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

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1. ABBREVIATIONS

HTx – heart transplantation
BSD – brain stem death
CSS – cold static storage
LV – left ventricular
RV – right ventricular
PRSW – preload recruitable stroke work
T3 - triiodothyronine
M – male
F – female
Y – yes
BCI – Balloon catheter inflation
EEG – electroencephalogram
BP – blood pressure
HR – heart rate
CVP – central venous pressure
NE – norepinephrine
MAP – mean arterial pressure
NA – noradrenaline
A – adrenaline
NIH – National Institute of Health
MP – methylprednisolone
CPB – cardiopulmonary bypass
VVI – ventricular demand pacing
LAD – left anterior descending coronary artery
PVR – pressure volume relationship

CO – cardiac output

dP/dt – rate pressure change

PCWP – pulmonary capillary wedge pressure

%LVEF - % left ventricular ejection fraction

TTE – transthoracic echocardiography
2. ABSTRACT

Despite advances in mechanical circulatory devices and pharmacological therapies, heart transplantation 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. While 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 heart transplantation, and assessed studies for scientific rigor and clinical relevance. Following literature screening via MEDLINE and Embase, 29 studies were assessed. Analysis of included studies identified marked heterogeneity in animal models of donor brain death coupled to heart transplantation, 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 is potentially hindering advancement of this field of investigation.
5. MAIN BODY TEXT

1. INTRODUCTION:

End-stage heart failure is an irreversible and progressive condition associated with high morbidity and mortality, and is increasing in frequency worldwide. Globally, approximately 38 million people are affected by heart failure, and an estimated 17-45% patients with heart failure die within 1 year from hospital admission. The gold standard treatment for end-stage heart failure is heart transplantation (HTx), however the donor pool is grossly inadequate and, increasingly unable to satisfy the growing demand. While 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. Despite advances in technological, surgical and pharmacological measures to broaden donor heart availability, 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. 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 hours 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.
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, e.g., 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. While 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 heart transplantation. In addition, scientific rigor and clinical relevance of these previous models are characterized and suggestions for future methodological improvements are provided.

2. MATERIALS AND METHODS:

2.1 Design

We developed a systematic review protocol, which was reported on the systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) website (https://issuu.com/radboudumc/docs/animal_models_of_heart_transplantation_1467589717c6f9a8) on February 21, 2017, and published in May 2017. This protocol aligns with the Preferred Reporting Items for Systematic Review and Meta-analysis Protocols (PRISMA-P). Following publication, the protocol was adjusted to remove language restrictions that excluded non-English language publications.
2.2 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 MEDLINE (via PubMed) and Embase (via Ovid SP) online databases were searched in order to retrieve studies over any time period up until June 2019. Please refer to Supplemental Digital Content 1 for the full search strategy.

2.3 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 multiorgan transplantation (including heart/lung transplantation). Regarding heart/lung transplantation, studies have been included if the technique (eg. heterotopic HTx) required transplantation of the heart and lungs en bloc, but the lungs remained nonfunctional.

2.4 Literature Search and Screening

After removal of duplicates, all retrieved studies were screened in 2 separate phases (Fig 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 where 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.
2.5 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.

2. General animal characteristics for each included study: Details regarding animal breed, age, weight, gender, and the use of anticoagulation were 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. Heart transplantation model details: Data were recorded on several aspects of HTx procedures, including heart preservation (preservation method, duration and solution used), HTx surgical 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 was 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.
3. RESULTS:

3.1 Description of included studies

A summary of included studies is detailed in Table 1. Following screening via the search strategy (Figure 1), 29 papers were included in the systematic review. The studies were published between 1993 and 2017 (24 years), with most of the studies performed in the USA (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).

3.2 General study parameters

General study parameters are reported in Table 2. Breed was predominantly reported and consistent within each species. Age (in weeks/months/years) was generally not reported (83%), however all mouse studies (n=3) reported use of young mice aged 8-12 weeks. 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). 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.
3.3 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 technique used to induce BSD (93%). One rat and 1 pig study used a combination of balloon catheter inflation and carotid artery ligation, and a study in pigs used decapitation between the 2nd and 3rd cervical vertebrae. Rodent studies typically reported catheter size (3-4F), however times and volumes to induce BSD varied. Mouse studies induced BSD over 10-15 mins. Rat studies either used smaller volumes (200 ± 25 μL) over 1-5 mins, or larger volumes (600-750 μL) over 40-50 mins. Studies in dogs utilized 15-20 mL to inflate the catheter and induce BSD. Induction methods in pigs were typically consistent, using 20-24 mL water for balloon catheter inflation over 3 mins (8/12). One pig study reported a slightly higher volume for balloon catheter inflation (30 mL saline) over an unknown period of time.

All studies except 2 reported at least 1 parameter for confirming BSD (27/29). The most common criteria enlisted for confirming BSD was loss of corneal reflexes (13/29), followed by typical BSD-related hemodynamic changes (12/29), loss of pupillary reflexes (12/29), apnea (11/29), absence of response to stimuli after cessation of anesthesia (8/26), and an isoelectric electroencephalogram (EEG) (7/29). Some studies reported confirmation of BSD with nonspecific details, namely absence of spinal/brain stem/deep pain reflexes (7/29)). 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, 24/29 used more than 1 test listed in the ANZICS statement to confirm BSD.

The duration of BSD to transplant in rodent studies ranged from 1-6 hrs, with most studies choosing 5-6 hrs (9/13). Of the dog and pig models that reported of the duration of BSD (13/16) to transplant, most utilized a period of 1 hr donor BSD (8/13), with no uniformity among remaining studies, incorporating 1.5, 4, 6, 18 or up to 24 hrs BSD. Most rodent and pig
studies (17/25) 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 hrs BSD) and 5 pig (1 hr BSD) studies specifically reported
no use of these drugs during BSD. The 3 studies with the longest periods from BSD to graft
procurement (6 hrs, 18 hrs, and 24 hrs) used additional pharmacological support in the
donor (norepinephrine, methylprednisolone, T3, vasopressin, hydrocortisone, or in-house
developed ‘brain death cocktail’).

3.4 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) (25/29), and remaining studies utilized warm (40°C) static storage, or
compared CSS vs. ex-vivo machine perfusion storage at 8°C. Interestingly, 21/29 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 mins, 3/29), followed by ≈ 1 hr (2/29),
3.5-4 hrs (2/29), and 24 hrs (1/29). Preservation solutions were highly heterogeneous across all
studies, and included specific supplementations depending on the purpose of each study.
Commercially available crystalloid cardioplegia (14/29) and in-house developed cardioplegia
(8/29) were the primary types of preservation solutions used. Supplemented additives
comprised hydrogen chloride, intralipid, lazaroid U74389G, cariporide, glyceryl trinitrate,
lidocaine, erythropoietin and zoniporide.

All rodent studies used models of heterotopic HTx, and all remaining studies (dog and
pig) used models with orthotopic HTx. For the heterotopic transplants undertaken in rodents,
donor hearts were predominantly (9/13) positioned in the recipient abdomen. Studies in dogs
reported use of both biatrial (1/4) and bicaval (2/4) HTx techniques. All pig studies used a
model of biatrial implantation as described by Lower and Shumway. 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 mins, followed by 1.5-4 hrs for dog studies, and 2-14 hours 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 buffer²⁰). Immunosuppression was commonly employed in all dog (methylprednisolone, solumedrol and azathioprine) and most pig (8/12) studies, but only in 2 rodent studies.²⁶,²⁹ Vasopressor support for the HTx recipient was not used in rodent studies, yet reported in dog studies (2/4 studies reported use isoprenaline or noradrenaline). Conversely, pig studies largely used dobutamine for inotropic support following HTx (9/12), however some studies also used noradrenaline,¹⁷ adrenaline,¹⁹,²⁰ dopamine, atropine, lidocaine and furosemide.¹⁹ Most pig studies employed ventricular pacing at 120 bpm. Antibiotics use was reported in some rat²⁶ and dog studies,³²,³⁴ but never in mouse or pig studies.

Following HTx, 27/29 studies continued monitoring after weaning from cardiopulmonary bypass (orthotopic studies) or graft reperfusion (heterotopic studies). The post-HTx monitoring period for rodents was variable, ranging from 90 mins to 120 days. For the dog and pig models, recipients were typically monitored post-HTx for 1-3 hrs, with the exception of 1 study that extended to 24 hrs,²⁰ and 2 studies where animals were either not weaned at all,¹⁵ or the study ended immediately after weaning.¹⁶

3.5 Cardiac function assessment

Methods used to assess cardiac function in the included studies are outlined in Table 5. Several studies (7/29) did not assess graft function post-HTx, predominantly in rodents²³,²⁵-²⁷,²⁹,³⁵ and 1 pig study.¹⁵ 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 (SWI), preload recruitable stroke work (PRSW), end-diastolic pressure-volume relationship (EDPVR), cardiac output (CO), cardiac index (CI), left (LV) and right ventricular (RV) systolic and diastolic pressures and volumes, systemic and pulmonary vascular resistances, aortic and mean arterial pressures, heart rate (HR), ejection fraction (EF), and the minimum (dP/dt<sub>min</sub>) and maximum (dP/dt<sub>max</sub>) rate of pressure change in the ventricle. Two rodent studies assessed cardiac function via manual palpation of the graft.\textsuperscript{18,24} In addition to PVR analyses, ‘successful weaning off bypass’ was reported as a measure of transplant success in 9/16 (dog and pig) studies. Other less frequent analyses used for cardiac function were cine cardiac magnetic resonance imaging (pig\textsuperscript{16}), an adrenaline stress test (pig\textsuperscript{20}), and %EF using transthoracic echocardiography (rat\textsuperscript{28} and pig\textsuperscript{19}).

4. 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.\textsuperscript{7} Preclinical research models that accurately mimic the clinical scenario are essential for groundbreaking advancements in donor management strategies, surgical procedures, novel pharmacological 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, 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 hr) relative to metabolic scaling,\(^3\) and nonworking heterotopic models.

4.1 The influence of age, weight and gender upon HTx outcomes

The ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) were developed and published in 2010 to improve and standardize the reporting of animal research\(^3\) (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). While body weight is considered for donor heart allocation for HTx, weight matching is a poor predictor of HTx outcomes.\(^3\) 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.\(^1,13,3,4,0,4\) The 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).\(^{42}\) 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 where feasible. Additionally, the consideration, inclusion and reporting of females in basic and preclinical research is now a requirement for NIH-funded research.\(^{43,44}\)

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.\(^{45}\) While 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).
4.2 BSD model diversity

The complex pathophysiological changes that occur with brain death undoubtedly impair donor heart function. Studies in this review typically used balloon catheter inflation (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. Here, the authors note that the pig has a robust tentorium cerebelli, and spinal arteries that ascend to the brainstem and are a source of extracranial blood supply. The authors concluded balloon catheter inflation in the pig skull would be insufficient to induce complete brain stem death due to the unique anatomy of the pig brain and skull, but ensured complete BSD with decapitation. According to the ANZICS statement on death and organ donation, 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 brain stem death. Differing injuries, such as traumatic brain injury (TBI), intracranial hemorrhage (ICH), and cerebral ischemia, can lead to brain death. The actual cause of brain death (eg. TBI, ICH, trauma) is a notable risk factor for primary graft failure (PGF), which is one of the greatest contributors to early mortality following HTx. However, a study in the UK found that medium term (30 days – 3 years) 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. Cantin et al 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 to gradual elevations, has been previously shown to significantly impair myocardial function, and adversely affect HTx outcomes in dogs. 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. No study included in this review utilized tests that covered all 3 of these criteria. Absence of responsiveness was often not performed, whilst 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. Whilst informative, this criterion is isolated to animal models of BSD since hemodynamic data in the patient is often absent prior to 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 hours of hospital admission (median time was 22 hours). The length of BSD in the reviewed animal models (~ 1-6 hours predominantly) generally fell significantly shorter than times reported by other transplant centers. Clinically, the time that donor organs are subject to BSD has been shown to influence HTx outcomes with mixed results. Extended donor management (> 14 hrs and > 72 hours) time prior to HTx has been associated with poorer recipient survival in humans. However, Marasco et al. reported no effect of donor brain death time (approximately 19 hours) upon recipient survival. Furthermore, a period of 4-17 days 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. Indeed, Borbely et al. 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.

4.3 Donor heart preservation

Cold static storage (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. While 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-June 2016) was 3.2 hours (1.5-5 hours). 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 hours). Allograft ischemic time and viability are intricately entwined, and in clinical transplantation, an ischemic time beyond 4 hrs significantly impairs both short- and long-term recipient survival.

Ischemic time is one of the challenges of organ retrieval and HTx, and can be a determinant of acceptance of a donor heart. Due to 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 a) extend the ischemic (or ex vivo/in transit) time, b) assess function of ‘marginal’ donor hearts prior to HTx (depending on the machine), and/or c)
recondition donor hearts to improve outcomes in the recipient. Indeed, Steen et al. demonstrated experimentally that hearts can be safely transplanted (as assessed 24 hrs post-HTx) following exposure to 24 hours BSD, followed by 24 hours cold machine-perfused storage. The PROCEED II trial demonstrated that donor heart storage using the clinically approved Organ Care System (OCS, warm machine perfusion) was noninferior to typical CSS, despite a significantly longer ex-vivo storage time.

In addition to novel storage techniques, pharmacological 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 pharmacological preconditioning agent, alone and in combination with glyceryl trinitrate. Cariporide was however removed from further use following the EXPEDITION trial, as it was associated with an increase perioperative stroke related mortality, although cariporide did improve postischemic myocardial function. 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). Ryan et al. have also demonstrated that inhibition of lipid peroxidation (using lazaroizd U74389G), while beneficial in other animal models of cardiac I-R injury and HTx 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, and is no doubt complicated by the pathophysiological processes associated with BSD and HTx.

4.4 Heart transplantation and postoperative support in animal models
This review identified a clear delineation between animal HTx techniques, where heterotopic HTx (HHTx) was employed in rodents, and orthotopic HTx (OHTx) in dog and pig models, suggesting the complexity of OHTx renders it unfeasible in smaller animals such as rodents. Clinical heterotopic heart transplantation 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\textsuperscript{68,69}), several studies have reported similar long-term survival between biatrial vs. bicaval techniques\textsuperscript{68,70}.

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)\textsuperscript{71,72}. Rodent studies commonly assessed inflammation and rejection post-HTx using matched and unmatched breeds where 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 was 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 ug/kg/min) that greatly overcame the recommended ISHLT doses (1-10 ug/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 seems 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 bpm) 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 hrs) for the reviewed studies, both of which are notable mechanisms of arrhythmias post-HTx.73,74

4.5 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 cardio-pulmonary bypass (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.75 While 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).71 Only 2 studies in this review used any measure of echocardiography to examine cardiac function.19,28 Despite being a powerful means of characterizing ventricular properties, PVR analysis has not been used in clinical practice, because 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. ejection fraction compared to dP/dtmax).76 Echocardiographically-derived ejection fraction, has however not been validated
in animal studies, despite its widespread use in clinical transplant settings. In 2018, Chowdhury et al. conducted the first comprehensive study evaluating the association between echocardiographic measures of systolic function and a composite measure of pressure-volume derived contractility.\textsuperscript{77} 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, while conventional measures such as ejection fraction and fractional shortening were significantly associated with LV load and mass, but not contractility. Schroeder et al. had reported that no single clinical characteristic was statistically significant when correlated with pressure-volume loop data in a cohort of 18 transplant patients.\textsuperscript{78} 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.

4.6 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 and collaborators. 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 (i.e. no research group attempted development of more than 1 animal model). As detailed above, undertaking large animal (i.e. dog, pig, sheep, 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.

4.7 Limitations

Each study combined models of donor BSD with HTx, assessed different outcomes and showed slight variations in models when published form 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, due to the heterogenous 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.

5. CONCLUSION

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.

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5. FIGURE LEGEND

Fig 1 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 following donor brain stem death.

6. TABLES

TABLE 1: General study information sorted by publication year

TABLE 2: General animal characteristics

TABLE 3: Methodological features of BSD development/management

TABLE 4: Heart transplantation model details

TABLE 5: Outcome measures of cardiac function in donor and recipient
| Author, Year (Country) | Species | Study type | Aims |
|-----------------------|---------|------------|------|
| Shivalkar B., 1993 (Belgium)²¹ | Dog | Physiological | Assess effects of sudden vs. gradual intracranial pressure increases pre- and post-HTx upon catecholamine levels, hemodynamics and myocardial structure. |
| Bittner H., 1995 (USA)³⁴ | Dog | Physiological | Examine effects of BSD and CSS upon LV and RV function post-HTx |
| Votapka T., 1996 (USA)¹⁸ | Rat | Interventional | Determine the effect of triiodothyronine treatment (in donor) upon graft function |
| Kim Y., 1998 (Belgium)²² | Dog | Interventional | Assess the effects of Na⁺/H⁺ exchange inhibition upon graft function post-HTx |
| Bittner H., 1999 (USA)³² | Dog | Physiological | Determine if post-HTx RV dysfunction is due to recipient pulmonary hypertension or donor BSD-induced cardiac injury |
| Ryan J., 2000 (Australia)⁷⁹ | Pig | Physiological | Develop a large animal model of orthotopic HTx incorporating donor BSD |
| Wilhelm M., 2000 (USA)²⁵ | Rat | Physiological | Examine acute rejection of hearts from BSD donors |
| Wilhelm M., 2001 (USA)²⁹ | Rat | Physiological | Determine the effect of donor brain death upon inflammatory cell population changes and myocardial fibrosis in chronic cardiac allograft rejection |
| Ryan J., 2002 (Australia)⁸⁰ | Pig | Physiological | Determine the PRSW relationship as an index of graft LV contractility post-HTx following CSS |
| Wilhelm M., 2002 (USA)²⁶ | 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 J., 2003a (Australia)⁸¹ | Pig | Physiological | Determine if early cardiac troponin I release predicts effectiveness of cardiac protection during preservation |
| Ryan J., 2003b (Australia)⁶⁰ | Pig | Interventional | Examine the effects of preconditioning the donor heart with cariporide could reduce ischemia-reperfusion injury post-HTx |
| Ryan J., 2003c (Australia)⁶⁵ | Pig | Interventional | Determine the effects of lazaroid U74389G supplementation in cardioplegia upon post-HTx graft function |
| Ryan J., 2003d (Australia)⁹⁹ | Pig | Interventional | Examine effects of cariporide and BMS180448 supplementation in cardioplegia HTx |
| Name                        | Year       | Species | Study Type | Summary                                                                |
|-----------------------------|------------|---------|------------|-------------------------------------------------------------------------|
| Konstantinov I., 2005       | (Canada)   | Pig     | Intervential | Determine if remote ischemic preconditioning induces cardioprotection via a circulator effector in a model of HTx |
| Hing A., 2009 (Australia)   |            | Pig     | Intervential | Compare the effects of different cardioplegic strategies via supplementation with glyceryl trinitrate and/or cariporide |
| Ali A., 2011 (Canada)       |            | Pig     | Physiological | Assess whether hearts retrieved following circulatory death are suitable for transplantation (compared to BSD hearts) |
| Floerchinger B., 2012       | (USA)      | Mouse   | Physiological | Investigate graft-specific inflammatory immune responses following BSD in donors and recipients |
| Atkinson C., 2013 (USA)     |            | Mouse   | Both        | Investigate the effects of BSD upon graft ischemia-reperfusion injury and the effect of targeted complement inhibition |
| Watson A., 2013 (Australia) |            | Pig     | Intervential | Evaluate the effects of erythropoietin, glyceryl trinitrate, and Zoniporide treatment in a porcine model of HTx |
| Li S., 2015a (Germany)      |            | Rat     | Physiological | Investigate the functional, gene, protein and histopathological changes in heart grafts (post-HTx) following donor BSD vs circulatory death |
| Li S., 2015b (Germany)      |            | Rat     | Physiological | Investigate different periods of BSD and effect upon post-HTx graft function |
| Spindler R., 2015 (Germany) |            | Rat     | Intervential | Examine the effects of n-octanoyl dopamine administration during brain death upon cardiac allografts |
| Hegedus P., 2016 (Germany) |            | Rat     | Intervential | Determine if dimethylxalylglycine improves post-HTx graft function |
| Ritschl P., 2016 (Germany)  |            | Mouse   | Physiological | Investigate the effect of BSD upon immune activation and ischemia-reperfusion injury in transplant settings |
| Steen S., 2016 (Sweden)     |            | Pig     | Interventional | Assess efficacy of novel cold machine perfusion system upon post-HTx graft function |
| Li S., 2017 (Germany)       |            | Rat     | Intervential | Examine the effect of n-octanoyl dopamine on early post-HTx graft function |
| Yip H., 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 T., 2017 (USA)        |            | Pig     | Interventional | Determine the effect of T3 upon post-HTx cardiac function in immature pigs |
**Table 1 abbreviations**: USA – United States of America; BSD – brain stem death; CSS – cold static storage; LV – left ventricular; RV – right ventricular; Na⁺ - sodium; H⁺- hydrogen; PRSW – preload recruitable stroke work; T3 - triiodothyronine.
| Study                  | Breed                                      | Age           | Weight      | Gender | Anticoagulation |
|-----------------------|--------------------------------------------|---------------|-------------|--------|-----------------|
| Mouse                 |                                            |               |             |        |                 |
| Floerchinger B., 2012 | C57BL6 (WT and Rag2/Il2rg double knockout) | 8-12 weeks    | 20-30 g     | M      | -               |
| Atkinson C., 2013     | C57BL6, BALB/c and C57BL6 pan-GFP          | 8-12 weeks    | 20-30 g     | M      | -               |
| Ritschl P., 2016     | C57BL6 (H-2b) and BALB/c (H-2d)            | 8-12 weeks    | -           | M      | -               |
| Rat                   |                                            |               |             |        |                 |
| Votapka T., 1996     | Lewis (inbred)                             | -             | 0.27-0.32 kg| M      | Y               |
| Wilhelm M., 2000     | Lewis RT1 and Fisher 344 RT11v1            | 8-10 weeks    | 0.2-0.22 kg | M      | -               |
| Wilhelm M., 2001     | Lewis and Fisher 344                       | -             | -           | -      | -               |
| Wilhelm M., 2002     | Lewis and Fisher 344                       | -             | -           | -      | -               |
| Li S., 2015a         | Lewis                                      | -             | 0.25-0.35 kg| M      | Y               |
| Li S., 2015b         | Lewis                                      | -             | 0.25-0.35 kg| M      | Y               |
| Spindler R., 2015    | Lewis/Crl and Fisher 344/DuCrl             | -             | 0.25-0.3 kg | M      | -               |
| Hegedus P., 2016     | Lewis                                      | -             | 0.25-0.35 kg| M      | Y               |
| Li S., 2017          | Lewis                                      | -             | 0.25-0.35 kg| M      | Y               |
| Yip H., 2017         | Fisher 344                                 | Adult         | 250-280 g   | M      | Y               |
| Dog                   |                                            |               |             |        |                 |
| Shivalkar B., 1993   | Mongrel                                    | Adult         | 28 ± 4 kg   | -      | -               |
| Bittner H., 1995     | Mongrel                                    | Adult         | 22-31 kg    | -      | Y               |
| Kim Y., 1998         | Mongrel                                    | Adult         | 22-37 kg    | -      | Y               |
| Bittner H., 1999     | Mongrel                                    | Adult         | 23-33 kg    | -      | Y               |
| Pig                   |                                            |               |             |        |                 |
| Reference | Year | Breed | Gender | Age | Weight Range | Place of Birth | Inbreeding |
|-----------|------|-------|--------|-----|--------------|----------------|------------|
| Ryan J., 2000<sup>79</sup> | 2000 | Westran<sup>a</sup> | - | 20-50 kg | - | Y | Y |
| Ryan J., 2002<sup>80</sup> | 2002 | Westran<sup>a</sup> | - | 22-57 kg | - | Y | Y |
| Ryan J., 2003a<sup>81</sup> | 2003 | Westran<sup>a</sup> | - | 36-68 kg | - | Y | Y |
| Ryan J., 2003b<sup>60</sup> | 2003 | Westran<sup>a</sup> | - | 36-68 kg | - | Y | Y |
| Ryan J., 2003c<sup>65</sup> | 2003 | Westran<sup>a</sup> | - | 20-57 kg | - | Y | Y |
| Ryan J., 2003d<sup>59</sup> | 2003 | Westran<sup>a</sup> | - | 37.5-63 kg | - | Y | Y |
| Konstantinov I., 2005<sup>15</sup> | 2005 | Yorkshire | - | 26-34.2 kg | M | - | - |
| Hing A., 2009<sup>31</sup> | 2009 | Westran<sup>a</sup> or Landrace<sup>a</sup> | Juvenile | 40-60 kg | - | Y | Y |
| Ali A., 2011<sup>16</sup> | 2011 | Domestic | - | 62 ± 5 kg | F | - | - |
| Watson A., 2013<sup>17</sup> | 2013 | Landrace<sup>a</sup> | Juvenile | 40-60 kg | Mixed gender | Y | Y |
| Steen S., 2016<sup>20</sup> | 2016 | Swedish pigs of native breed | - | 35-63 kg | - | Y | Y |
| Kumar T., 2017<sup>19</sup> | 2017 | - | 6-9 weeks | 20-27 kg | - | Y | Y |

**Table 2 abbreviations:** M – male; F – female; Y – yes

<sup>a</sup>Highly inbred siblings obtained in pairs
**Table 3: Methodological features of BSD development/management**

| Author, Year | Induction | Fluid & drugs | Follow-up | Confirmation criteria |
|--------------|-----------|---------------|-----------|-----------------------|
| **Mouse**    |           |               |           |                       |
| Floechinger B., 2012<sup>27</sup> | 3F BCI over 10 mins | Thymoglobulin (25 mg/kg) bolus at BSD confirmation<sup>a</sup> | 3 hrs | - |
| Atkinson C., 2013<sup>24</sup> | 4F BCI (82 ± 27 μL saline) over 10 mins | Volume resuscitation with 0.9% saline | 3 hrs | Initial blood pressure peak (Cushing reflex), transient spontaneous muscular fasciculation of the rear limbs during brainstem compression, and subsequent absence of spinal reflexes |
| Ritschl P., 2016<sup>23</sup> | No. 3 Fogarty BCI over 15 mins<sup>24,86</sup> | Volume resuscitation with 0.9% saline | 4 hrs | Initial blood pressure peak, subsequent transient spontaneous muscular fasciculations of the rear limbs and the absence of spinal reflexes |
| **Rat**      |           |               |           |                       |
| Votapka T., 1996<sup>18</sup> | Combination of bilateral carotid ligation, and BCI with 0.5 mL saline maintained for 20 mins before release<sup>87,88</sup> | Saline ± T3<sup>a</sup> | 2 hrs | Apnea and loss of deep pain reflexes |
| Wilhelm M., 2000<sup>25</sup> | 3F BCI over 5 mins (200 ± 25 μL) | - | 6 hrs | Isoelectric EEG, apnea, absence of brain stem reflexes |
| Wilhelm M., 2001<sup>29</sup> | BCI Fogarty catheter | - | 6 hrs | Isoelectric EEG, apnea, absence of brain stem reflexes |
| Wilhelm M., 2002<sup>26</sup> | 3F BCI over 5 mins (200 ± 25 μL) | - | 6 hrs | Isoelectric EEG, apnea, absence of brain stem reflexes |
| Author, Year | Description | Maintenance | Time | Outcome |
|--------------|-------------|-------------|------|---------|
| Li S., 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 | 5 hrs | BP stabilization |
| Li S., 2015b | 4F BCI at 15 μL/min to a total volume of 750 μL | Volume resuscitation with fluid (undefined) | 1 or 5 hrs | BP stabilization |
| Spindler R., 2015 | 3F BCI over 1 min (200 μL of saline) | 2ml/hr saline ± n-octanoyl dopamine | 6 hrs | BP stabilization, the loss of corneal reflexes, apnea |
| Hegedus P., 2016 | 4F BCI (15 μL/min to a total volume of 750 μL) | Volume resuscitation with 0.9% saline | 5 hrs | Loss of corneal reflexes, apnea |
| Li S., 2017 | 4F BCI (15 μL/min to a total volume of 600 μL) | Continuous i.v. saline (or n-octanoyl dopamine) after BSD confirmation | 5 hrs | Loss of corneal reflexes, apnea |
| Yip H., 2017 | 4F BCI with 0.5 mL distilled water | Allogeneic adipose-derived mesenchymal stem cells 3 hr post-BSD induction in some groups | 6 hrs | Apnea, Irreversible deep coma (lack of response and reflex to pain), Absence of pupillary reflex (fixed and dilated pupils without reflex to light) |
**Dog**

| Author          | Methodology                                                                 | Duration   | Effects                                                                 |
|-----------------|-----------------------------------------------------------------------------|------------|------------------------------------------------------------------------|
| Shivalkar B., 1993<sup>21</sup> | Sudden intracranial pressure increase – BCI using 4 mL boluses of saline each hour until BSD established<br>Gradual intracranial pressure increase – BCI using an infusion pump to inflate catheter at 4 mL/hr slowly until BSD established. | Up to 4 hrs | Loss of pupillary and corneal reflexes, cerebral perfusion pressure ≤ 0 mmHg, isoelectric EEG |
| Bittner H., 1995<sup>34</sup> | BCI over 2-3 mins (15-18 mL saline)<sup>90</sup> | -          | Loss of pupillary and corneal reflexes, isoelectric EEG, apnea, neuropathology (end of experiment), absence of response to stimuli after cessation of anesthesia |
| Kim Y., 1998<sup>22</sup> | Rapid BCI (15 mL saline)<sup>21</sup> | 1 hr       | Loss of pupillary and corneal reflexes, cerebral perfusion pressure ≤ 0 mmHg |
| Bittner H., 1999<sup>32</sup> | BCI over 2-3 mins (15-18 mL saline)<sup>90</sup> | -          | Loss of pupillary and corneal reflexes, isoelectric EEG, apnea, absence of response to stimuli after cessation of anesthesia |

**Pig**

| Author          | Methodology                                                                 | Duration   | Effects                                                                 |
|-----------------|-----------------------------------------------------------------------------|------------|------------------------------------------------------------------------|
| Ryan J., 2000<sup>79</sup> | 20 cc BCI over 3 mins (20 mL water)                                        | 1 hr       | Hemodynamic changes                                                    |
| Ryan J., 2002<sup>80</sup> | BCI (3mL water/30 secs to a total of 21 mL)                                | 1 hr       | Hemodynamic instability, loss of pupillary and corneal reflexes, absence of response to stimuli after cessation of anesthesia |
| Study | Method | Fluid Details | Inotrope Support | Time to Hemodynamic Instability | Cause of Hemodynamic Instability |
|-------|--------|---------------|-----------------|-------------------------------|----------------------------------|
| Ryan J., 2003a | BCI (3mL water/30 secs to a total of 21 mL) | No additional fluid or inotrope support | Saline (10 mL/kg in first hour, followed by 5 mL/kg/hr) | 1 hr | Hemodynamic instability, loss of pupillary and corneal reflexes, absence of response to stimuli after cessation of anesthesia |
| Ryan J., 2003b | BCI (3mL water/30 secs to a total of 21 mL) | No additional fluid or inotrope support | Saline (10 mL/kg in first hour, followed by 5 mL/kg/hr) | 1 hr | Hemodynamic instability, loss of pupillary and corneal reflexes, absence of response to stimuli after cessation of anesthesia |
| Ryan J., 2003c | BCI (3mL water/30 secs to a total of 21 mL) | No additional fluid or inotrope support | Saline (10 mL/kg in first hour, followed by 5 mL/kg/hr) | 1 hr | Hemodynamic instability, loss of pupillary and corneal reflexes, absence of response to stimuli after cessation of anesthesia |
| Ryan J., 2003d | BCI (3mL water/30 secs to a total of 21 mL) | No additional fluid or inotrope support | Saline (10 mL/kg in first hour, followed by 5 mL/kg/hr) | 1 hr | Hemodynamic instability, loss of pupillary and corneal reflexes, absence of response to stimuli after cessation of anesthesia |
| Konstantinov I., 2005 | BCI to 20 mL | No additional fluid or inotrope support | - | 1.5 hrs | Hemodynamic instability (increased HR and BP), loss of pupillary reflex |
| Hing A., 2009 | BCI over 3 mins (24 mL water) | - | Saline (10 mL/kg in first hour, followed by 5 mL/kg/hr titrated to CVP 0-5 mmHg.) | 6 hrs | Hemodynamic instability, no response to painful stimuli, loss of pupillary, gag, cough and corneal reflexes |
| Author, Year | Procedure | Fluid | Hypotensive Agents | Additional Treatments | Observations |
|--------------|-----------|-------|---------------------|-----------------------|-------------|
| Ali A., 2011<sup>16</sup> | 14F BCI (30 mL saline) | - | i.v. NE (20 μg/mL) to maintain MAP 60-70 mmHg | After 3 hrs BSD - Methylprednisolone, T3, vasopressin | Apnea, hemodynamic instability, EEG monitoring, magnetic resonance imaging of the brain |
| Watson A., 2013<sup>17</sup> | BCI over 3 mins (24 mL water over 3 mins)<sup>79,80</sup> | Saline titrated to maintain CVP 0-5 mmHg. | 1 hr | Hemodynamic instability, intracranial pressure in excess of MAP, absence of brainstem reflexes following cessation of anesthesia |
| Steen S., 2016<sup>20</sup> | Decapitation between the 2nd and 3rd cervical vertebrae<sup>46</sup> | Continuous i.v. infusion of cocaine, NA, A, cortisol, T3, thyroxine, desmopressin started after 30 mins BSD | 24 hrs | Hemodynamic changes, hormonal dysregulation and plasma catecholamine levels (postexperiment) |
| Kumar T., 2017<sup>19</sup> | Carotid artery ligation and BCI (12 or 14F Foley catheter, 25 cc saline over 20 sec) | Saline throughout | Inotrope support where required to maintain normal BP | 18 hrs | - |
T3 (0.2 ug/kg/dose, 3 doses every hr from 12 hrs post-BSD induction in half of the donors + 1 mg/kg hydrocortisone)

**Table 3 abbreviations:** BCI – Balloon catheter inflation; EEG – electroencephalogram; BP – blood pressure; BSD – brain stem death; HR – heart rate; CVP – central venous pressure; NE – norepinephrine; MAP – mean arterial pressure; NA – noradrenaline; A – adrenaline; T3 – triiodothyronine

*aSpecific to study intervention groups.*
| Author, Year                  | Preservation | Method       | Solution                        | Specific technique/details                             | Total Ischemic Time | Recipient Support                                      | Duration of reperfusion |
|------------------------------|--------------|--------------|----------------------------------|--------------------------------------------------------|---------------------|--------------------------------------------------------|--------------------------|
| **Mouse**                    |              |              |                                  |                                                        |                     |                                                        |                          |
| Floerchinger B., 2012\(^{27}\) | CSS          |              | Heparinized saline              | Corry et al., 1973 Nonworking model\(^{95}\)           |                     |                                                        | 72 hrs                   |
|                              |              |              | (abdominal)                     |                                                        | 95                  | Phosphate buffered saline (± respective study treatment)|                          |
| Atkinson C., 2013\(^{24}\)   | CSS          | 35 mins      | Phosphate buffered saline       | Corry et al., 1973 Nonworking model\(^{95,96}\)       | 65 mins             | Phosphate buffered saline                               | 48 hrs                   |
|                              |              |              | (abdominal)                     |                                                        |                     |                                                        |                          |
| Ritschl P., 2016\(^{23}\)    | CSS          | 20 mins      | Custodial solution              | Oberhuber et al., 2014\(^{97}\)                      | 35 mins             | 0.3 mL saline postoperatively i.p.                      | 20 hrs                   |
|                              |              |              | (cervical)                      |                                                        |                     |                                                        |                          |
|                              |              |              |                                  | Using modified cuff technique, technique modified from Maruyama et al., 1991\(^{98}\) |                     |                                                        |                          |
| Rat                          |              |              |                                  |                                                        |                     |                                                        |                          |
| Votapka T., 1996\(^{18}\)    | CSS          |              | Saline + 20 mEq/L potassium     | Maruyama et al., 1994\(^{99,100}\)                    |                     |                                                        | 48 hrs                   |
|                              |              |              | (abdominal)                     | Working heart model                                    |                     |                                                        |                          |
| Wilhelm M., 2000\(^{25}\)    | CSS          | ≈ 2-3 mins   | Saline                          |                                                        | ≈ 25 mins           |                                                        | Up to 19 days            |
| Authors | Year | CSS | Technique | Positioning | Treatment | Time Period |
|---------|------|-----|-----------|-------------|-----------|-------------|
| Wilhelm M., 2001 | 29 | - | Heterotopic (-) | Abdominal | - | Cyclosporine (5 mg/kg/day for 30 days then every other day) |
| Wilhelm M., 2002 | 26 | CSS | Heterotopic (-) | - | - | Cyclosporine (5 mg/kg/day) |
| Li S., 2015a | 82 | CSS | Custodial Heterotopic (abdominal) | - | - | Crystalloid volume substitution (Ringer’s solution) |
| Li S., 2015b | 83 | CSS | Custodial Heterotopic (abdominal) | - | - | Crystalloid volume substitution (Ringer’s solution) |
| Spindler R., 2015 | 35 | CSS | Heterotopic (abdominal) | - | - | Peter Terness method |
| Hegedus P., 2016 | 84 | CSS | Custodial Heterotopic (abdominal) | - | - | Crystalloid volume substitution (Ringer’s solution) |
| Li S., 2017 | 85 | CSS | Custodial Heterotopic (abdominal) | - | - | - |
| Study                  | Method         | Time 1 | Time 2 | Time 3 | Time 4 | Time 5 |
|-----------------------|----------------|--------|--------|--------|--------|--------|
| Yip H., 2017<sup>28</sup> | Heterotopic (cervical) | (adapted from Ono et al., 1969<sup>102</sup>) | Yip et al, 2017<sup>28</sup> | - | - | 5 days |
| Dog                   |                |        |        |        |        |        |
| Shivalkar B., 1993<sup>21</sup> | CSS | 55 ± 7 mins | NIH | Orthotopic (thoracic) | - | 240 ± 20 mins | Isoprenaline 0.5 g calcium chloride 250 mg solumedrol 20 mg xylocaine 0.8 M bicarbonate as required | 1 hr (post-CPB weaning) |
| Bittner H., 1995<sup>34</sup> | CSS | - | St Thomas' | Orthotopic (thoracic) | Bicaval technique | 1.5 or 4 hours | 1 mg NA bolus as needed on CPB Oral Cyclosporine (10 mg/kg), Oral azathioprine (2 mg/kg), methylprednisolone (25 mg/kg i.v.) | 1 hr (post-CPB weaning) |
| Kim Y., 1998<sup>22</sup> | CSS | 4 hrs | NIH-2 | Orthotopic (thoracic) | Biatrial technique | N/A | Ventricular pacing 110 bpm methylprednisolone (5 mg/kg) | 1 hr (post-CPB weaning) |
| Bittner H., 1999<sup>32</sup> | Warm (40°C) static storage | 40°C Preservation solution (formulated at Duke) | Orthotopic (thoracic) | Bicaval technique | 2-4 hrs (depending on group) | Oral Cyclosporine (10 mg/kg) Oral azathioprine (2 mg/kg) methylprednisolone (25 mg/kg i.v.) | 1 hr (post-CPB weaning) |
| Pig | Ryan J., 2000<sup>79</sup> | CSS  | - | Cold cardioplegia supplemented with HCl, Intralipid, U74389G | Orthotopic (thoracic) | Lower and Shumway, 1960<sup>33</sup> | 6 hrs | Dobutamine (10-20 ug/kg/min) 45 mins after reperfusion | Up to 6 hrs (post-CPB weaning) |
|-----|--------------------------|------|---|-------------------------------------------------------------|----------------------|-------------------------------------|------|------------------------------------------------|-----------------------------------|
|     | Ryan J., 2002<sup>80</sup> | CSS  | - | St Vincent's Cold crystalloid cardioplegia | Orthotopic (thoracic) | Lower and Shumway, 1960<sup>33</sup> | 4, 6 or 14 hr | Dobutamine (10 ug/kg/min) 45 mins after reperfusion | Pacing – VVI 120 bpm |
|     |                          |      |   | St Vincent's Cold crystalloid cardioplegia | Orthotopic (thoracic) | Lower and Shumway, 1960<sup>33</sup> | 4 hrs and 14 hrs | Dobutamine (10-20 ug/kg/min) 45 mins after reperfusion | Pacing – VVI 120 bpm |
|     |                          |      |   | St Vincent's Cold crystalloid cardioplegia | Orthotopic (thoracic) | Lower and Shumway, 1960<sup>33</sup> | 4 hrs and 14 hrs | Dobutamine (10-20 ug/kg/min) 45 mins after reperfusion | Pacing – VVI 120 bpm |

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| Ryan J., 2003b<sup>60</sup> | CSS | St Vincent's | Orthotopic (thoracic) | Lower and Shumway, 1960<sup>33</sup> | 4 hrs |
|-----------------------------|-----|-------------|----------------------|---------------------------------|-------|
|                             |     | crystalloid cardioplegia |                     |                                 |       |
|                             |     |                        | Dobutamine (10 μg/kg/min) 45 mins after reperfusion | Up to 3 hrs (post-CPB weaning) |
|                             |     |                        | Pacing – VVI 120 bpm |                                 |
|                             |     |                        | methylprednisolone (500 mg) on anaesthetic induction and 15 mins before reperfusion | |

| Ryan J., 2003c<sup>65</sup> | CSS | St Vincent's | Orthotopic (thoracic) | Lower and Shumway, 1960<sup>33</sup> | 6 hrs |
|-----------------------------|-----|-------------|----------------------|---------------------------------|-------|
|                             |     | crystalloid cardioplegia |                     |                                 |       |
|                             |     |                        | Dobutamine (10 μg/kg/min) 45 mins after reperfusion | Up to 3 hrs (post-CPB weaning) |
|                             |     |                        | Pacing – VVI 120 bpm |                                 |
|                             |     |                        | methylprednisolone (500 mg) on anaesthetic induction and 15 mins before reperfusion | |
| Study | Methodology | Cardioplegia | Pacing | Reperfusion | Additional Treatments |
|-------|-------------|--------------|--------|-------------|----------------------|
| Ryan J., 2003d | CSS - | St Vincent’s Cold crystalloid cardioplegia (supplemented with UG4389 G ± cariporide or vehicle) | Orthotopic (thoracic) | Lower and Shumway, 1960 | 14 hrs |
| Konstantinov I., 2005 | CSS 1 hr | Crystalloid | Orthotopic (thoracic) | Biatrial technique | 120 mins |

- Saline (10 mL/kg in first hour, followed by 5 mL/kg/hr)
- Dobutamine (10-20 ug/kg/min) 45 mins after reperfusion
- Pacing – VVI 120 bpm
- Methylprednisolone (500 mg) on anesthetic induction and 15 mins before reperfusion
- Saline (10 mL/kg in first hour, followed by 5 mL/kg/hr)

- Did not wean animals. Performed LAD ligation and reperfusion on cardiac allograft while on bypass
| Authors           | Year | CSS | Time | Temperature | Blood Flow | Pacing | Sedation | Inotropic Support | Other Medicine | Additional Details |
|-------------------|------|-----|------|-------------|------------|--------|----------|-------------------|----------------|------------------|
| Hing A., 2009     |      | CSS |      | Celsior ± glyceryl trinitrate and/or cariporide | Orthotopic (thoracic) | Lower and Shumway, 1960 | 14 hrs | Dobutamine (10-20 ug/kg/min) 45 mins after reperfusion | methylprednisolone (500 mg IV at anesthetic induction and 15 mins prior to reperfusion) | Saline (10 mL/kg in first hour, followed by 5 mL/kg/hr titrated to CVP 0-5 mmHg. Up to 3 hrs (post-CPB weaning)) |
| Ali A., 2011      |      | CSS |      | Crystallloid + lidocaine | Orthotopic (thoracic) | Biatrial technique | - | Dobutamine (2.5 ug/kg/min) | Immediately after CPB weaning measurements collected (CPB weaning after 30 mins reperfusion) |
| Watson A., 2013   |      | CSS | 210 mins | Celsior ± erythropo | Orthotopic (thoracic) | Lower and Shumway, 1960 | 5 hrs | Dobutamine (10-20 ug/kg/min) after 45 mins reperfusion | Up to 3 hrs (post- |
| Study Authors, Year | Study Design | Duration | Technique | Solution | Time | Treatments |
|---------------------|--------------|----------|-----------|----------|------|------------|
| Steen S., 2016<sup>20</sup> | CSS vs. cold (8°C) machine perfusion | 24 hrs | Orthotopic (thoracic) Biatrial technique | Krebs solution (3 ml/kg/hr) | 24 hrs | Adrenaline (10 ug boluses and i.v. 0.05 ug/kg/min) for first 6 hours methylprednisolone (1g) |
| Kumar T., 2017<sup>19</sup> | CSS Custodial | 3-3.5 hrs | Biatrial technique | MP (10 mg/kg) Dopamine, epinephrine, atropine, lidocaine, furosemide, 8.4% sodium bicarbonate | 2-3 hrs | Noradrenaline (as needed) Pacing – VVI 120 bpm methylprednisolone (500 mg) Saline titrated to maintain CVP 0-5 mmHg |

<sup>a</sup>Specific to study intervention groups

**Table 4 abbreviations:** CSS – cold static storage; NIH – National Institute of Health; CPB – cardiopulmonary bypass; NA – noradrenaline; VVI – ventricular demand pacing; LAD – left anterior descending coronary artery; CVP – central venous pressure
| Study | DONOR | RECIPIENT (Graft) |
|-------|-------|-------------------|
| **Mouse** | | |
| Floerchinger B., 2012<sup>27</sup> | - | - |
| Atkinson C., 2013<sup>24</sup> | MAP | Manual palpation for graft function |
| Ritschl P., 2016<sup>23</sup> | MAP | - |
| **Rat** | | |
| Votapka T., 1996<sup>18</sup> | - | Manual palpation for graft function |
| Wilhelm M., 2000<sup>25</sup> | MAP | LV PVR analyses |
| Wilhelm M., 2001<sup>29</sup> | - | Graft survival |
| Wilhelm M., 2002<sup>26</sup> | - | - |
| Li S., 2015<sup>82</sup> | LV PVR analyses | LV PVR analyses |
| Li S., 2015<sup>83</sup> | LV PVR analyses | LV PVR analyses |
| Spindler R., 2015<sup>35</sup> | MAP and HR | - |
| Hegedus P., 2016<sup>84</sup> | LV PVR analyses | LV PVR analyses |
| Li S., 2017<sup>85</sup> | LV PVR analyses | LV PVR analyses |
| Yip H., 2017<sup>28</sup> | %LVEF (TTE) | %LVEF (TTE) |
| **Dog** | | |
| Shivalkar B., 1993<sup>21</sup> | LVP, MAP, HR, CO, dP/dt, electrocardiogram | Successful weaning from bypass, MAP, CO, LVP, dP/dt, electrocardiogram |
| Bittner H., 1995<sup>34</sup> | LV and RV PVR analyses | LV and RV PVR analyses |
| Authors                | Year  | Measurements/Analyses                                                                 | Results                                      |
|-----------------------|-------|---------------------------------------------------------------------------------------|----------------------------------------------|
| Kim Y., 1998          | 22    | MAP, CVP, CO, pulmonary artery pressure, PCWP, HR                                     | LV PVR analyses                              |
| Bittner H., 1999      | 32    | LV and RV PVR analyses                                                               | LV and RV PVR analyses                       |
| Pig                   |       |                                                                                      |                                              |
| Ryan J., 2000         | 79    | LV and RV PVR analyses                                                               | LV and RV PVR analyses                       |
| Ryan J., 2002         | 80    | LV PVR analyses                                                                      | Successful weaning from bypass              |
| Ryan J., 2003a        | 81    | -                                                                                    | Successful weaning from bypass              |
| Ryan J., 2003b        | 60    | LV PVR analyses                                                                      | Successful weaning from bypass              |
| Ryan J., 2003c        | 65    | LV PVR analyses                                                                      | LV PVR analyses                              |
| Ryan J., 2003d        | 59    | LV PVR analyses                                                                      | Successful weaning from bypass              |
| Konstantinov I., 2005 | 15    | Blood pressure and HR                                                                 | LV PVR analyses                              |
| Hing A., 2009         | 31    | LV PVR analyses                                                                      | MAP, CO, LAD coronary flow                   |
|                       |       | MAP, CO, LAD coronary flow                                                           | Successful weaning off bypass               |
| Ali A., 2011          | 16    | LV and RV PVR analyses                                                               | MAP, CO, LAD coronary flow                   |
|                       |       | Cine cardiac magnetic resonance imaging to assess biventricular chamber volumes and function | Successful weaning off bypass               |
| Source: Watson A., 2013<sup>17</sup> | LV PVR analyses | Cine cardiac magnetic resonance imaging to assess biventricular chamber volumes and function |
| --- | --- | --- |
| Source: Steen S., 2016<sup>20</sup> | Left atrial pressure, pulmonary artery pressure, CVP, aortic pressure | Successful weaning off bypass and ability to maintain CO for duration of study |
| Source: Kumar T., 2017<sup>19</sup> | Hemodynamic parameters (nonspecific), %LVEF (TTE) | LV PVR analyses |

**Table 5 abbreviations:**
- MAP – Mean arterial pressure
- LV – left ventricle
- PVR – pressure volume relationship
- HR – heart rate
- CO – cardiac output
- dP/dt – rate pressure change
- CVP – central venous pressure
- PCWP – pulmonary capillary wedge pressure
- LAD – left anterior descending artery
- %LVEF - % left ventricular ejection fraction
- TTE – transthoracic echocardiograph
Figure 1