI	.532**	.589**	% RVSWI	.617**	.680**
RV FAC	-0.407	-0.445	% RV FAC	-0.115	-0.139

CI: cardiac index. dP/dt: rate of developed pressure. Ees: end-systolic elastance. EDPVR: end-diastolic pressure-volume relationship. PRSW: preload recruitable stroke work. EDV: end-diastolic volume. Emax: maximum elastance. RVSWI: right ventricular stroke work index. RV-FAC: right ventricular fractional area change. %: represents percent value relative to baseline measurements. *p<0.05, **p<0.01.

Table S2. Spearman correlation between metabolic variables measured during Ex Situ Heart Perfusion and post-transplant cardiac function.

	DP	% DP	Maximum dP/dt	% Maximum dP/dt	Minimum dP/dt	% Minimum dP/dt	SW	% SW	PRSW	% PRSW	RVSWI	% RVSWI	CI	% CI
CVR 30min	-0.11	-0.14	-0.23	-0.23	0.34	-0.09	-0.09	-0.29	-0.09	-0.08	-.525*	-0.27	-0.43	-0.43
CVR 1h	-0.05	-0.12	-0.11	-0.05	0.25	-0.05	-0.01	-0.03	-0.13	-0.19	-0.45	-0.14	-0.36	-0.31
CVR 2h	-0.08	-0.15	-0.09	-0.14	0.30	-0.15	0.09	-0.13	-0.01	-0.14	-0.35	-0.08	-0.38	-0.30
CVR 3h	-0.16	-0.20	-0.23	-0.27	0.50	-0.43	-0.13	-0.37	-0.22	-0.25	-.503*	-0.28	-0.48	-0.47
MVO2 30min	-0.11	-0.21	0.15	0.14	-0.05	-0.23	0.13	-0.02	0.35	0.29	0.07	0.02	0.17	0.26
MVO21h	0.06	0.08	0.36	0.30	-0.10	-0.14	0.42	0.25	0.61	0.56	0.26	0.23	0.37	0.37
MVO2 2h	0.09	-0.05	0.31	0.29	-0.16	0.00	0.42	0.10	0.42	0.19	0.22	0.31	0.31	0.38
MVO2 3h	0.04	0.05	0.31	0.24	-0.23	0.24	0.33	0.10	0.52	0.53	.600*	.650**	0.31	0.41
O2 Extraction 30min	-0.29	-0.36	-0.09	-0.16	0.11	-0.21	-0.19	-0.48	0.08	-0.01	-0.09	-0.05	-0.14	0.00
O2 Extraction 1h	0.04	0.04	0.40	0.31	-0.24	0.14	0.37	0.13	0.47	0.22	0.23	0.38	0.21	0.31
O2 Extraction 2h	0.34	0.35	0.38	0.31	-0.11	0.13	0.26	0.21	0.16	0.07	0.33	0.48	0.18	0.33
O2 Extraction 3h	0.03	0.09	0.07	-0.04	0.03	0.09	-0.07	-0.19	-0.12	-0.03	0.25	0.36	-0.09	0.03
Lactate 30min	-0.24	-0.27	-0.34	-0.36	0.17	-0.20	-0.52	-0.52	-0.25	-0.13	-0.36	-0.44	-0.46	-0.42
Lactate 1h	-0.14	-0.20	-0.33	-0.33	0.12	-0.12	-0.58	-0.53	-0.31	-0.18	-0.40	-.484*	-0.45	-0.44
Lactate 2h	-0.19	-0.25	-0.44	-0.41	0.04	0.04	-0.46	-0.38	-0.14	-0.03	-0.30	-0.40	-0.43	-0.44

Lactate 3h	-0.07	-0.09	-0.39	-0.35	0.03	0.08	-0.48	-0.36	-0.19	-0.07	-0.32	-0.40	-.495*	-.490*
Lactate Extraction 30min	-0.37	-0.37	-0.31	-0.28	0.34	-0.28	0.07	0.07	0.21	0.28	-0.17	-0.34	-0.05	-0.07
Lactate Extraction 1h	-0.04	0.07	-0.11	-0.11	0.31	-0.24	0.09	0.26	0.09	0.26	0.10	-0.08	0.00	-0.03
Lactate Extraction 2h	-0.41	-0.46	-0.30	-0.25	0.35	-0.46	-0.52	-0.52	-0.29	-0.52	-.608*	-.573*	-0.33	-0.40
Lactate Extraction 3h	-0.34	-0.28	-0.31	-0.25	0.22	-0.06	-0.07	-0.07	0.07	0.21	-0.10	-0.12	-0.29	-0.32

CI: cardiac index. CVR: coronary vascular resistance. dP: developed pressure. dP/dt: rate of developed pressure. MVO2: myocardial oxygen consumption. PRSW: preload recruitable stroke work. RVSWI: right ventricular stroke work index. SW: stroke work. %: represents percent value relative to baseline measurements. *p<0.05, **p<0.01.

Table S3. Spearman correlation between left ventricular parameters and post-transplant cardiac function.

Steady Beats	DP	% DP	Max dP/dt	% Max dP/dt	Min dP/dt	% Min dP/dt	SW	% SW	PRSW	% PRSW	CI	% CI
Developed Pressure	0.295	0.284	0.503	0.465	-0.218	-0.013	.650*	0.553	0.347	0.049	.649**	.611**
Maximum dPdt	.545*	0.479	.707**	.714**	-.657**	.654**	.794**	.648*	.867**	.673*	.664**	.620**
Minimum dPdt	-0.261	-0.243	-0.432	-0.436	0.143	-0.104	-.661*	-0.418	-0.612	-0.358	-0.316	-0.328
Tau	-0.125	-0.138	-0.464	-0.496	0.252	-0.193	-.699*	-0.517	-.760*	-.669*	-0.409	-0.441
Stroke work	0.248	0.467	0.297	0.321	0.345	-0.442	0.552	0.527	0.164	0.067	0.373	0.364
Ees	-0.115	-0.333	0.079	-0.055	-0.2	0.055	0.055	-0.309	0.273	0.248	0.209	0.236
EDPVR	-0.407	-0.587	-.743*	-.826*	0.18	-0.347	-.814*	-.946**	-.790*	-.778*	-0.577	-.678*
PRSW	0.479	0.394	.673*	0.576	-0.321	0.067	.782**	0.37	.709*	0.6	.770**	.782**
Maximum dP/dt vs EDV	0.103	-0.115	0.152	0.115	-0.491	0.455	-0.139	-0.309	0.224	0.103	0.036	0.027
Emax	0.018	-0.139	0.139	0.042	-0.091	-0.139	0.127	-0.188	0.176	0.115	0.255	0.264
NI Emax	0.304	0.179	.618*	.536*	-.589*	0.325	.636*	0.273	.661*	0.37	.674**	.706**
NI PRSW	0.257	0.186	.661**	.632*	-.521*	0.293	.685*	0.491	0.539	0.358	.686**	.730**

CI: cardiac index. dP/dt: rate of developed pressure. Ees: end-systolic elastance. EDPVR: end-diastolic pressure-volume relationship. NI: non-invasive. PRSW: preload recruitable stroke work. EDV: end-diastolic volume. Emax: maximum elastance. %: represents percent value relative to baseline measurements. *p<0.05, **p<0.01.

Table S4. Spearman correlation between right ventricular parameters and post-transplant cardiac function.

	RVSWI	% RVSWI	CI	% Ci
Developed pressure	0.516	.560*	0.473	0.505
Maximum dP/dt	0.253	0.275	0.505	0.511
Minimum dP/dt	-0.225	-0.401	-0.451	-0.44
Tau	-0.197	-0.176	-0.085	-0.148
Stroke work	.571*	0.396	0.313	0.324
Ees	0.341	0.341	0.411	0.42
EDPVR	0.259	-.629*	0.371	0.434
PRSW	0.464	0.332	0.305	0.341
Emax	0.495	0.473	0.385	0.433
NI Emax	0.159	0.297	0.129	0.162
NI PRSW	.688**	.597*	0.303	0.329

CI: cardiac index. dP/dt: rate of developed pressure. Ees: end-systolic elastance. EDPVR: end-diastolic pressure-volume relationship. PRSW: preload recruitable stroke work. EDV: end-diastolic volume. Emax: maximum elastance. NI: non-invasive. RVSWI: right ventricular stroke work index. %: represents percent value relative to baseline measurements. *p<0.05, **p<0.01.

Table S5. Spearman correlation between echocardiographic parameters and post-transplant cardiac function.

	DP	% DP	Maximum dP/dt	% Maximum dP/dt	Minimum dP/dt	% Minimum dP/dt	SW	% SW	PRSW	% PRSW	CI	% CI
LV EF	0.046	0.032	0.275	0.152	-0.263	0.249	0.298	0.176	0.395	0.486	0.267	0.265
LV-FAC	-0.179	-0.146	0.032	0	0	-0.018	-0.333	-0.115	-0.382	-0.261	-0.074	-0.044
GLS	-0.216	-0.218	-0.196	-0.096	0.104	0.011	-0.2	-0.248	-0.394	-0.358	-0.076	-0.047
GCS	0.145	0.202	-0.079	0.009	0.415	-0.374	0.212	0.358	-0.224	-0.321	-0.052	0.007
	RVSWI	% RVSWI	CI	% CI								
RV-FAC	-0.232	-0.256	0.053	0.056								

CI: cardiac index. dP: developed pressure. dP/dt: rate of developed pressure. EF: ejection fraction. LV: Left ventricular. RV: right ventricular. GLS: global longitudinal strain. GCS: global circumferential strain. PRSW: preload recruitable stroke work. SW: stroke work. RVSWI: right ventricular stroke work index. FAC: fractional area change. %: represents percent value relative to baseline measurements. *p<0.05, **p<0.01.