Heart Transplantation From Brain Dead Donors: A Systematic Review of Animal Models

Louise E. See Hoe, PhD,[1,2] Matthew A. Wells,[1,3] Nicole Bartnikowski, PhD,[1,4] Nchafatso G. Obonyo, MD,[1,5] Jonathan E. Millar, MD,[1,2,6] Aimee Khoo,[1,2] Katrina K. Ki, PhD,[1,2] Tristan Shuker,[1,2] Alessandro Ferraioli,[1,2] Sebastiano M. Colombo, MD,[1,2,7] Wandy Chan, MD, PhD,[1,2] David C. McGiffin, MD,[1,8] Jacky Y. Suen, PhD,[1,2] and John F. Fraser, MD, PhD[1,2]

Abstract. Despite advances in mechanical circulatory devices and pharmacologic therapies, heart transplantation (HTx) is the definitive and most effective therapy for an important proportion of qualifying patients with end-stage heart failure. However, the demand for donor hearts significantly outweighs the supply. Hearts are sourced from donors following brain death, which exposes donor hearts to substantial pathophysiological perturbations that can influence heart transplant success and recipient survival. Although significant advances in recipient selection, donor and HTx recipient management, immunosuppression, and pretransplant mechanical circulatory support have been achieved, primary graft dysfunction after cardiac transplantation continues to be an important cause of morbidity and mortality. Animal models, when appropriate, can guide/inform medical practice, and fill gaps in knowledge that are unattainable in clinical settings. Consequently, we performed a systematic review of existing animal models that incorporate donor brain death and subsequent HTx and assessed studies for scientific rigor and clinical relevance. Following literature screening via the U.S National Library of Medicine bibliographic database (MEDLINE) and Embase, 29 studies were assessed. Analysis of included studies identified marked heterogeneity in animal models of donor brain death coupled to HTx, with few research groups worldwide identified as utilizing these models. General reporting of important determinants of heart transplant success was mixed, and assessment of posttransplant cardiac function was limited to an invasive technique (pressure-volume analysis), which is limitedly applied in clinical settings. This review highlights translational challenges between available animal models and clinical heart transplant settings that are potentially hindering advancement of this field of investigation.

(*Transplantation* 2020;104: 2272–2289).

Received 22 October 2019. Revision received 13 January 2020.

Accepted 22 January 2020.

[1] *Critical Care Research Group, The Prince Charles Hospital, Brisbane, QLD, Australia.*

[2] *Faculty of Medicine, University of Queensland, Brisbane, QLD, Australia.*

[3] *School of Medical Science, Griffith University, Gold Coast, QLD, Australia.*

[4] *Faculty of Science and Engineering, Queensland University of Technology, Brisbane, QLD, Australia.*

[5] *Wellcome Trust Centre for Global Health Research, Imperial College London, London, United Kingdom.*

[6] *Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, United Kingdom.*

[7] *Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy.*

[8] *Cardiothoracic Surgery and Transplantation, The Alfred Hospital, Melbourne, VIC, Australia.*

This work and the authors are supported by the University of Queensland, the Prince Charles Hospital Foundation, Queensland Health (Bionics), the Alfred Foundation, the Metro North Hospital and Health Service, The Donald and Joan Wilson Foundation, the National Health and Medical Research Council (APP1145761—The Dead Heart Project), and the Centre for Research Excellence for Advanced Cardio-respiratory Therapies Improving Organ Support (CRE ACTIONS). L.E.S.H. and N.B. are recipients of a Prince Charles Hospital Foundation Postdoctoral Fellowship. J.F.F. is the recipient of an Office of Health and Medical Research Fellowship from the Queensland Government.

Outside of the submitted work, J.F.F. provides consultancy services to Fisher and Paykel Healthcare, BiVACOR, De Motu Cordis Pty Limited, and the Quantum Medical Innovation Fund. J.F.F. and his research group have received grant funding from the National Health and Medical Research Council, The Prince Charles Hospital Foundation, and the Queensland Government (Department of Health, Metro North Hospital and Health Service). Additionally, J.F.F. and his research group work with Fisher and Paykel Healthcare, Mallinckrodt Pharmaceuticals, CSL, BiVACOR, De Motu Cordis Pty Limited, and Australian Red Cross Blood service. This has no direct impact on the contents of the article. The other authors declare no conflicts of interest.

L.E.S.H., M.A.W., A.K., and J.E.M. participated in the conceptualization of the article. L.E.S.H., M.A.W., N.B., N.G.O., A.K., K.K.K., T.S., A.F., W.C., D.C.M., and J.Y.S. participated in the data collection of the article. L.E.S.H., M.A.W., N.B., and N.G.O. participated in the writing (original draft preparation) of the article. L.E.S.H., M.A.W., N.B., N.G.O., J.E.M., K.K.K., S.M.C., W.C., D.C.M., J.Y.S., and J.F.F. participated in the writing (review, editing, and interpretation) of the article.

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantjournal.com).

Correspondence: Louise E. See Hoe, PhD, Room 13, Level 3, Clinical Sciences Bldg, The Prince Charles Hospital (The Critical Care Research Group and University of Queensland), 627, Rode Rd, Chermside 4032 QLD, Australia. (l.seehoe@uq.edu.au).

ISSN: 0041-1337/20/10411-2272

DOI: 10.1097/TP.0000000000003217

<zdoi;10.1097/TP.0000000000003217>

INTRODUCTION

End-stage heart failure is an irreversible and progressive condition associated with high morbidity and mortality and is increasing in frequency worldwide.[1] Globally, approximately 38 million people are affected by heart failure,[2,3] and an estimated 17%–45% patients with heart failure die within 1 y from hospital admission.[4] The gold standard treatment for end-stage heart failure is heart transplantation (HTx);[1,5] however, the donor pool is grossly inadequate and, increasingly unable to satisfy the growing demand. Although in the last decade the number of deceased donors has increased globally, in 2017 only 22% of this donor pool was a source of viable hearts for transplant.[6] Despite advances in technological, surgical, and pharmacologic measures to broaden donor heart availability,[7] death on the waitlist for transplant is a frequent occurrence.

Currently, hearts for transplantation are mainly procured from donors exposed to brain stem death (BSD), although experience with donation after circulatory determination of death is slowly increasing.[8-11] The donor heart is subject to a number of injuries that adversely affect posttransplant cardiac function. Initially, risk of cardiac injury is due to the systemic derangement associated with brain death, then, cold and warm ischemia during procurement and implant, and finally associated with reperfusion injury. The injuries sustained by the donor heart following implantation may manifest as primary graft dysfunction (PGD), which is an important cause of recipient mortality and morbidity in the immediate postoperative period. Prolongation of the ischemic time beyond 4 h increases the risk of PGD. Several other factors potentiate this risk, including older donor age, left ventricular hypertrophy, ventricular dysfunction (particularly a discrete left ventricular wall abnormality), and donor/recipient size mismatch.[12,13]

The clinical need to improve the availability and quality of donor hearts has driven preclinical research toward the understanding and mitigation of the pathophysiological mechanisms underlying profound neurologic-induced cardiac injury. The complexities, for example, timing and sampling, and sensitive nature of performing clinical-based BSD studies can hinder the interpretation of research outcomes. Conversely, animal models provide a platform to obtain in vivo physiological data ranging from whole organs to organelle and can detail changes in mechanistic pathways of disease development, management, and treatment. To facilitate clinical translation of research findings and ensure clinical relevance, the animal model should mimic the human/clinical situation as closely as possible. Although a number of models exist that have examined BSD and/or heart transplant, the number of animal models that closely simulate human donor BSD followed by heart transplant are limited.

Hence, the primary aim of this review is to identify and comprehensively describe the experimental animal models that have been used to investigate donor BSD followed by HTx. In addition, scientific rigor and clinical relevance of these previous models are characterized and suggestions for future methodological improvements are provided.

MATERIALS AND METHODS

Design

We developed a systematic review protocol, which was reported on the Systematic Review Center for Laboratory Animal Experimentation website (https://www.radboudumc.nl/getmedia/892c264e-24a2-445d-8364-23adae6364c2/Animal-models-of-heart-transplantation-from-brain-dead-donors.aspx) on February 21, 2017, and published in May 2017. This protocol aligns with the Preferred Reporting Items for Systematic Review and Meta-analysis Protocols.[14] Following publication, the protocol was adjusted to remove language restrictions that excluded non-English language publications.

Search Strategy

Following consultation with a trained medical librarian (University of Queensland), individual search strategies using nomenclature compatible with the PubMed and Embase engines were generated. The U.S National Library of Medicine bibliographic database (MEDLINE, via PubMed) and Embase (via Ovid SP) online databases were searched to retrieve studies over any time period up until June 2019. Please refer to Supplemental Digital Content 1 for the full search strategy.

Inclusion and Exclusion Criteria

This systematic review included all nonhuman, in vivo animal studies that described or used a model of donor BSD for HTx. Studies were excluded if they involved any of the following: clinical (human), in vitro and/or ex vivo studies, any studies that did not incorporate or proceed to actual HTx (orthotopic or heterotopic), animal and/or clinical (human) models of donation after circulatory determination of death as a single experimental group, and multiple organ transplantation (including heart/lung transplantation). Regarding heart/lung transplantation, studies have been included if the technique (eg, heterotopic HTx [HHTx]) required transplantation of the heart and lungs en bloc, but the lungs remained nonfunctional.

Literature Search and Screening

After removal of duplicates, all retrieved studies were screened in 2 separate phases (Figure 1). Phase I screening of search results was undertaken by 3 independent reviewers based upon title and abstract only (please refer to **Figure S1** for flowchart demonstrating abstract screening, **SDC**, http://links.lww.com/TP/B898). Phase II screening was undertaken by 5 independent reviewers in which full-text articles were evaluated for eligibility, based upon the inclusion and exclusion criteria listed above. Disagreements were resolved by a senior author. All reference lists of studies identified by Phase I screening were reviewed to identify publications not found in the initial search strategy.

Data Extraction

During Phase II, reviewers collected data collated into 5 major categories, which have been recorded in Tables 1–5:

1. General study features: Information tabulated includes publication year, authors, country of origin, species, general study type, and study aim.

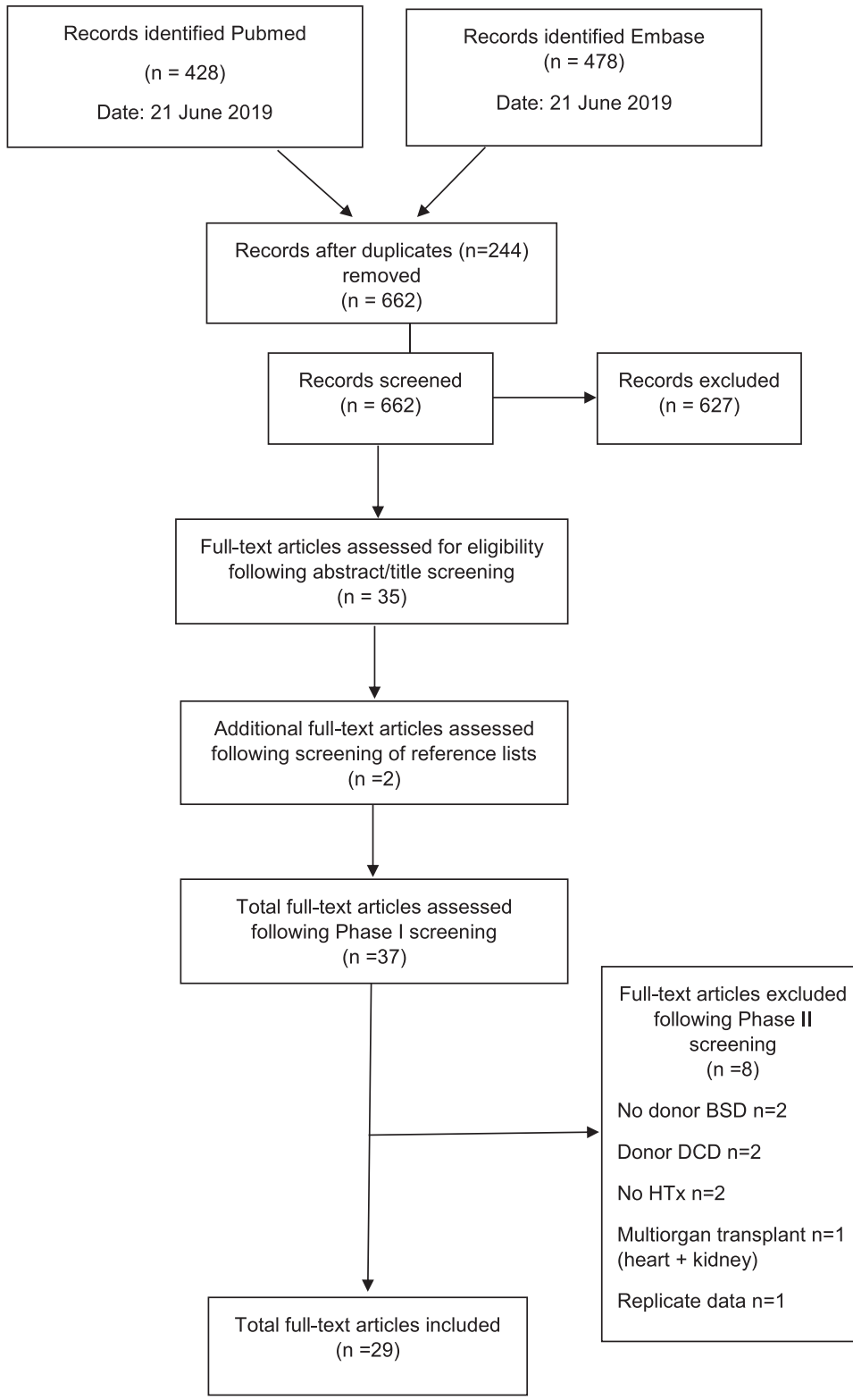

FIGURE 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram. This flow diagram depicts the method used to identify included and excluded studies for the systematic review of animal models of heart transplantation (HTx) following donor brain stem death (BSD). DCD, donation after circulatory death.

2. General animal characteristics for each included study: Details regarding animal breed, age, weight, gender, and the use of anticoagulation was reported.
3. Methodological features of BSD development and management: Specific data regarding the induction method of BSD, particular fluids and drugs used during BSD management, follow-up time following BSD confirmation, and specific BSD confirmation criteria were recorded.
4. HTx model details: Data were recorded on several aspects of HTx procedures, including heart preservation (preservation method, duration, and solution used), HTx surgical

TABLE 1.

General study information sorted by publication y

Author, y (country)	Species	Study type	Aims
Shivalkar et al,[15] 1993 (Belgium)	Dog	Physiological	Assess effects of sudden vs gradual intracranial pressure increases pre- and post-HTx upon catecholamine levels, hemodynamics, and myocardial structure
Bittner et al,[16] 1995 (United States)	Dog	Physiological	Examine effects of BSD and CSS upon LV and RV function post-HTx
Votapka et al,[17] 1996 (United States)	Rat	Interventional	Determine the effect of triiodothyronine treatment (in donor) upon graft function
Kim et al,[18] 1998 (Belgium)	Dog	Interventional	Assess the effects of Na^+/H^+ exchange inhibition upon graft function post-HTx
Bittner et al,[19] 1999 (United States)	Dog	Physiological	Determine if post-HTx RV dysfunction is due to recipient pulmonary hypertension or donor BSD-induced cardiac injury
Ryan et al,[20] 2000 (Australia)	Pig	Physiological	Develop a large animal model of orthotopic HTx incorporating donor BSD
Wilhelm et al,[21] 2000 (United States)	Rat	Physiological	Examine acute rejection of hearts from BSD donors
Wilhelm et al,[22] 2001 (United States)	Rat	Physiological	Determine the effect of donor brain death upon inflammatory cell population changes and myocardial fibrosis in chronic cardiac allograft rejection
Ryan et al,[23] 2002 (Australia)	Pig	Physiological	Determine the PRSW relationship as an index of graft LV contractility post-HTx following CSS
Wilhelm et al,[24] 2002 (United States)	Rat	Physiological	Examine the effect of donor BSD upon the inflammatory response in recipients towards cardiac graft (in model of chronic cardiac allograft rejection)
Ryan et al,[25] 2003a (Australia)	Pig	Physiological	Determine if early cardiac troponin I release predicts effectiveness of cardiac protection during preservation
Ryan et al,[26] 2003b (Australia)	Pig	Interventional	Examine the effects of preconditioning the donor heart with cariporide could reduce ischemia-reperfusion injury post-HTx
Ryan et al,[27] 2003c (Australia)	Pig	Interventional	Determine the effects of lazaroid U74389G supplementation in cardioplegia upon post-HTx graft function
Ryan et al,[28] 2003d (Australia)	Pig	Interventional	Examine effects of cariporide and BMS180448 supplementation in cardioplegia HTx
Konstantinov et al,[29] 2005 (Canada)	Pig	Interventional	Determine if remote ischemic preconditioning induces cardioprotection via a circulator effector in a model of HTx
Hing et al,[30] 2009 (Australia)	Pig	Interventional	Compare the effects of different cardioplegic strategies via supplementation with glyceryl trinitrate and/or cariporide
Ali et al,[31] 2011 (Canada)	Pig	Physiological	Assess whether hearts retrieved following circulatory death are suitable for transplantation (compared to BSD hearts)
Floerchinger et al,[32] 2012 (United States)	Mouse	Physiological	Investigate graft-specific inflammatory immune responses following BSD in donors and recipients
Atkinson et al,[33] 2013 (United States)	Mouse	Both	Investigate the effects of BSD upon graft ischemia-reperfusion injury and the effect of targeted complement inhibition
Watson et al,[34] 2013 (Australia)	Pig	Interventional	Evaluate the effects of erythropoietin, glyceryl trinitrate, and zoniporide treatment in a porcine model of HTx
Li et al,[35] 2015a (Germany)	Rat	Physiological	Investigate the functional, gene, protein, and histopathologic changes in heart grafts (post-HTx) following donor BSD vs circulatory death
Li et al,[36] 2015b (Germany)	Rat	Physiological	Investigate different periods of BSD and effect upon post-HTx graft function
Spindler et al,[37] 2015 (Germany)	Rat	Interventional	Examine the effects of N-octanoyl dopamine administration during brain death upon cardiac allografts
Hegedus et al,[38] 2016 (Germany)	Rat	Interventional	Determine if dimethyloxalylglycine improves post-HTx graft function
Ritschl et al,[39] 2016 (Germany)	Mouse	Physiological	Investigate the effect of BSD upon immune activation and ischemia-reperfusion injury in transplant settings
Steen et al,[40] 2016 (Sweden)	Pig	Interventional	Assess efficacy of novel cold machine perfusion system upon post-HTx graft function
Li et al,[41] 2017 (Germany)	Rat	Interventional	Examine the effect of N-octanoyl dopamine on early post-HTx graft function
Yip et al,[42] 2017 (Taiwan)	Rat	Interventional	Assess the effects of allogeneic adipose-derived mesenchymal stem cells upon brain death-induced injury and acute rejection post-HTx
Kumar et al,[43] 2017 (United States)	Pig	Interventional	Determine the effect of T3 upon post-HTx cardiac function in immature pigs

BSD, brain stem death; CSS, cold static storage; H^+, hydrogen; HTx, heart transplantation; LV, left ventricular; Na^+, sodium; PRSW, preload recruitable stroke work; RV, right ventricular; T3, triiodothyronine.

aspects (surgical method and positioning, technique, and total ischemic time), recipient support following HTx (fluids, drugs, pacing), and the duration of reperfusion following HTx.

5. Outcome measures of cardiac function in donor and recipients: Here, the specific parameters relating to outcomes of cardiac function in these studies were recorded.

Given the aim of this review being descriptive in nature, and the heterogeneity of the animal studies included, no meta-analysis was performed. Summary statistics were used as appropriate. Results are reported in alignment with Tables 1–5.

RESULTS

Description of Included Studies

A summary of included studies is detailed in Table 1. Following screening via the search strategy (Figure 1), 29

TABLE 2.

General animal characteristics

Study	Breed	Age	Weight	Gender	Anticoagulation	
					Donor	Recipient
Mouse						
Floerchinger et al,[32] 2012	C57BL6 (WT and Rag2/Il2rg double knockout)	8–12 wk	20–30 g	M	–	–
Atkinson et al,[33] 2013	C57BL6, BALB/c, and C57BL6 pan-GFP	8–12 wk	20–30 g	M	–	–
Ritschl et al,[39] 2016	C57BL6 (H-2^b) and BALB/c (H-2^d)	8–12 wk	–	M	–	–
Rat						
Votapka et al,[17] 1996	Lewis (inbred)	–	0.27–0.32 kg	M	Y	Y
Wilhelm et al,[21] 2000	Lewis RT^1 and Fisher 344 $RT1^{1v1}$	8–10 wk	0.2–0.22 kg	M	–	–
Wilhelm et al,[22] 2001	Lewis and Fisher 344	–	–	–	–	–
Wilhelm et al,[24] 2002	Lewis and Fisher 344	–	–	–	–	–
Li et al,[35] 2015a	Lewis	–	0.25–0.35 kg	M	Y	Y
Li et al,[36] 2015b	Lewis	–	0.25–0.35 kg	M	Y	Y
Spindler et al,[37] 2015	Lewis/Crl and Fisher 344/DuCrl	–	0.25–0.3 kg	M		
Hegedus et al,[38] 2016	Lewis	–	0.25–0.35 kg	M	Y	Y
Li et al,[41] 2017	Lewis	–	0.25–0.35 kg	M	Y	Y
Yip et al,[42] 2017	Fisher 344	Adult	250–280 g	M	Y	–
Dog						
Shivalkar et al,[15] 1993	–	–	28 ± 4 kg	–	–	–
Bittner et al,[16] 1995	Mongrel	Adult	22–31 kg	–	Y	Y
Kim et al,[18] 1998	Mongrel	Adult	22–37 kg	–	Y	Y
Bittner et al,[19] 1999	Mongrel	Adult	23–33 kg	–	Y	Y
Pig						
Ryan et al,[20] 2000	Westran[a]	–	20–50 kg	–	Y	Y
Ryan et al,[23] 2002	Westran[a]	–	22–57 kg	–	Y	Y
Ryan et al,[25] 2003a	Westran[a]	–	36–68 kg	–	Y	Y
Ryan et al,[26] 2003b	Westran[a]	–	36–68 kg	–	Y	Y
Ryan et al,[27] 2003c	Westran[a]	–	20–57 kg	–	Y	Y
Ryan et al,[28] 2003d	Westran[a]	–	37.5–63 kg	–	Y	Y
Konstantinov et al,[29] 2005	Yorkshire	–	26–34.2 kg	M	–	–
Hing et al,[30] 2009	Westran[a] or Landrace[a]	Juvenile	40–60 kg	–	Y	Y
Ali et al,[31] 2011	Domestic	–	62 ± 5 kg	F	–	–
Watson et al,[34] 2013	Landrace[a]	Juvenile	40–60 kg	Mixed gender	Y	Y
Steen et al,[40] 2016	Swedish pigs of native breed	–	35–63 kg	–	Y	Y
Kumar et al,[43] 2017	–	6–9 wk	20–27 kg	–	Y	Y

[a]Highly inbred siblings obtained in pairs.
F, female; M, male; Y, yes.

papers were included in the systematic review. The studies were published between 1993 and 2017 (24 y), with most of the studies performed in the United States (n = 9), Australia (n = 8), and Germany (n = 6), followed by Canada (n = 2), Belgium (n = 2), Sweden (n = 1), and Taiwan (n = 1). Studies were performed mostly in pigs (n = 12), rodents (rats n = 10; mice n = 3), and dogs (n = 4). Among the 29 studies identified, 80% of the publications were from 6 research groups across all identified animal models. The primary purpose of each study varied, but generally observational (n = 14) or interventional (n = 14) in nature, with 1 study incorporating both purposes (n = 1).

General Study Parameters

General study parameters are reported in Table 2. Breed was predominantly reported and consistent within each species. Age (in wk/mo/y) was generally not reported (83%); however, all mouse studies (n = 3) reported use of young mice aged 8–12 wk. Conversely, weight was typically recorded for all studies (87%), and the reported weight range varied across all studies and species. Gender was consistently reported for mouse and rat studies, yet was largely unreported in dog and pig studies (exception of 3 pig studies[29,31,34]). Studies predominantly used male animals. Anesthetic regimes varied between animal models. Rodent models predominantly used sodium pentobarbital (30–60 mg/kg) for both the donor and recipient; however, combinations of ketamine (100–120 mg/kg) and xylazine (3–12 mg/kg) were also utilized. Anesthetic protocols were most consistent in pig studies, with anesthesia largely maintained with 1%–4% isoflurane (all pig studies). The greatest variation in anesthetic protocols existed in the dog models. The majority of studies used heparin for anticoagulation for both the donor and recipient. No studies in mice used anticoagulation.

BSD Methods

Methods to develop BSD in the donor animals are detailed in Table 3. Inflation of a balloon catheter, inserted through a burr hole in the skull, was the most common

TABLE 3.

Methodological features of BSD development/management

Author, y	Induction	Fluid and drugs	Follow-up	Confirmation criteria
Mouse				
Floerchinger et al,[32] 2012	3F BCI over 10 min	Thymoglobulin (25 mg/kg) bolus at BSD confirmation[a] Volume resuscitation with 0.9% saline	3 h	–
Atkinson et al,[33] 2013	4F BCI (82 ± 27 µL saline) over 10 min	Volume resuscitation with 0.9% saline	3 h	Initial blood pressure peak (Cushing reflex), transient spontaneous muscular fasciculation of the rear limbs during brain stem compression, and subsequent absence of spinal reflexes
Ritschl et al,[39] 2016	No. 3 Fogarty BCI over 15 min[33,44]	Volume resuscitation with 0.9% saline	4 h	Initial blood pressure peak, subsequent transient spontaneous muscular fasciculations of the rear limbs and the absence of spinal reflexes
Rat				
Votapka et al,[17] 1996	Combination of bilateral carotid ligation, and BCI with 0.5 mL saline maintained for 20 min before release[45,46]	Saline ± T3[a]	2 h	Apnea and loss of deep pain reflexes
Wilhelm et al,[21] 2000	3F BCI over 5 min (200 ± 25 µL)	–	6 h	Isoelectric EEG, apnea, absence of brain stem reflexes
Wilhelm et al,[22] 2001	BCI Fogarty catheter	–	6 h	Isoelectric EEG, apnea, absence of brain stem reflexes
Wilhelm et al,[24] 2002	3F BCI over 5 min (200 ± 25 µL)	–	6 h	Isoelectric EEG, apnea, absence of brain stem reflexes
Li et al,[35] 2015a	4F BCI at 15 µL/min to a total volume of 750 µL	Ringer solution (5.5 ± 0.9 mL) boluses to maintain BP No inotropic or vasoactive support	5 h	BP stabilization
Li et al,[36] 2015b	4F BCI at 15 µL/min to a total volume of 750 µL	Volume resuscitation with fluid (undefined) No inotropic or vasoactive support	1 or 5 h	BP stabilization
Spindler et al,[37] 2015	3F BCI over 1 min (200 µL of saline)[47]	2 mL/h saline ± N-octanoyl dopamine[a]	6 h	BP stabilization, the loss of corneal reflexes, apnea
Hegedus et al,[38] 2016	4F BCI (15 µL/min to a total volume of 750 µL)	Volume resuscitation with 0.9% saline No inotropic or vasoactive support	5 h	Loss of corneal reflexes, apnea
Li et al,[41] 2017	4F BCI (15 µL/min to a total volume of 600 µL)[35]	Continuous IV saline (or N-octanoyl dopamine[a]) after BSD confirmation No inotropic or vasoactive support	5 h	Loss of corneal reflexes, apnea
Yip et al,[42] 2017	4F BCI with 0.5 mL distilled water	Allogeneic adipose-derived mesenchymal stem cells 3 h post-BSD induction in some groups[a]	6 h	Apnea Irreversible deep coma (lack of response and reflex to pain) Absence of pupillary reflex (fixed and dilated pupils without reflex to light) Hemodynamic changes (immediate fall in blood pressure and power density of low-frequency components of BP)
Dog				
Shivalkar et al,[15] 1993	Sudden intracranial pressure increase—BCI using 4 mL boluses of saline each h until BSD established Gradual intracranial pressure increase—BCI using an infusion pump to inflate catheter at 4 mL/h slowly until BSD established	–	Up to 4 h	Loss of pupillary and corneal reflexes, cerebral perfusion pressure ≤0 mm Hg, isoelectric EEG

(*Continued next page*)

TABLE 3. (Continued)

Author, y	Induction	Fluid and drugs	Follow-up	Confirmation criteria
Bittner et al,[16] 1995	BCI over 2–3 min (15–18 mL saline)[48]	–	–	Loss of pupillary and corneal reflexes, isoelectric EEG, apnea, neuropathology (end of experiment), absence of response to stimuli after cessation of anesthesia
Kim et al,[18] 1998	Rapid BCI (15 mL saline)[15]	–	1 h	Loss of pupillary and corneal reflexes, cerebral perfusion pressure ≤0 mm Hg
Bittner et al,[19] 1999	BCI over 2–3 min (15–18 mL saline)[48]	–	–	Loss of pupillary and corneal reflexes, isoelectric EEG, apnea, absence of response to stimuli after cessation of anesthesia
Pig				
Ryan et al,[20] 2000	20 cc BCI over 3 min (20 mL water)	Saline (10 mL/kg in first h, followed by 5 mL/kg/h)	1 h	Hemodynamic changes
Ryan et al,[23] 2002	BCI (3 mL water/30 s to a total of 21 mL)	Saline (10 mL/kg in first h, followed by 5 mL/kg/h) No additional fluid or inotrope support	1 h	Hemodynamic instability, loss of pupillary and corneal reflexes, absence of response to stimuli after cessation of anesthesia
Ryan et al,[25] 2003a	BCI (3 mL water/30 s to a total of 21 mL)[23]	Saline (10 mL/kg in first h, followed by 5 mL/kg/h) No additional fluid or inotrope support	1 h	Hemodynamic instability, loss of pupillary and corneal reflexes, absence of response to stimuli after cessation of anesthesia
Ryan et al,[26] 2003b	BCI (3 mL water/30 s to a total of 21 mL)[48]	Saline (10 mL/kg in first h, followed by 5 mL/kg/h) No additional fluid or inotrope support	1 h	Hemodynamic instability, loss of pupillary and corneal reflexes, absence of response to stimuli after cessation of anesthesia
Ryan et al,[27] 2003c	BCI (3 mL water/30 s to a total of 21 mL)	Saline (10 mL/kg in first h, followed by 5 mL/kg/h) No additional fluid or inotrope support	1 h	Hemodynamic instability, loss of pupillary and corneal reflexes, absence of response to stimuli after cessation of anesthesia
Ryan et al,[28] 2003d	BCI (3 mL water/30 s to a total of 21 mL)	Saline (10 mL/kg in first h, followed by 5 mL/kg/h) No additional fluid or inotrope support	1 h	Hemodynamic instability, loss of pupillary and corneal reflexes, absence of response to stimuli after cessation of anesthesia
Konstantinov et al,[29] 2005	BCI to 20 mL[49-51]	–	1.5 h	Hemodynamic instability (increased HR and BP), loss of pupillary reflex
Hing et al,[30] 2009	BCI over 3 min (24 mL water)[20,52]	Saline (10 mL/kg in first h, followed by 5 mL/kg/h titrated to CVP 0–5 mm Hg) IV NE (20 μg/mL) to maintain MAP 60–70 mm Hg) After 3 h BSD—methylprednisolone, T3, vasopressin	6 h	Hemodynamic instability, no response to painful stimuli, loss of pupillary, gag, cough, and corneal reflexes
Ali et al,[31] 2011	14F BCI (30 mL saline)	–	–	Apnea, hemodynamic instability, EEG monitoring, MRI of the brain
Watson et al,[34] 2013	BCI over 3 min (24 mL water over 3 min)[20,23]	Saline titrated to maintain CVP 0–5 mm Hg	1 h	Hemodynamic instability, intracranial pressure in excess of MAP, absence of brain stem reflexes following cessation of anesthesia
Steen et al,[40] 2016	Decapitation between the second and third cervical vertebrae[53]	Continuous IV infusion of cocaine, NA, A, cortisol, T3, thyroxine, desmopressin started after 30 min BSD Krebs solution (3 mL/kg/h)	24 h	Hemodynamic changes, hormonal dysregulation, and plasma catecholamine levels (postexperiment)
Kumar et al,[43] 2017	Carotid artery ligation and BCI (12F or 14F Foley catheter, 25 cc saline over 20 s)	Saline throughout Inotrope support where required to maintain normal BP T3 (0.2 ug/kg/dose, 3 doses every hour from 12 h post-BSD induction in half of the donors + 1 mg/kg hydrocortisone)	18 h	–

[a]Specific to study intervention groups.
A, adrenaline; BCI, balloon catheter inflation; BP, blood pressure; BSD, brain stem death; CVP, central venous pressure; EEG, electroencephalogram; HR, heart rate; MAP, mean arterial pressure; NA, noradrenaline; NE, norepinephrine; T3, triiodothyronine.

TABLE 4.

Heart transplantation model details

	Preservation			HTx				
Author, y	Method	Duration	Solution	Method	Specific technique/details	Total ischemic time	Recipient support	Duration of reperfusion
Mouse								
Floerchinger et al,[32] 2012	CSS	–	Heparinized saline	Heterotopic (abdominal)	Corry et al,[54] 1973 (Nonworking model)	–	–	72 h
Atkinson et al,[33] 2013	CSS	35 min	PBS	Heterotopic (abdominal)	Corry et al,[54] 1973 (Nonworking model[55])	65 min	PBS (± respective study treatment)	48 h
Ritschl et al,[39] 2016	CSS	20 min	Custodial solution	Heterotopic (cervical)	Oberhuber et al,[56] 2014 (Using modified cuff technique, technique modified from Matsuura et al,[57] 1991)	35 min	0.3 mL saline postoperatively IP	20 h
Rat								
Votapka et al,[17] 1996	CSS	–	Saline + 20 mEq/L potassium	Heterotopic (abdominal)	Maruyama et al,[58] 1994 (Working heart model[59])	–	–	48 h
Wilhelm et al,[21] 2000	CSS	≈2–3 min	Saline	Heterotopic (abdominal)	– Abdominal positioning	≈25 min	–	Up to 19 d
Wilhelm et al,[22] 2001	–	–	–	Heterotopic (–)	–	–	Cyclosporine (5 mg/kg/d for 30 d then every other d)	15, 30, 60, 90, and 120 d
Wilhelm et al,[24] 2002	CSS	–	–	Heterotopic (–)	–	–	Cyclosporine (5 mg/kg/d)	Up to 90 d
Li et al,[35] 2015a	CSS	–	Custodial	Heterotopic (abdominal)	Szabo et al,[60] 1998 (adapted from Ono et al,[61] 1969)	1 h	Crystalloid volume substitution (Ringer's solution)	90 min
Li et al,[36] 2015b	CSS	–	Custodial	Heterotopic (abdominal)	Szabo et al,[60] 1998 (adapted from Ono et al,[61] 1969)	1 h	Crystalloid volume substitution (Ringer's solution)	90 min
Spindler et al,[37] 2015	CSS	–	–	Heterotopic (abdominal)	Peter Terness method[62] (adapted from Ono et al,[61] 1969)	–	–	7 d
Hegedus et al,[38] 2016	CSS	–	Custodial	Heterotopic (abdominal)	Szabo et al,[60] 1998 (adapted from Ono et al,[61] 1969)	1 h	Crystalloid volume substitution (Ringer's solution)	90 min
Li et al,[41] 2017	CSS	–	Custodial	Heterotopic (abdominal)	Szabo et al,[60] 1998 (adapted from Ono et al,[61] 1969)	1 h	–	90 min
Yip et al,[42] 2017	–	–	–	Heterotopic (cervical)	Yip et al,[42] 2017	–	–	5 d
Dog								
Shivalkar et al,[15] 1993	CSS	55 ± 7 min	NIH	Orthotopic (thoracic)	–	240 ± 20 min	Isoprenaline 0.5 g calcium chloride 250 mg solumedrol 20 mg xylocaine 0.8 M bicarbonate as required	1 h (post-CPB weaning)

(*Continued next page*)

TABLE 4. (Continued)

	Preservation			HTx				
Author, y	Method	Duration	Solution	Method	Specific technique/details	Total ischemic time	Recipient support	Duration of reperfusion
Bittner et al,[16] 1995	CSS	–	St Thomas'	Orthotopic (thoracic)	Bicaval technique Bittner et al,[63] 1995 Complete atrioventricular	1.5 or 4 h	1 mg NA bolus as needed on CPB Oral cyclosporine (10 mg/kg) Oral azathioprine (2 mg/kg) Methylprednisolone (25 mg/kg IV)	1 h (post-CPB weaning)
Kim et al,[18] 1998	CSS	4 h	NIH-2	Orthotopic (thoracic)	Biatrial technique Replaced the aortic and mitral valves with Bjork-Shiley valves	–	Ventricular pacing 110 bpm Methylprednisolone (5 mg/kg)	1 h (post-CPB weaning)
Bittner et al,[19] 1999	Warm (40°C) static storage	–	40°C Preservation solution (formulated at Duke University)	Orthotopic (thoracic)	Bicaval technique Bittner et al,[63] 1995	2–4 h (depending on group)	Oral cyclosporine (10 mg/kg) Oral azathioprine (2 mg/kg) Methylprednisolone (25 mg/kg IV)	1 h (post-CPB weaning)
Pig								
Ryan et al,[20] 2000	CSS	–	Cold cardioplegia supplemented with HCl, intralipid, U74389G	Orthotopic (thoracic)	Lower and Shumway,[64] 1960	6 h	Dobutamine (10–20 ug/kg/min) 45 min after reperfusion Saline (10 mL/kg in first h, followed by 5 mL/kg/h)	Up to 6 h (post-CPB weaning)
Ryan et al,[23] 2002	CSS	–	St Vincent's cold crystalloid cardioplegia	Orthotopic (thoracic)	Lower and Shumway,[64] 1960	4, 6, or 14 h	Dobutamine (10 ug/kg/min) 45 min after reperfusion Pacing—VVI 120 bpm Saline (10 mL/kg in first h, followed by 5 mL/kg/h)	Up to 3 h (post-CPB weaning)
Ryan et al,[25] 2003a	CSS	–	St Vincent's cold crystalloid cardioplegia	Orthotopic (thoracic)	Lower and Shumway,[64] 1960	4 and 14 h	Dobutamine (10–20 ug/kg/min) 45 min after reperfusion Pacing—VVI 120 bpm Methylprednisolone (500 mg) on anesthetic induction and 15 min before reperfusion Saline (10 mL/kg in first h, followed by 5 mL/kg/h)	Up to 3 h (post-CPB weaning)
Ryan et al,[26] 2003b	CSS	–	St Vincent's crystalloid cardioplegia	Orthotopic (thoracic)	Lower and Shumway,[64] 1960	4 h	Dobutamine (10 ug/kg/min) 45 min after reperfusion Pacing—VVI 120 bpm Methylprednisolone (500 mg) on anesthetic induction and 15 min before reperfusion Saline (10 mL/kg in first h, followed by 5 mL/kg/h)	Up to 3 h (post-CPB weaning)
Ryan J, 2003c[27]	CSS	–	St Vincent's crystalloid cardioplegia	Orthotopic (thoracic)	Lower and Shumway,[64] 1960	6 h	Dobutamine (10 ug/kg/min) 45 min after reperfusion Pacing—VVI 120 bpm Methylprednisolone (500 mg) on anesthetic induction and 15 min before reperfusion Saline (10 mL/kg in first h, followed by 5 mL/kg/h)	Up to 3 h (post-CPB weaning)

(*Continued next page*)

TABLE 4. (Continued)

	Preservation			HTx				
Author, y	Method	Duration	Solution	Method	Specific technique/details	Total ischemic time	Recipient support	Duration of reperfusion
Ryan et al,[28] 2003d	CSS	–	St Vincent's cold crystalloid cardioplegia (supplemented with UG4389G ± cariporide or vehicle)[a]	Orthotopic (thoracic)	Lower and Shumway,[64] 1960	14 h	Dobutamine (10–20 ug/kg/min) 45 min after reperfusion Pacing—VVI 120 bpm Methylprednisolone (500 mg) on anesthetic induction and 15 min before reperfusion Saline (10 mL/kg in first h, followed by 5 mL/kg/h)	Up to 3 h (post-CPB weaning)
Konstantinov et al,[29] 2005	CSS	1 h	Crystalloid	Orthotopic (thoracic)	Biatrial technique	120 min	None used	Did not wean animals. Performed LAD ligation and reperfusion on cardiac allograft while on bypass
Hing et al,[30] 2009	CSS	–	Celsior ± glyceryl trinitrate and/or cariporide	Orthotopic (thoracic)	Lower and Shumway,[64] 1960	14 h	Dobutamine (10–20 ug/kg/min) 45 min after reperfusion Pacing—VVI 120 bpm Methylprednisolone (500 mg IV at anesthetic induction and 15 min before reperfusion) Saline (10 mL/kg in first h, followed by 5 mL/kg/h titrated to CVP 0–5 mm Hg)	Up to 3 h (post-CPB weaning)
Ali et al,[31] 2011	CSS	–	Crystalloid + lidocaine	Orthotopic (thoracic)	Biatrial technique	–	Dobutamine (2.5 ug/kg/min)	Immediately after CPB weaning measurements collected (CPB weaning after 30 min reperfusion)
Watson et al,[34] 2013	CSS	210 min	Celsior (± erythropoietin, glyceryl trinitrate, and zoniporide)	Orthotopic (thoracic)	Lower and Shumway,[64] 1960	5 h	Dobutamine (10–20 ug/kg/min) after 45 min reperfusion Noradrenaline (as needed) Pacing—VVI 120 bpm Methylprednisolone (500 mg) Saline titrated to maintain CVP 0–5 mm Hg	Up to 3 h (post-CPB weaning)
Steen et al,[40] 2016	CSS vs cold (8°C) machine perfusion	24 h	St Thomas (CSS) vs in-house developed perfusion solution	Orthotopic (thoracic)	Biatrial technique	–	Adrenaline (10 ug boluses and IV 0.05 ug/kg/min) for first 6 h Methylprednisolone (1 g) Krebs solution (3 mL/kg/h)	24 h
Kumar et al,[43] 2017	CSS		Custodial		Biatrial technique	3–3.5 h	Methylprednisolone (10 mg/kg) Dopamine, epinephrine, atropine, lidocaine, furosemide, 8.4% sodium bicarbonate	2–3 h

[a]Specific to study intervention groups.

bpm, beats per minute; CPB, cardiopulmonary bypass; CSS, cold static storage; CVP, central venous pressure; HTx, heart transplantation; LAD, left anterior descending coronary artery; NA, noradrenaline; NIH, National Institute of Health; VVI, ventricular demand pacing.

technique used to induce BSD (93%). One rat and 1 pig study used a combination of balloon catheter inflation (BCI) and carotid artery ligation,[17,43] and a study in pigs used decapitation between the second and third cervical vertebrae.[40] Rodent studies typically reported catheter size (3F–4F); however, times and volumes to induce BSD varied. Mouse studies induced BSD over 10–15 min. Rat studies either used smaller volumes (200 ± 25 μL) over 1–5 min or larger volumes (600–750 μL) over 40–50 min. Studies in dogs utilized 15–20 mL to inflate the catheter and induce BSD.[15,18] Induction methods in pigs were typically consistent, using 20–24 mL water for BCI over 3 min (8 of 12). One pig study reported a slightly higher volume for BCI (30 mL saline) over an unknown period of time.[31]

All studies except 2 reported at least 1 parameter for confirming BSD (27 of 29). The most common criteria enlisted for confirming BSD was loss of corneal reflexes (13 of 29), followed by typical BSD-related hemodynamic changes (12 of 29), loss of pupillary reflexes (12 of 29), apnea (11 of 29), absence of response to stimuli after cessation of anesthesia (8 of 26), and an isoelectric electroencephalogram (7 of 29). Some studies reported confirmation of BSD with nonspecific details, namely absence of spinal/brain stem/deep pain reflexes[17,21,22,24,32,33,39,42] (7 of 29). No study used all clinical testing criteria outlined in the Australia and New Zealand Intensive Care Society (ANZICS) statement on death and organ donation; however, 24 of 29 used more than one test listed in the ANZICS statement to confirm BSD.[65]

The duration of BSD to transplant in rodent studies ranged from 1–6 h, with most studies choosing 5–6 h (9 of 13). Of the dog and pig models that reported of the duration of BSD (13 of 16) to transplant, most utilized a period of 1 h donor BSD (8 of 13), with no uniformity among remaining studies, incorporating 1.5, 4, 6, 18, or up to 24 h BSD. Most rodent and pig studies (17 of 25) reported the administration of saline for volume support in donor animals. No details were provided regarding fluid support for canine studies. Regarding inotropic or vasoactive support, only 4 rat (5 h BSD) and 5 pig (1 h BSD) studies specifically reported no use of these drugs during BSD. The 3 studies with the longest periods from BSD to graft procurement (6 h,[30] 18 h,[43] and 24 h[40]) used additional pharmacologic support in the donor (norepinephrine, methylprednisolone, triiodothyronine, vasopressin, hydrocortisone, or in-house developed "brain death cocktail."

Heart Transplant Methods

Methods involved in the HTx component of included studies are recorded in Table 4. The type of donor heart preservation employed by all studies was predominantly cold static storage (CSS) (25 of 29), and remaining studies utilized warm (40°C) static storage,[19] or compared CSS versus ex vivo machine perfusion storage at 8°C.[40] Interestingly, 21 of 29 did not report length of heart preservation (cold ischemic time/ex vivo time). As expected, rodent studies employed the shortest periods of CSS (≈3–35 min, 3 of 29), followed by ≈1 h (2 of 29), 3.5–4 h (2 of 29), and 24 h (1 of 29). Preservation solutions were highly heterogeneous across all studies and included specific supplementations depending on the purpose of each study. Commercially available crystalloid cardioplegia (14 of 29) and in-house developed cardioplegia (8 of 29) were the primary types of preservation solutions used. Supplemented additives comprised of hydrogen chloride, intralipid, lazaroid U74389G, cariporide, glyceryl trinitrate, lidocaine, erythropoietin, and zoniporide.

All rodent studies used models of HHTx, and all remaining studies (dog and pig) used models with orthotopic HTx (OHTx). For the heterotopic transplants undertaken in rodents, donor hearts were predominantly (9 of 13) positioned in the recipient abdomen. Studies in dogs reported use of both biatrial (1 of 4) and bicaval (2 of 4) HTx techniques. All pig studies used a model of biatrial implantation as described by Lower and Shumway.[64] Unlike length of heart preservation time, the total ischemic time was typically standardized and recorded for 69% of studies. Rodent studies reported the shortest total ischemic times, ranging from ≈25–65 min, followed by 1.5–4 h for dog studies and 2–14 h for pig studies.

Regarding recipient fluid and postoperative support, 55% of studies did not report any basic fluid support following HTx. Rat studies primarily reported the use of crystalloid Ringer's solution for volume substitution, and 67% of pig studies used saline for fluid support (1 study used Krebs buffer[40]). Immunosuppression was commonly employed in all dogs (methylprednisolone, solumedrol, and azathioprine) and most pig (8 of 12) studies, but only in 2 rodent studies.[22,24] Vasopressor support for the HTx recipient was not used in rodent studies, yet reported in dog studies (2 of 4 studies reported use isoprenaline or noradrenaline). Conversely, pig studies largely used dobutamine for inotropic support following HTx (9 of 12; however, some studies also used noradrenaline,[34] adrenaline,[40,43] dopamine, atropine, lidocaine, and furosemide.[43] Most pig studies employed ventricular pacing at 120 beats per minute. Antibiotics use was reported in some rat[24] and dog studies,[16,19] but never in mouse or pig studies.

Following HTx, 27 of 29 studies continued monitoring after weaning from cardiopulmonary bypass (CPB) (orthotopic studies) or graft reperfusion (heterotopic studies). The post-HTx monitoring period for rodents was variable, ranging from 90 min to 120 d. For the dog and pig models, recipients were typically monitored post-HTx for 1–3 h, with the exception of 1 study that extended to 24 h,[40] and 2 studies where animals were either not weaned at all,[29] or the study ended immediately after weaning.[31]

Cardiac Function Assessment

Methods used to assess cardiac function in the included studies are outlined in Table 5. Several studies (7 of 29) did not assess graft function post-HTx, predominantly in rod ents[21,22,24,32,37,39] and 1 pig study.[29] Cardiac hemodynamic function in rat, dog, and pig studies (both donor and recipient) was primarily reported using invasive left ventricular pressure-volume relationship (PVR) analyses. A number of parameters were derived including stroke work index, preload recruitable stroke work, end-diastolic pressure-volume relationship, cardiac output, cardiac index, left ventricular and right ventricular systolic and diastolic pressures and volumes, systemic and pulmonary vascular resistances, aortic and mean arterial pressures, heart rate, ejection fraction (EF), and the minimum and maximum

TABLE 5.

Outcome measures of cardiac function in donor and recipient

Study	Donor	Recipient (graft)
Mouse		
Floerchinger et al,[32] 2012	–	–
Atkinson et al,[33] 2013	MAP	Manual palpation for graft function
Ritschl et al,[39] 2016	MAP	–
Rat		
Votapka et al,[17] 1996	–	Manual palpation for graft function LV PVR analyses
Wilhelm et al,[21] 2000	MAP	Graft survival
Wilhelm et al,[22] 2001	–	–
Wilhelm et al,[24] 2002	–	–
Li et al,[35] 2015a	LV PVR analyses	LV PVR analyses
Li et al,[36] 2015b	LV PVR analyses	LV PVR analyses
Spindler et al,[37] 2015	MAP and HR	–
Hegedus et al,[38] 2016	LV PVR analyses	LV PVR analyses
Li et al,[41] 2017	LV PVR analyses	LV PVR analyses
Yip et al,[42] 2017	%LVEF (TTE)	%LVEF (TTE)
Dog		
Shivalkar et al,[15] 1993	LVP, MAP, HR, CO, dP/dt, electrocardiogram	Successful weaning from bypass, MAP, CO, LVP, dP/dt, electrocardiogram
Bittner et al,[16] 1995	LV and RV PVR analyses	LV and RV PVR analyses
Kim et al,[18] 1998	MAP, CVP, CO, pulmonary artery pressure, PCWP, HR	LV PVR analyses
Bittner et al,[19] 1999	LV and RV PVR analyses	LV and RV PVR analyses
Pig		
Ryan et al,[20] 2000	LV and RV PVR analyses	LV and RV PVR analyses
Ryan et al,[23] 2002	LV PVR analyses	LV PVR analyses Successful weaning from bypass
Ryan et al,[25] 2003a	–	Successful weaning from bypass
Ryan et al,[26] 2003b	LV PVR analyses	Successful weaning from bypass LV PVR analyses
Ryan et al,[27] 2003c	LV PVR analyses	Successful weaning from bypass LV PVR analyses
Ryan et al,[28] 2003d	LV PVR analyses	Successful weaning from bypass LV PVR analyses
Konstantinov et al,[29] 2005	Blood pressure and HR	–
Hing et al,[30] 2009	LV PVR analyses MAP, CO, LAD coronary flow	LV PVR analyses Successful weaning off bypass MAP, CO, LAD coronary flow
Ali et al,[31] 2011	LV and RV PVR analyses Cine cardiac MRI to assess biventricular chamber volumes and function	Successful weaning off bypass LV and RV PVR analyses Cine cardiac MRI to assess biventricular chamber volumes and function
Watson et al,[34] 2013	LV PVR analyses	Successful weaning off bypass and ability to maintain CO for duration of study LV PVR analyses
Steen et al,[40] 2016	Left atrial pressure, pulmonary artery pressure, CVP, aortic pressure	Left atrial pressure, pulmonary artery pressure, CVP, aortic pressure Adrenaline stress test (HR, systolic and diastolic blood pressures in response to increasing adrenaline doses)
Kumar et al,[43] 2017	Hemodynamic parameters (nonspecific), %LVEF (TTE)	Hemodynamic parameters (nonspecific), survival off CPB, vasoactive inotrope score, %LVEF (via TTE)

CO, cardiac output; CPB, cardiopulmonary bypass; CVP, central venous pressure; dP/dt, rate pressure change; HR, heart rate; LAD, left anterior descending artery; LV, left ventricle; %LVEF, % left ventricular ejection fraction; MAP, mean arterial pressure; PCWP, pulmonary capillary wedge pressure; PVR, pressure-volume relationship; RV, right ventricular; TTE, transthoracic echocardiography.

rate of pressure change in the ventricle. Two rodent studies assessed cardiac function via manual palpation of the graft.[17,33] In addition to PVR analyses, "successful weaning off bypass" was reported as a measure of transplant success in 9 of 16 (dog and pig) studies. Other less frequent analyses used for cardiac function were cine cardiac MRI (pig[31]), an adrenaline stress test (pig[40]), and %EF using transthoracic echocardiography (rat[42] and pig[43]).

DISCUSSION

In the clinical field of HTx, brain dead donors form the majority of the donor pool. In Australia, approximately 95% of transplanted hearts are sourced from brain dead donors.[7] Preclinical research models that accurately mimic the clinical scenario are essential for groundbreaking advancements in donor management strategies, surgical procedures, novel pharmacologic therapies, and donor heart perfusion technology, ultimately translating valuable experimental results into effective interventions for human HTx recipients. A number of animal models of BSD or HTx are available, but currently, there is still a lack of complex models incorporating both BSD and HTx, accurately simulating clinical HTx. Consequently, this review specifically focused on identifying and characterizing these composite animal models.

We successfully identified several animal models of HTx incorporating donor BSD, albeit with marked heterogeneity. Interestingly there is an almost equal distribution of studies reviewed that were largely aimed at understanding the physiological mechanisms in BSD and HTx or interventional assessment of various therapeutic modalities to modify HTx (and related injury) outcomes (Table 1). While large animal models (ie, dog, pig, sheep, and primate) can be used to closely resemble clinical settings and related human pathophysiology, they have many limiting factors: these models are expensive, resource-intensive, often require complex clinical technologies and facilities, and depending on the model used, are not well supported in specific biochemical assays available. Without the ability of a research group to address these complex factors, many must settle with a small animal model (ie, rodents) to address their research question. Biomedical research is largely skewed towards small animal models in the abundance of biological assays and genetically modified models available, in addition to the cost-, resource- and time-effective benefits. This is evident in the post-HTx monitoring periods reported for the included studies, with smaller more manageable (both financially and technically) animal models having longer postoperative monitoring periods. This, however, was in the context of shorter organ storage durations (<1 h) relative to metabolic scaling[66] and non-working heterotopic models.

The Influence of Age, Weight, and Gender Upon HTx Outcomes

The Animal Research: Reporting of In Vivo Experiments guidelines were developed and published in 2010 to improve and standardize the reporting of animal research[67] (ie, to include age, weight, gender, housing, husbandry, etc), and are being increasingly incorporated by journals as part of their mandatory reporting for publication. In this review, weight was largely reported; however, gender, and predominantly age, were not (except in mouse models). Although bodyweight is considered for donor heart allocation for HTx, weight matching is a poor predictor of HTx outcomes.[68,69] More importantly, a gender mismatch between the donor and recipient (predominantly a small female donor and a large male recipient) is largely associated with impaired survival post-HTx and may be related to the associated mismatch of cardiac size between genders.[12,13,68,70,71] The International Society for Heart and Lung Transplantation (ISHLT) Registry reports that since 1992, both the donor (68%–70%) and recipient (75%–80%) pools are largely comprised of males (75%–80%). Interestingly, this review identified that rodent studies consistently reported the use of males; however, the dog and pig models predominantly did not report gender. The field of medical and scientific research is becoming increasingly aware of the gender bias towards studies using males only. Considering that basic science, translational, and clinical studies guide medical practice and treatment, the inclusion of female animals in studies is vital for our understanding of disease and relevant therapeutics in this cohort. Ischemic heart disease, a large precursor to heart failure, is responsible for the most deaths worldwide in both males and females (together and separately).[72] Thus, while females may comprise a smaller percentage of the donor and recipient pools, animal studies should consider the use of both males and females (and related combinations) in their research design when feasible. Additionally, the consideration, inclusion, and reporting of females in basic and preclinical research is now a requirement for National Institute of Health-funded research.[73,74]

Older age of the donor and/or recipient significantly increases the risk of mortality following HTx. However, due to a severe shortage in available donor hearts, the use of marginal donor organs is increasing. The inherent changes that occur with age (as well as comorbidities and chronic pharmacotherapy) have precluded the effective clinical translation of several promising preconditioning and postconditioning strategies targeting ischemia-reperfusion injury.[75] Although the impact of donor and recipient age is well reported and comprehensively studied, perhaps what requires more attention, is understanding how to condition or improve the quality of marginal hearts to extend the donor pool and improve recipient post-HTx outcomes. Considering the detrimental impact that gender mismatching and older age can have upon post-HTx outcomes, these variables should be clearly reported, particularly in larger animal models (ie, dog and pig).

BSD Model Diversity

The complex pathophysiological changes that occur with brain death undoubtedly impair donor heart function. Studies in this review typically used BCI for BSD induction and reported using at least 1 criterion for confirming BSD. Most protocols varied, however, in volume and time reported for BCI and the specific confirmation criteria enlisted. One pig study utilized decapitation to ensure a reproducible and severe sympathetic storm and subsequent hemodynamic collapse as occurs with brain death.[40,53] Here, the authors note that the pig has a robust tentorium cerebelli, and spinal arteries that ascend to the brain stem and are a source of extracranial blood supply.[40] The authors concluded BCI in the pig skull would be insufficient to induce complete BSD due to the unique anatomy of the pig brain and skull but ensured complete BSD with decapitation.[40,53] According to the ANZICS statement on death and organ donation,[65] BSD occurs in the setting of severe brain injury resulting in elevated intracranial pressures, leading to the inevitable cessation of cerebral blood flow and consequently, whole brain and BSD. Differing injuries, such as traumatic brain injury, intracranial hemorrhage, and cerebral ischemia, can lead to brain death.[76] The actual cause of brain death (eg, traumatic brain injury,

intracranial hemorrhage, trauma) is a notable risk factor for primary graft failure,[77] which is one of the greatest contributors to early mortality following HTx.[78,79] However, a study in the United Kingdom found that medium-term (30 d to 3 y) survival post-HTx is not significantly impacted by modality of donor death, once corrected for confounding effects such as donor and recipient characteristics, donor management, organ ischemic time, etc.[80] Cantin et al[81] also identified that the cause of brain death did not influence post-HTx survival, but may increase the rate of rejection. Conversely, rapid increases in intracranial pressure, compared with gradual elevations, have been previously shown to significantly impair myocardial function and adversely affect HTx outcomes in dogs.[15] Thus, differences in BSD induction methods of experimental models likely govern BSD-related injury development, and influence variation in post-HTx outcomes and resultant myocardial function, but potentially have less impact on survival outcomes.

According to the ANZICS statement on death and organ donation, BSD must be confirmed using the following criteria: (1) unresponsive coma, (2) absence of brain stem reflexes, and (3) apnea.[65] No study included in this review utilized tests that covered all 3 of these criteria. Absence of responsiveness was often not performed, whereas the latter 2 criteria were included in the majority of studies. In lieu of 1 criterion, most studies utilized hemodynamic stability after further inflation of a balloon catheter, implying a loss of cardiorespiratory control and thus brain death. Although informative, this criterion is isolated to animal models of BSD since hemodynamic data in the patient is often absent before admission. Clinically, the time from donor hospital admission to aortic cross-clamp can be as long as several days. In Australia (2016), 24% of donors were declared brain dead within 24 h of hospital admission (median time was 22 h).[76] The length of BSD in the reviewed animal models (~1–6 h predominantly) generally fell significantly shorter than times reported by other transplant centers.[82,83] Clinically, the time that donor organs are subject to BSD has been shown to influence HTx outcomes with mixed results. Extended donor management (>14 h[84] and >72 h[81]) time before HTx has been associated with poorer recipient survival in humans.[81,84] However, Marasco et al[82] reported no effect of donor brain death time (approximately 19 h) upon recipient survival. Furthermore, a period of 4–17 d between the time of brain injury to BSD confirmation in pediatric patients led to improved rejection-free survival post-HTx but had no significant effect upon mortality.[85] Indeed, Borbely et al[86] demonstrated that extending the donor management time to optimize cardiac function, and using serial transthoracic echocardiograms to monitor functional changes, resulted in 52% of donor hearts, considered initially as functionally limited, being ultimately transplanted. The complexities of conducting animal studies that replicate similar clinical timeframes, particularly in large animal models (ie, dog, pig, sheep), likely influence experimental design and feasibility. However, it is clear that the majority of animal models mimicking donor BSD followed by HTx use a time frame that is significantly shorter than the clinical scenario.

Donor Heart Preservation

CSS using crystalloid cardioplegia has been the predominant method of heart preservation for decades and was the key preservation method utilized by studies in this review. Although cold ischemic time was typically not recorded, total ischemic time was more commonly reported, yet quite variable across the spectrum of studies. The median ischemic time for clinical HTx recently reported by the ISHLT (2009 to June 2016) was 3.2 h (1.5–5 h).[79] Several studies employed total ischemic times that corresponded to the ISHLT data; however, some pig studies used ischemic times that extended well beyond (up to 14 h). Allograft ischemic time and viability are intricately entwined, and in clinical transplantation, an ischemic time beyond 4 h significantly impairs both short- and long-term recipient survival.[1,79]

Ischemic time is one of the challenges of organ retrieval and HTx and can be a determinant of acceptance of a donor heart. Because of this type of limitation that occurs with clinical HTx and the severe shortage of donor hearts available worldwide, many avenues are being investigated to improve donor heart quality and quantity. Development of ex vivo perfusion devices that can preserve donor hearts and continue oxygen perfusion to the donor organ (using warm or cold preservation solutions) is expanding in the hope that these machines can be used for growth of the donor pool. Preservation of donor hearts via machine perfusion (warm or cold) could be used to (1) extend the ischemic (or ex vivo/in transit) time, (2) assess function of "marginal" donor hearts before HTx (depending on the machine), and/or (3) recondition donor hearts to improve outcomes in the recipient. Indeed, Steen et al[40] demonstrated experimentally that hearts can be safely transplanted (as assessed 24 h post-HTx) following exposure to 24 h BSD, followed by 24 h cold machine-perfused storage. The PROCEED II trial (prospective, multicenter, randomized, clinical investigation of TransMedics Organ Care System [OCS] for cardiac use) demonstrated that donor heart storage using the clinically approved Organ Care System (warm machine perfusion) was noninferior to typical CSS, despite a significantly longer ex vivo storage time.[87]

In addition to novel storage techniques, pharmacologic variations to storage solutions (and related methods) to improve donor heart preservation has been a key interest of the field. Cariporide, an agent used to block sodium-hydrogen exchange in the heart, has been shown in relevant experimental models of HTx (incorporating donor BSD) to improve myocardial function and reduce troponin I release post-HTx when used as a pharmacologic preconditioning agent,[26,28,30] alone and in combination with glyceryl trinitrate.[30] Cariporide was however removed from further use following the EXPEDITION (sodium-proton exchange inhibition to prevent coronary events in acute cardiac conditions) trial, as it was associated with an increase perioperative stroke-related mortality, although cariporide did improve postischemic myocardial function.[88] Alternatively, post-HTx myocardial contractility and troponin I release were improved in a pig study that supplemented Celsior cardioplegia with erythropoietin, glyceryl trinitrate, and zoniporide (an alternative sodium-hydrogen exchange inhibitor).[34] Ryan et al[27] have also demonstrated that inhibition of lipid peroxidation (using lazaroid U74389G), although beneficial in other[89] animal models of cardiac ischemia-reperfusion injury and HTx[90,91] protection, did not translate to a porcine model of donor BSD and HTx. Clinical translation

of cardioprotective agents has been a consistent issue for this field,[75,92,93] and is no doubt complicated by the pathophysiological processes associated with BSD and HTx.

Heart Transplantation and Postoperative Support in Animal Models

This review identified a clear delineation between animal HTx techniques, where in HHTx was employed in rodents, and OHTx in dog and pig models, suggesting the complexity of OHTx renders it unfeasible in smaller animals such as rodents. Clinical HHTx is now rarely if ever required as patients with indications that in the past may have been considered for this procedure can be managed by continuous-flow ventricular assist devices. OHTx, in this review, predominantly used the biatrial technique. Despite the clinical disadvantages with biatrial implantation (valvular, hemodynamic, and electrophysiological disturbances[94,95]), several studies have reported similar long-term survival between biatrial versus bicaval techniques.[94,96]

Key components of postoperative care for HTx recipients include fluid status management, immunosuppression, and inotropic/vasoactive support to maintain stable hemodynamic function (weaned as tolerated).[97,98] Rodent studies commonly assessed inflammation and rejection post-HTx using matched and unmatched breeds when appropriate, thus potentially reflecting the choice to avoid immunosuppressive therapies in these studies. Alternatively, dog and pig studies were typically oriented towards investigation of post-HTx graft contractility (with and without specific interventions). The use of immunosuppressive therapy in the dog and pig studies were more commonly employed. Vasoactive/inotropic support is vital for early postoperative care of the HTx recipient. Several pig studies used levels of dobutamine (10–20 μg/kg/min) that greatly overcame the recommended ISHLT doses (1–10 μg/kg/min). Limited data is available regarding the influence of high dose dobutamine use post-HTx upon short- and long-term graft function. However, pigs seem to require higher doses than in humans for optimal hemodynamic function and management post-HTx. Additionally, as most pig (and some dog) studies employed ventricular pacing perioperatively (110–120 beats per min) to maintain sufficient heart rates, it is evident that these transplanted hearts required additional electrical support post-HTx for optimal cardiac function. This consequence in pig and dog models may reflect the predominant choice of biatrial OHTx technique and extended graft ischemic periods (4–14 h) for the reviewed studies, both of which are notable mechanisms of arrhythmias post-HTx.[99,100]

Lack of Correlation in Assessing Cardiac Function Post-HTx With the Clinical Scenario

Many of the dog and pig BSD-HTx studies in this review primarily aimed at successful weaning from CPB. When a patient fails to wean from CPB following a heart transplant, invariably due to PGD, mechanical circulatory support is available to support the circulation, until myocardial contractile failure reverses. The ability to wean off bypass is a wholly definitive outcome and clearly delineates differences between groups; however, without additional mechanical circulatory support information regarding the contractile function and recovery of these hearts in these experimental settings cannot be measured.

The contractility of successfully weaned cardiac grafts were commonly assessed using PVR analysis, which has become the benchmark technique for measuring cardiac contractility. Technological advances have facilitated expansion of this technique from humans to animals.[101] Although significant informative data was generated from the animal studies that employed PVR analysis, contractility of the transplanted heart is generally monitored using transesophageal echocardiography in clinical settings (coupled with hemodynamic monitoring and management).[97] Only 2 studies in this review used any measure of echocardiography to examine cardiac function.[42,43] Despite being a powerful means of characterizing ventricular properties, PVR analysis has not been used in clinical practice because it requires highly specialized and invasive techniques for accurate measurement. In particular, techniques for measuring volume have been difficult and imprecise and the majority of decision-making information can be achieved using less invasive parameters (eg, EF compared to dP/dtmax).[102] Echocardiographically derived EF, has however not been validated in animal studies, despite its widespread use in clinical transplant settings. In 2018, Chowdhury et al[103] conducted the first comprehensive study evaluating the association between echocardiographic measures of systolic function and a composite measure of pressure-volume derived contractility. This study showed that longitudinal strain derived from speckle-tracking echocardiography had a moderate relationship with the invasive composite contractility index derived from pressure-volume analyses, whereas conventional measures such as EF and fractional shortening were significantly associated with left ventricular load and mass, but not contractility. Schroeder et al[104] had reported that no single clinical characteristic was statistically significant when correlated with pressure-volume loop data in a cohort of 18 transplant patients. In view of this previous evidence, validation of speckle-tracking echocardiography alongside pressure-volume loops in HTx animal studies should be a primary focus in future studies to improve outcomes utilizing less invasive techniques and clinical translatability.

The Impact of Research Group Diversity

Interestingly, the majority of the BSD-HTx studies identified by this review came from just a limited number of research groups. As an example, 73% of preclinical HTx studies incorporating donor BSD (in pigs) were performed by Ryan et al.[20,23,25–28] Thus, despite BSD and HTx being intensively investigated separately, few research groups have identified the clinically relevant value in assessing pertinent pathophysiology and protective/treatment strategies utilizing these composite models. Additionally, methods appeared to be relatively consistent within research groups, and research groups published 1 species only (ie, no research group attempted development of more than one animal model). As detailed above, undertaking large animal (ie, dog, pig, sheep, and primate) research requires highly skilled personnel, facilities, logistical consideration, and financial support, which are obvious considerations for feasibility of a study. These challenges may ultimately direct paths taken by research groups to simplify studies to

a smaller animal model (ie, rodents), which may/may not incorporate both donor BSD and HTx. Regardless, it is clear that a large field of preclinical research is dominated by a small number of groups worldwide.

Limitations

Each study combined models of donor BSD with HTx, assessed different outcomes and showed slight variations in models when published from the same group. However, in certain instances, investigators used components of previous models of donor BSD or HTx to generate their animal models. All attempts were made to retrieve relevant data in these instances where studies were referenced. Additionally, because of the heterogeneous nature of the animal models identified, in conjunction with the primary aim to identify and describe these models, no formal risk of bias was undertaken.

CONCLUSIONS

This review highlighted that animal models comprising both donor BSD and HTx are available, but they are fairly heterogeneous in design and methods. In addition, general reporting of important model components that influence HTx outcomes was diverse and often limited. Thus, it seems that no uniform consensus exists on the preferred BSD-HTx animal model. This could be also driven by the limited number of research groups that are leading this field of investigation. There appears to be a lack of consistent parameters used to assess BSD and HTx outcomes, making comparison between studies difficult. Finally, it is evident from the animal studies presented that preclinical models are marginally mimicking the real clinical scenario, creating obstacles for translation of valuable results into practice, and impeding progression of the field as whole.

ACKNOWLEDGMENTS

The authors would like to acknowledge Dr Gianluigi Li Bassi for his critical appraisal of this study.

REFERENCES

1. Ramani GV, Uber PA, Mehra MR. Chronic heart failure: contemporary diagnosis and management. *Mayo Clin Proc*. 2010;85:180–195. doi:10.4065/mcp.2009.0494
2. Vos T, Flaxman AD, Naghavi M, et al. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380:2163–2196. doi:10.1016/S0140-6736(12)61729-2
3. Savarese G, Lund LH. Global public health burden of heart failure. *Card Fail Rev*. 2017;3:7–11. doi:10.15420/cfr.2016:25:2
4. Ponikowski P, Anker SD, AlHabib KF, et al. Heart failure: preventing disease and death worldwide. *ESC Heart Fail*. 2014;1:4–25. doi:10.1002/ehf2.12005
5. Australian Institute of Health and Welfare. *Cardiovascular Disease Series*. Canberra, ACT, Australia: Australian Institute of Health and Welfare; 2011. Cardiovascular disease: Australian facts 2011.
6. Global Observatory on Donation and Transplantation. *Global Observatory on Donation and Transplantation (GODT) data* [Global Observatory on Donation and Transplantation Website]. 2017. Available at http://www.transplant-observatory.org/export-database/. Accessed October 11, 2018.
7. ANZOD Registry. *2017 Annual Report, Section 7: Deceased Donor Heart Donation*. Adelaide, SA, Australia: Australia and New Zealand Dialysis and Transplant Registry; 2017.
8. Iyer A, Gao L, Doyle A, et al. Normothermic ex vivo perfusion provides superior organ preservation and enables viability assessment of hearts from DCD donors. *Am J Transplant*. 2015;15:371–380. doi:10.1111/ajt.12994
9. Chew HC, Iyer A, Connellan M, et al. Outcomes of donation after circulatory death heart transplantation in Australia. *J Am Coll Cardiol*. 2019;73:1447–1459. doi:10.1016/j.jacc.2018.12.067
10. Messer S, Page A, Axell R, et al. Outcome after heart transplantation from donation after circulatory-determined death donors. *J Heart Lung Transplant*. 2017;36:1311–1318. doi:10.1016/j.healun.2017.10.021
11. Messer SJ, Axell RG, Colah S, et al. Functional assessment and transplantation of the donor heart after circulatory death. *J Heart Lung Transplant*. 2016;35:1443–1452. doi:10.1016/j.healun.2016.07.004
12. Kirklin JK, Young JB, McGiffin DC. Chapter sixteen: survival after heart transplantation. In: *Heart Transplantation*. Vol 1. Churchill Livingstone; 2002:587–614.
13. Kirklin JK, Young JB, McGiffin DC. Chapter nine: the donor heart. In: *Heart Transplantation*. Vol 1. Churchill Livingstone, New York, NY; 2003:293–338.
14. Moher D, Shamseer L, Clarke M, et al; PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev*. 2015;4:1. doi:10.1186/2046-4053-4-1
15. Shivalkar B, Van Loon J, Wieland W, et al. Variable effects of explosive or gradual increase of intracranial pressure on myocardial structure and function. *Circulation*. 1993;87:230–239. doi:10.1161/01.cir.87.1.230
16. Bittner HB, Kendall SW, Chen EP, et al. Myocardial performance after graft preservation and subsequent cardiac transplantation from brain-dead donors. *Ann Thorac Surg*. 1995;60:47–54.
17. Votapka TV, Canvasser DA, Pennington DG, et al. Effect of triiodothyronine on graft function in a model of heart transplantation. *Ann Thorac Surg*. 1996;62:78–82. doi:10.1016/0003-4975(96)00238-x
18. Kim YI, Herijgers P, Van Lommel A, et al. Na+/H+ exchange inhibition improves post-transplant myocardial compliance in 4-hour stored donor hearts. *Cardiovasc Surg*. 1998;6:67–75. doi:10.1016/s0967-2109(97)00080-x
19. Bittner HB, Chen EP, Biswas SS, et al. Right ventricular dysfunction after cardiac transplantation: primarily related to status of donor heart. *Ann Thorac Surg*. 1999;68:1605–1611. doi:10.1016/s0003-4975(99)00987-x
20. Ryan JB, Wilson MK, Hicks M, et al. A brain dead donor model of porcine orthotopic cardiac transplantation for assessment of cardiac allograft preservation. *Heart Lung Circ*. 2000;9:78–81. doi:10.1046/j.1444-2892.2000.00028.x
21. Wilhelm MJ, Pratschke J, Beato F, et al. Activation of the heart by donor brain death accelerates acute rejection after transplantation. *Circulation*. 2000;102:2426–2433. doi:10.1161/01.cir.102.19.2426
22. Wilhelm MJ, Pratschke J, Laskowski IA, et al. Altered alloimmune response toward grafts from brain-dead donors in chronic rat cardiac allograft rejection. *Transplant Proc*. 2001;33:366–367. doi:10.1016/s0041-1345(00)02050-9
23. Ryan JB, Hicks M, Cropper JR, et al. The preload recruitable stroke work relationship as a measure of left ventricular contractile dysfunction in porcine cardiac allografts. *Eur J Cardiothorac Surg*. 2002;22:738–745. doi:10.1016/s1010-7940(02)00526-2
24. Wilhelm MJ, Pratschke J, Beato F, et al. Activation of proinflammatory mediators in heart transplants from brain-dead donors: evidence from a model of chronic rat cardiac allograft rejection. *Transplant Proc*. 2002;34:2359–2360. doi:10.1016/s0041-1345(02)03283-9
25. Ryan JB, Hicks M, Cropper JR, et al. The initial rate of troponin I release post-reperfusion reflects the effectiveness of myocardial protection during cardiac allograft preservation. *Eur J Cardiothorac Surg*. 2003;23:898–906. doi:10.1016/s1010-7940(03)00114-3
26. Ryan JB, Hicks M, Cropper JR, et al. Cariporide (HOE-642) improves cardiac allograft preservation in a porcine model of orthotopic heart transplantation. *Transplantation*. 2003;75:625–631. doi:10.1097/01.TP.0000054619.13962.30
27. Ryan JB, Hicks M, Cropper JR, et al. Lazaroid (U74389G)-supplemented cardioplegia: results of a double-blind, randomized, controlled trial in a porcine model of orthotopic heart transplantation. *J Heart Lung Transplant*. 2003;22:347–356. doi:10.1016/s1053-2498(02)00555-7
28. Ryan JB, Hicks M, Cropper JR, et al. Sodium-hydrogen exchanger inhibition, pharmacologic ischemic preconditioning, or both for extended cardiac allograft preservation. *Transplantation*. 2003;76:766–771. doi:10.1097/01.TP.0000079254.81264.6D
29. Konstantinov IE, Li J, Cheung MM, et al. Remote ischemic preconditioning of the recipient reduces myocardial ischemia-reperfusion injury of the denervated donor heart via a Katp channel-dependent

mechanism. *Transplantation*. 2005;79:1691–1695. doi:10.1097/01.tp.0000159137.76400.5d
30. Hing AJ, Watson A, Hicks M, et al. Combining cariporide with glyceryl trinitrate optimizes cardiac preservation during porcine heart transplantation. *Am J Transplant*. 2009;9:2048–2056. doi:10.1111/j.1600-6143.2009.02736.x
31. Ali AA, White P, Xiang B, et al. Hearts from DCD donors display acceptable biventricular function after heart transplantation in pigs. *Am J Transplant*. 2011;11:1621–1632. doi:10.1111/j.1600-6143.2011.03622.x
32. Floerchinger B, Ge X, Lee YL, et al. Graft-specific immune cells communicate inflammatory immune responses after brain death. *J Heart Lung Transplant*. 2012;31:1293–1300. doi:10.1016/j.healun.2012.09.005
33. Atkinson C, Floerchinger B, Qiao F, et al. Donor brain death exacerbates complement-dependent ischemia/reperfusion injury in transplanted hearts. *Circulation*. 2013;127:1290–1299. doi:10.1161/CIRCULATIONAHA.112.000784
34. Watson AJ, Gao L, Sun L, et al. Enhanced preservation of pig cardiac allografts by combining erythropoietin with glyceryl trinitrate and zoniporide. *Am J Transplant*. 2013;13:1676–1687. doi:10.1111/ajt.12249
35. Li S, Loganathan S, Korkmaz S, et al. Transplantation of donor hearts after circulatory or brain death in a rat model. *J Surg Res*. 2015;195:315–324. doi:10.1016/j.jss.2014.12.038
36. Li S, Korkmaz S, Loganathan S, et al. Short- and long-term effects of brain death on post-transplant graft function in a rodent model. *Interact Cardiovasc Thorac Surg*. 2015;20:379–386. doi:10.1093/icvts/ivu403
37. Spindler RS, Schnuelle P, Nickels L, et al. N-octanoyl dopamine for donor treatment in a brain-death model of kidney and heart transplantation. *Transplantation*. 2015;99:935–941. doi:10.1097/TP.0000000000000577
38. Hegedűs P, Li S, Korkmaz-Icöz S, et al. Dimethyloxalylglycine treatment of brain-dead donor rats improves both donor and graft left ventricular function after heart transplantation. *J Heart Lung Transplant*. 2016;35:99–107. doi:10.1016/j.healun.2015.06.016
39. Ritschl PV, Ashraf MI, Oberhuber R, et al. Donor brain death leads to differential immune activation in solid organs but does not accelerate ischaemia-reperfusion injury. *J Pathol*. 2016;239:84–96. doi:10.1002/path.4704
40. Steen S, Paskevicius A, Liao Q, et al. Safe orthotopic transplantation of hearts harvested 24 hours after brain death and preserved for 24 hours. *Scand Cardiovasc J*. 2016;50:193–200. doi:10.3109/14017431.2016.1154598
41. Li S, Korkmaz-Icöz S, Radovits T, et al. Donor preconditioning after the onset of brain death with dopamine derivate n-octanoyl dopamine improves early posttransplant graft function in the rat. *Am J Transplant*. 2017;17:1802–1812. doi:10.1111/ajt.14207
42. Yip HK, Lee MS, Sun CK, et al. Therapeutic effects of adipose-derived mesenchymal stem cells against brain death-induced remote organ damage and post-heart transplant acute rejection. *Oncotarget*. 2017;8:108692–108711. doi:10.18632/oncotarget.21433
43. Kumar TKS, Mathis C, Sathanandam S, et al. Effect of thyroid hormone on cardiac function following orthotopic heart transplantation in piglets. *Pediatr Transplant*. 2017;21:e13002. doi:10.1111/petr.13002
44. Pomper G, Trescher K, Santer D, et al. Introducing a mouse model of brain death. *J Neurosci Methods*. 2010;192:70–74. doi:10.1016/j.jneumeth.2010.07.019
45. Pulsinelli WA, Brierley JB. A new model of bilateral hemispheric ischemia in the unanesthetized rat. *Stroke*. 1979;10:267–272. doi:10.1161/01.str.10.3.267
46. Schwartz I, Bird S, Lotz Z, et al. The influence of thyroid hormone replacement in a porcine brain death model. *Transplantation*. 1993;55:474–476. doi:10.1097/00007890-199303000-00003
47. Hoeger S, Gottmann U, Liu Z, et al. Dopamine treatment in brain-dead rats mediates anti-inflammatory effects: the role of hemodynamic stabilization and D-receptor stimulation. *Transpl Int*. 2007;20:790–799. doi:10.1111/j.1432-2277.2007.00510.x
48. Bittner HB, Kendall SW, Campbell KA, et al. A valid experimental brain death organ donor model. *J Heart Lung Transplant*. 1995;14:308–317.
49. Kirsch M, Farhat F, Garnier JP, et al. Acute brain death abolishes the cardioprotective effects of ischemic preconditioning in the rabbit. *Transplantation*. 2000;69:2013–2019. doi:10.1097/00007890-200005270-00007
50. Farhat F, Loisance D, Garnier JP, et al. Norepinephrine release after acute brain death abolishes the cardioprotective effects of ischemic preconditioning in rabbit. *Eur J Cardiothorac Surg*. 2001;19:313–320. doi:10.1016/s1010-7940(00)00659-x
51. de Zeeuw S, Lameris TW, Duncker DJ, et al. Cardioprotection in pigs by exogenous norepinephrine but not by cerebral ischemia-induced release of endogenous norepinephrine. *Stroke*. 2001;32:767–774. doi:10.1161/01.str.32.3.767
52. Hing AJ, Hicks M, Garlick SR, et al. The effects of hormone resuscitation on cardiac function and hemodynamics in a porcine brain-dead organ donor model. *Am J Transplant*. 2007;7:809–817. doi:10.1111/j.1600-6143.2007.01735.x
53. Steen S, Sjöberg T, Liao Q, et al. Pharmacological normalization of circulation after acute brain death. *Acta Anaesthesiol Scand*. 2012;56:1006–1012. doi:10.1111/j.1399-6576.2012.02721.x
54. Corry RJ, Winn HJ, Russell PS. Primarily vascularized allografts of hearts in mice. The role of H-2D, H-2K, and non-H-2 antigens in rejection. *Transplantation*. 1973;16:343–350. doi:10.1097/00007890-197310000-00010
55. Atkinson C, He S, Morris K, et al. Targeted complement inhibitors protect against posttransplant cardiac ischemia and reperfusion injury and reveal an important role for the alternative pathway of complement activation. *J Immunol*. 2010;185:7007–7013. doi:10.4049/jimmunol.1001504
56. Oberhuber R, Cardini B, Kofler M, et al. Murine cervical heart transplantation model using a modified cuff technique. *J Vis Exp*. 2014;92:e50753. doi:10.3791/50753
57. Matsuura A, Abe T, Yasuura K. Simplified mouse cervical heart transplantation using a cuff technique. *Transplantation*. 1991;51:896–898. doi:10.1097/00007890-199104000-00031
58. Maruyama T, Swartz MT, McBride LR, et al. Working heart model of heterotopic heart-lung transplantation in rats. *J Thorac Cardiovasc Surg*. 1994;107:210–215. doi:10.1016/S0022-5223(94)70472-4
59. Konertz W, Semik M, Bernhard A. Heart, lung and heart-lung transplantation in rats. *J Heart Transplant*. 1985;4:426–430.
60. Szabó G, Bährle S, Stumpf N, et al. Poly(ADP-ribose) polymerase inhibition reduces reperfusion injury after heart transplantation. *Circ Res*. 2002;90:100–106. doi:10.1161/hh0102.102657
61. Ono K, Lindsey ES. Improved technique of heart transplantation in rats. *J Thorac Cardiovasc Surg*. 1969;57:225–229.
62. Wang D, Kleist C, Ehser S, et al. Ex vivo perfusion with mitomycin C containing solution prolongs heart graft survival in rats. *Transplantation*. 2006;82:1537–1540. doi:10.1097/01.tp.0000235439.39872.f8
63. Bittner HB, Kendall SW, Chen EP, et al. Complete atrioventricular cardiac transplantation: improved performance compared with the standard technique. *Ann Thorac Surg*. 1995;60:275–282; discussion 282–283. doi:10.1016/0003-4975(95)00364-q
64. Lower RR, Shumway NE. Studies on orthotopic homotransplantation of the canine heart. *Surg Forum*. 1960;11:18–19.
65. Australian and New Zealand Intensive Care Society. *The ANZICS Statement on Death and Organ Donation*. 3.2 ed. Melbourne, VIC, Australia: Australian and New Zealand Intensive Care Society; 2013.
66. Dobson GP, Headrick JP. Bioenergetic scaling: metabolic design and body-size constraints in mammals. *Proc Natl Acad Sci U S A*. 1995;92:7317–7321. doi:10.1073/pnas.92.16.7317
67. Kilkenny C, Browne WJ, Cuthill IC, et al. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol*. 2010;8:e1000412. doi:10.1371/journal.pbio.1000412
68. Khush KK, Kubo JT, Desai M. Influence of donor and recipient sex mismatch on heart transplant outcomes: analysis of the International Society for Heart and Lung Transplantation Registry. *J Heart Lung Transplant*. 2012;31:459–466. doi:10.1016/j.healun.2012.02.005
69. Patel ND, Weiss ES, Nwakanma LU, et al. Impact of donor-to-recipient weight ratio on survival after heart transplantation: analysis of the United Network for Organ Sharing Database. *Circulation*. 2008;118(14 Suppl):S83–S88. doi:10.1161/CIRCULATIONAHA.107.756866
70. Reed RM, Netzer G, Hunsicker L, et al. Cardiac size and sex-matching in heart transplantation: size matters in matters of sex and the heart. *JACC Heart Fail*. 2014;2:73–83. doi:10.1016/j.jchf.2013.09.005
71. Weiss ES, Allen JG, Patel ND, et al. The impact of donor-recipient sex matching on survival after orthotopic heart transplantation: analysis of 18 000 transplants in the modern era. *Circ Heart Fail*. 2009;2:401–408. doi:10.1161/CIRCHEARTFAILURE.108.844183
72. World Health Organization. *Global health estimates 2016: disease burden by cause, age, sex, by country and by region, 2000-2016* [World Health Organization Website]. 2018. Global summary estimates. Available at https://www.who.int/healthinfo/global_burden_disease/estimates/en/. Accessed October 8, 2018.
73. Clayton JA. Studying both sexes: a guiding principle for biomedicine. *Faseb J*. 2016;30:519–524. doi:10.1096/fj.15-279554
74. Clayton JA, Collins FS. Policy: NIH to balance sex in cell and animal studies. *Nature*. 2014;509:282–283. doi:10.1038/509282a

75. Peart JN, Headrick JP. Clinical cardioprotection and the value of conditioning responses. *Am J Physiol Heart Circ Physiol*. 2009;296:H1705–H1720. doi:10.1152/ajpheart.00162.2009
76. ANZOD Registry. *2017 Annual Report, Section 3: Deceased Organ Donor Pathway*. Adelaide, SA, Australia: Australia and New Zealand Dialysis and Transplant Registry; 2017.
77. Iyer A, Kumarasinghe G, Hicks M, et al. Primary graft failure after heart transplantation. *J Transplant*. 2011;2011:175768. doi:10.1155/2011/175768
78. Lund LH, Edwards LB, Kucheryavaya AY, et al; International Society for Heart and Lung Transplantation. The registry of the International Society for Heart and Lung Transplantation: thirtieth official adult heart transplant report–2013; focus theme: age. *J Heart Lung Transplant*. 2013;32:951–964. doi:10.1016/j.healun.2013.08.006
79. Lund LH, Khush KK, Cherikh WS, et al; International Society for Heart and Lung Transplantation. The registry of the International Society for Heart and Lung Transplantation: thirty-fourth adult heart transplantation report-2017; focus theme: allograft ischemic time. *J Heart Lung Transplant*. 2017;36:1037–1046. doi:10.1016/j.healun.2017.07.019
80. Ganesh JS, Rogers CA, Banner NR, et al. Donor cause of death and medium-term survival after heart transplantation: a United Kingdom national study. *J Thorac Cardiovasc Surg*. 2005;129:1153–1159. doi:10.1016/j.jtcvs.2004.09.029
81. Cantin B, Kwok BW, Chan MC, et al. The impact of brain death on survival after heart transplantation: time is of the essence. *Transplantation*. 2003;76:1275–1279. doi:10.1097/01.TP.0000093445.50624.5A
82. Marasco S, Kras A, Schulberg E, et al. Donor brain death time and impact on outcomes in heart transplantation. *Transplant Proc*. 2013;45:33–37. doi:10.1016/j.transproceed.2012.08.008
83. Venkateswaran RV, Lannon J, Wong E, et al. The interval between brain stem death and cardiac assessment influences the retrieval of hearts for transplantation. *J Heart Lung Transplantation*. 2016;35(4, Suppl):S218. doi:10.1016/j.healun.2016.01.615
84. Ramjug S, Hussain N, Yonan N. Prolonged time between donor brain death and organ retrieval results in an increased risk of mortality in cardiac transplant recipients. *Interact Cardiovasc Thorac Surg*. 2011;12:938–942. doi:10.1510/icvts.2010.252809
85. Odim J, Laks H, Banerji A, et al. Does duration of donor brain injury affect outcome after orthotopic pediatric heart transplantation? *J Thorac Cardiovasc Surg*. 2005;130:187–193. doi:10.1016/j.jtcvs.2005.02.038
86. Borbely XI, Krishnamoorthy V, Modi S, et al. Temporal changes in left ventricular systolic function and use of echocardiography in adult heart donors. *Neurocrit Care*. 2015;23:66–71. doi:10.1007/s12028-014-0101-x
87. Ardehali A, Esmailian F, Deng M, et al; PROCEED II trial investigators. Ex-vivo perfusion of donor hearts for human heart transplantation (PROCEED II): a prospective, open-label, multicentre, randomised non-inferiority trial. *Lancet*. 2015;385:2577–2584. doi:10.1016/S0140-6736(15)60261-6
88. Mentzer RM Jr, Bartels C, Bolli R, et al; EXPEDITION Study Investigators. Sodium-hydrogen exchange inhibition by cariporide to reduce the risk of ischemic cardiac events in patients undergoing coronary artery bypass grafting: results of the EXPEDITION study. *Ann Thorac Surg*. 2008;85:1261–1270. doi:10.1016/j.athoracsur.2007.10.054
89. Tanoue Y, Morita S, Ochiai Y, et al. Inhibition of lipid peroxidation with the lazaroid U74500A attenuates ischemia-reperfusion injury in a canine orthotopic heart transplantation model. *J Thorac Cardiovasc Surg*. 1996;112:1017–1026. doi:10.1016/S0022-5223(96)70103-4
90. Du ZY, Hicks M, Spratt P, et al. Cardioprotective effects of pinacidil pretreatment and lazaroid (U74500A) preservation in isolated rat hearts after 12-hour hypothermic storage. *Transplantation*. 1998;66:158–163. doi:10.1097/00007890-199807270-00003
91. Takahashi T, Takeyoshi I, Hasegawa Y, et al. Cardioprotective effects of lazaroid U-74389G on ischemia-reperfusion injury in canine hearts. *J Heart Lung Transplant*. 1999;18:285–291. doi:10.1016/s1053-2498(98)00068-0
92. Lefer DJ, Marbán E. Is cardioprotection dead? *Circulation*. 2017;136:98–109. doi:10.1161/CIRCULATIONAHA.116.027039
93. Heusch G. Critical issues for the translation of cardioprotection. *Circ Res*. 2017;120:1477–1486. doi:10.1161/CIRCRESAHA.117.310820
94. Dell'Aquila AM, Mastrobuoni S, Bastarrika G, et al. Bicaval versus standard technique in orthotopic heart transplant: assessment of atrial performance at magnetic resonance and transthoracic echocardiography. *Interact Cardiovasc Thorac Surg*. 2012;14:457–462. doi:10.1093/icvts/ivr084
95. Schnoor M, Schäfer T, Lühmann D, et al. Bicaval versus standard technique in orthotopic heart transplantation: a systematic review and meta-analysis. *J Thorac Cardiovasc Surg*. 2007;134:1322–1331.
96. Weiss ES, Nwakanma LU, Russell SB, et al. Outcomes in bicaval versus biatrial techniques in heart transplantation: an analysis of the UNOS database. *J Heart Lung Transplant*. 2008;27:178–183. doi:10.1016/j.healun.2007.11.003
97. Costanzo MR, Dipchand A, Starling R, et al; International Society of Heart and Lung Transplantation Guidelines. The International Society of Heart and Lung Transplantation Guidelines for the care of heart transplant recipients. *J Heart Lung Transplant*. 2010;29:914–956. doi:10.1016/j.healun.2010.05.034
98. Vega E, Schroder J, Nicoara A. Postoperative management of heart transplantation patients. *Best Pract Res Clin Anaesthesiol*. 2017;31:201–213. doi:10.1016/j.bpa.2017.06.002
99. Thajudeen A, Stecker EC, Shehata M, et al. Arrhythmias after heart transplantation: mechanisms and management. *J Am Heart Assoc*. 2012;1:e001461. doi:10.1161/JAHA.112.001461
100. Jacquet L, Ziady G, Stein K, et al. Cardiac rhythm disturbances early after orthotopic heart transplantation: prevalence and clinical importance of the observed abnormalities. *J Am Coll Cardiol*. 1990;16:832–837. doi:10.1016/s0735-1097(10)80330-4
101. Pacher P, Nagayama T, Mukhopadhyay P, et al. Measurement of cardiac function using pressure-volume conductance catheter technique in mice and rats. *Nat Protoc*. 2008;3:1422–1434. doi:10.1038/nprot.2008.138
102. Burkhoff D, Mirsky I, Suga H. Assessment of systolic and diastolic ventricular properties via pressure-volume analysis: a guide for clinical, translational, and basic researchers. *Am J Physiol Heart Circ Physiol*. 2005;289:H501–H512. doi:10.1152/ajpheart.00138.2005
103. Chowdhury SM, Butts RJ, Taylor CL, et al. Longitudinal measures of deformation are associated with a composite measure of contractility derived from pressure-volume loop analysis in children. *Eur Heart J Cardiovasc Imaging*. 2018;19:562–568. doi:10.1093/ehjci/jex167
104. Schroeder LW, Chowdhury SM, Burnette AL, et al. Longer ischemic time is associated with increased ventricular stiffness as measured by pressure-volume loop analysis in pediatric heart transplant recipients. *Pediatr Cardiol*. 2018;39:324–328. doi:10.1007/s00246-017-1758-7