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Rat Heterotopic Abdominal Heart/Single-Lung Transplantation in a Volume-Loaded Configuration.

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Abstract:	Herein, we describe a novel technique for heterotopic abdominal heart-lung transplantation (HAHLT) in rats. The configuration of the transplant graft involves anastomosis of donor inferior vena cava (IVC) to recipient IVC, and donor ascending aorto (Ao) to recipient abdominal Ao. The right lung is preserved and functions as a conduit for blood flow from right heart to left heart. There are several advantages to using this technique, and it lends itself to a broad range of applications. Because the graft is transplanted in a volume-loaded configuration, cardiac function may be directly assessed in vivo. The use of pressure-volume conductance catheters permits characterization of load-dependent and load-independent hemodynamic parameters. The graft may be converted to an unloaded configuration by applying a clamp to its IVC inflow. We describe modified surgical techniques for both donor and recipient operations, and an ideal myocardial protection strategy. Depending on the experimental aim, this model may be adapted for use in both acute and chronic studies of graft function, immunologic status, and variable ventricular loading conditions. The conducting airways to the transplanted lung are preserved, and allow for acute lung re-ventilation. This enables the study of heart-lung interactions in the transplant graft, and the effects of the mixed venous and arterial blood providing coronary perfusion to the graft. A limitation of this model is its technical complexity. There is a significant learning curve for new operators, who should ideally be mentored in the technique. A surgical training background is advantageous for those wishing to apply this model. Despite its complexity, we aim to present the model in a clear and easily applicable format. Because of the physiologic similarity of this model to orthotopic transplantation, and its broad range of study applications, the effort invested in learning the technique is likely

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Dear Sir/Madam,

Thank you for considering our work for publication.

Our team has developed a unique method of heterotopically transplanting heart/single-lung grafts into the abdomens of recipient rats. We have made important modifications to the donor and recipient operations, as well as to the myocardial protection protocol. Furthermore, our model provides a physiologic base for studying many unique aspects of heterotopic heart-lung transplant, not the least of which is the capacity for direct *in vivo* cardiac functional assessment. Our model is described at length in the manuscript.

Mark J. Kearns and Yingjin Wang performed all technical aspects of the work. Mark J. Kearns wrote the manuscript. John H. Boyd is Mark's PhD supervisor and together, they developed the model, and its applications, described in the manuscript.

I have been in contact with Nandita Singh throughout the submission process.

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I look forward to hearing from you.

Sincerely,

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SHORT ABSTRACT:
We describe a novel technique for heterotopic abdominal heart-lung transplantation (HAHLT) in rats. The transplant configuration results in a partially loaded graft circulation, allowing direct functional assessment. This model may be employed for acute or chronic studies of function and immunologic status of the transplanted graft.

LONG ABSTRACT:
Herein, we describe a novel technique for heterotopic abdominal heart-lung transplantation (HAHLT) in rats. The configuration of the transplant graft involves anastomosis of donor inferior vena cava (IVC) to recipient IVC, and donor ascending aorta (Ao) to recipient abdominal Ao. The

right lung is preserved and functions as a conduit for blood flow from right heart to left heart.

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A limitation of this model is its technical complexity. There is a significant learning curve for new operators, who should ideally be mentored in the technique. A surgical training background is advantageous for those wishing to apply this model. Despite its complexity, we aim to present the model in a clear and easily applicable format. Because of the physiologic similarity of this model to orthotopic transplantation, and its broad range of study applications, the effort invested in learning the technique is likely to be worthwhile.

INTRODUCTION:

The first rodent model of heterotopic abdominal heart transplantation (HAHT) was described by Abbott and colleagues in 1964[1]. This technique, and subsequent modifications have been widely applied to characterize transplant graft function and immunologic status. The majority of HAHT techniques described involve a non-volume loaded heart[2,3]. Models of HAHT involving volume-loaded ventricles have been described, but they are frequently limited in one or more respects.

Heterotopic abdominal heart-lung transplantation (HAHLT) with a volume-loaded left ventricle (LV) has been described previously. Chen and colleagues[4], and subsequently Ibrahim and colleagues[5] described HAHLT with a single aorto-aortic (donor ascending to recipient abdominal aorta) anastomosis. The only volume load presented to the ventricle in this circulation is the coronary venous return. Asfour and colleagues described a HAHT technique in which the lung circuit was eliminated by anastomosing donor pulmonary artery (PA) to donor left atrium (LA)[6]. In this circulation, venous inflow to right ventricle (RV) occurs via a donor SVC to recipient IVC anastomosis, and the subsequent LV load is ejected into the aorto-aortic anastomosis. Cardiac function was partially assessed *in vivo*, and also *in vitro* using a Langendorff rig. Figueiredo and colleagues described a HAHLT model similar to our own[7], but in mice. Venous inflow to the RV occurs via donor SVC to recipient IVC anastomosis. Blood subsequently passes through the single lung circulation and LV load is ejected into the aorto-aortic anastomosis. Cardiac function in their study was assessed by magnetic resonance imaging (MRI). Wen and colleagues described a unique HAHT technique in which the LV is loaded by means of a recipient aorta to

donor LA anastomosis[8]. The LV, therefore, fills at systemic pressures. Cardiac function, and whether LV stroke volume is ejected antegradely in their model was not assessed.

Many of the techniques referenced above involve non-physiologic LV loading conditions, including the techniques whose partial LV load is represented only by coronary venous return. On the other hand, many techniques do approach physiologic LV loading. The majority of these techniques, as with the technique of Asfour and colleagues, omit the pulmonary circulation and utilize a donor PA to donor LA anastomosis[6,9]. The circulation described by Galinanes and colleagues[10] employs a direct recipient cava to donor LA anastomosis, omitting the pulmonary circulation and the right heart. Yokoyama and colleagues achieve the same effect by ligating the donor PA and creating an interatrial communication in the donor heart (omitting donor lung and right heart circulations)[11]. The circulation of Maruyama and colleagues[12] involves an anastomosis between donor left PA and recipient Ao, which permits LV filling via the pulmonary circulation as a conduit, but effectively excludes the right heart.

In cases where near physiologic loading conditions were met, we advance the technique of HAHLT in 2 major respects. First, to our knowledge, the exact configuration we report has not been described in rats. It is possibly the most versatile circulation for investigators wishing to study the physiology, structure, and immunology of the transplanted heart-lung graft. Second, we describe how the function of the transplant graft can be directly characterized *in vivo.* For this application, pressure-volume conductance catheters can be introduced directly into the LV apex of the transplant graft, which allows for complete cardiac functional characterization.

The technique described here can be applied to both acute and chronic studies of transplant graft function, while the functional assessment may be performed either *in vivo* or *in vitro*. We present a model in which the loading conditions are near physiologic, however the degree of ventricular loading may be manipulated both acutely and chronically by diverting venous return towards or away from the graft. Afterload conditions can also be manipulated. Because the lung and its airway are retained in this transplant configuration, investigators can re-ventilate the donor lung acutely. This allows characterization of transplant heart-lung interaction. Uniquely, lung re-ventilation also changes the composition of blood perfusing the transplant coronary arteries. Under non-ventilated conditions, blood ejected from the donor aorta is deoxygenated, and mixes with oxygenated blood in the recipient aorta. Under acutely ventilated conditions, ejected blood becomes oxygenated. Transplant graft function can be compared under ventilated and non-ventilated conditions, and also under variably stressed conditions.

The protocol below describes important modifications to previously described HAHLT donor and recipient operations. It also describes an optimal technique for protecting the transplant graft throughout the period of cold ischemia (time between donor explant and recipient implant). Advantages of this technique include physiologic conditions similar to an orthotopically transplanted graft, and a wide range of investigative applications. An important limitation is its technical complexity. With adequate mentoring and practice, the advantages of this technique will likely outweigh the challenges in adopting it.

PROTOCOL:
All animals were housed and cared for in accordance with National and Institutional guidelines for the care and use of laboratory animals. Ethics approval for this protocol was granted by the University of British Columbia's Animal Care Committee. Male, Sprague-Dawley rats weighing between 250-400g were used for this protocol.

1. Donor Operation

1.1) Have a large bucket of ice available. Place a 20mL syringe of sterile normal saline (NS) and a 10mL syringe of cardioplegic solution on ice. A blunt metal cannula (at least 16-Gauge) should be attached to the syringe containing cardioplegia.

1.2) To visualize structures adequately, use either a pair of surgical loupes or a dissecting microscope.

NOTE: We currently use surgical loupes with 3.5X magnification, and a binocular operating microscope with 3.4-21.3X magnification.

1.3) Keep a stack of surgical gauze in the ice bath (to be used later for topical myocardial cooling).

1.4) Place the donor in an anesthetic chamber and induce anesthesia with 4-5% isoflurane.

1.5) Transfer the rat to an operating platform and maintain anesthesia by nose-cone with 1-2% isoflurane. Apply vet ointment to the animal's eyes to prevent dryness. Administer ketamine (80mg/Kg), midazolam (2mg/Kg), and unfractionated heparin (500 IU) intra-peritoneally with a 25 Gauge needle.

1.6) Using surgical clippers, shave the donor from xiphisternum to mandible and prep the area with a povidone-iodine or chlorexidine based solution. Infiltrate the incisional sites with 0.1-0.5% lidocaine subcutaneously.

1.7) Secure the forelimbs and left hindlimb to the operating platform with adhesive tape, leaving the right hindlimb free for monitoring of anesthetic depth and vital signs.

1.8) After ensuring appropriate anesthetic depth by pedal pinch, tracheotomize the donor as follows:

1.8.1) Make a midline incision in the soft tissues between the jugular notch and mandible using Metzenbaum scissors. Penetrate the capsule of the thyroid gland in the midline using iris scissors, and separate its lobes using blunt forceps dissection.

1.8.2) Using blunt forceps dissection, separate the strap muscles of the neck in the midline to expose the anterior surface of the trachea.

1.8.3) Use a baby Lauer to bluntly dissect a circumferential plane around the trachea. Encircle the trachea with a 3-0 silk tie.

1.8.4) Using iris or tenotomy scissors, make a transverse incision in the anterior trachea, approximately 5mm inferior to the thyroid cartilage. Gently introduce the tracheal cannula (a 14-Gauge, blunt-tipped metal cannula) and secure it in place using the 3-0 silk tie.

1.8.5) Connect the tracheal cannula to a mechanical ventilator. Redirect the flow of oxygen and isoflurane through the ventilator circuit, and ventilate the donor at a rate and tidal volume predicted by its weight[9].

1.9) Expose the xiphisternum inferiorly by making a midline incision in the anterior abdominal wall.

1.10) Staying in the midline, Perform a median sternotomy using Metzenbaum scissors or a bone cutter. Retract the edges of the sternum using a self-retaining retractor. Enter the pericardium and pleural cavities.

1.11) Perform a thymectomy. It is easiest to first bluntly divide the thymus in the midline, and then separate it from surrounding structures using a combination of blunt and sharp dissection.

NOTE: The origin of the internal thoracic arteries may be injured when dissecting the thymus away from the superior sternal edges. To prevent bleeding, hemostatic clips may be applied before removing the thymus at these points.

1.12) Dissect the left vena cava free from surrounding structures and ligate it proximally and distally (with silk ties or surgical clips). Resect the intervening portion of cava to expose the aortic arch and left subclavian artery.

1.13) Using a baby Lauer, circumferentially free the aortic arch vessels, the superior vena cava (SVC) and inferior vena cava (IVC). Obtain as much length on the IVC as possible. Place a marking suture (7-0 or 8-0 prolene) distally on the anterior surface of the IVC to help orient the vessel during the recipient operation.

1.14) Once all the dissection is complete, prepare for an expedient but gentle harvest.

1.15) Ligate each of the aortic arch vessels with surgical clips. Next, ligate the IVC by placing a clip just above the diaphragm. Cannulate the SVC with a 24-26-Gauge intravenous (IV) catheter and connect it to the syringe containing ice-cold NS. Secure the IV cathteter in place with a 4-0 silk tie. An assistant is helpful to stabilize the IV catheter.

1.16) Next, transect the aortic arch distal to the left subclavian artery and flush the heart-lung circulation with cold NS. Approximately 10-15mL of NS is required over 10-20 seconds, until the

aortic run-off appears dilute, and the heart arrests. Euthanize the animal by exsanguination. Transect the SVC proximal to the IV cannula, then the IVC distal to the marking suture.

1.17) Following graft removal, cannulate the aortic arch *in situ,* using the 16-Gauge cannula attached to the cardioplegia syringe. Once it is cannulated, secure the cannula with a 4-0 silk tie.

1.18) Attempt to de-air the aorta prior to this step. Infuse cold cardioplegia into the aortic cannula. Initially infuse 5-10mL of cold cardioplegia over 30-45 seconds, applying gentle pressure. This step will flush the coronary arteries, cool the heart uniformly, and result in a prolonged cardioplegic arrest.

NOTE: A benefit to having the aortic cannula attached is that cardioplegia may be re-administered every 20 minutes or as desired, and at convenient intervals throughout the operation. We typically re-dose cardioplegia in 5mL boluses over 30-45 seconds.

1.19) Turn off the ventilator and disconnect the tracheal cannula. Transect the trachea proximally.

1.20) Grasp the transected trachea with forceps, and remove the heart and lungs en-bloc. This will require gentle traction with sharp dissection as the heart-lung graft is removed. Separate the trachea from the underlying esophagus.

2. Preparation of the Heart-lung Graft

NOTE: while completing this portion of the procedure, an assistant should be anesthetizing the recipient rodent and preparing for graft implantation.

2.1) Place the heart-lung graft on cold surgical gauze (taken from the ice bucket). Ligate the trachea proximally with a surgical clip.

2.2) Next, expose the left mainstem bronchus using blunt dissection, and ligate it proximally with a clip. Transect the left bronchus distal to the clip using iris scissors. Perform a left pneumonectomy by ligating the left pulmonary artery and veins with 3-0 silk ties. Transect distal to the ligature and remove the left lung.

2.3) Prepare the IVC for anastomosis by cleaning off any surrounding fat. It is usually necessary to remove some of the length of the IVC, and a clean oblique cut will help optimize the orientation of the implant. If the marking suture is removed in this process, it is helpful to replace it.

2.4) Prepare the ascending aorta by ensuring that it is circumferentially freed for anastomosis. Leave the aortic arch intact for ease of cardioplegia delivery, and resect it only at the time of aortic anastomosis.

2.5) At this point, the SVC IV catheter is left free and disconnected from any syringes or tubing. When the implant is almost complete the SVC can be ligated, but until then it will serve to vent away any coronary perfusate.

2.6) Place the heart in cardioplegic solution on ice or at 4 ^{o}C.

3. Recipient Operation

3.1) Anesthetize the recipient as per the donor operation, above. Use vet ointment to protect the animal's eyes from dryness. Confirm anesthesia frequently by pedal pinch.

3.2) Position the animal as per the donor operation. Shave and prep the abdomen from xiphisternum to penis.

3.3) Tracheotomize and ventilate the recipient as directed above. Alternatively, maintain the recipient under nose-cone anesthesia using volatile anesthetics.

3.4) Introduce a catheter into the femoral vein as demonstrated by Jespersen and colleagues[10].

NOTE: It is useful to have venous access for the purpose of volume resuscitation.

3.5) Perform a laparotomy by making a midline abdominal incision with Metzenbaum scissors from xiphisternum to penis. Retract the abdominal wall using a self-retaining retractor. Next, retract the bowels superiorly and to the left side. Wrap them in warm, NS-soaked gauze.

3.6) Expose the IVC and abdominal aorta by sharply dissecting through the overlying retro-peritoneal fat.

NOTE: Some operators recommend using cotton-tipped applicators to bluntly dissect around the IVC and aorta, but this can result in significant vessel spasm. In our experience, it is preferable to avoid directly touching the vessels as much as possible. The cava is also very fragile.

3.7) Have two vessel clamps available and ready. Clamps may consist of gentle metal bulldog-style clamps, thin silastic bands, surgical clips that can be removed without injuring the vessels, or simple silk ties. If using silk ties, they should be applied in a configuration that can be easily released. Employ both bulldog and Yasargil clamps for vascular control.

3.8) Circumferentially free the IVC and aorta only at the positions that clamps will be applied. There should be approximately 2-3cm of space between the clamp sites.

3.9) Identify any posterior branches on both IVC and aorta, and ligate them. If desired, the

branches may be clamped temporarily. Apply proximal and distal clamps (in that sequence) to the IVC and aortic clamp sites.

3.10) Make a small longitudinal incision in the anterior wall of the IVC with an 11-blade scalpel. The vessel should collapse after expelling its contents. If it continues to bleed, check the clamps, and search for any unidentified perforating branches (and ligate them).

3.11) Extend the incision with Potts scissors to match the length of the donor IVC orifice. If desired, administer another dose of cold cardioplegia.

3.12) Wrap the donor graft in cold surgical gauze. Position it in the abdomen, to the left of the cava.

3.13) Secure the heel and toe ends of the anastomosis with 8-0 prolene suture. Tie a secure knot at each end, leaving the needle attached to a long arm of suture, and a short end of suture to be tied to later.

3.14) In running fashion, complete one-half of the suture line and tie to the opposing short suture arm.

3.15) Complete the other half of the suture line and tie it down. Apply a gentle clamp to the donor IVC, above the newly created anastomosis.

3.16) Release the distal clamp and check for bleeding at the suture lines. If there is bleeding, repair the site with a single or figure-of-eight stitch (using 8-0 prolene).

3.17) After hemostasis is achieved, re-apply the distal clamp. Leave the donor IVC clamp in place. If desired, administer a final dose of cold cardioplegia into the aortic arch.

3.18) Using iris scissors, make a slightly oblique cut in the distal ascending aorta of the graft (just proximal to the innominate artery). Ensure that the aorta is prepared for anastomosis by clearing away any peri-aortic fat, and providing adequate separation between the aorta and pulmonary artery.

3.19) Gently adjust the heart-lung graft so that it lies to the right of the aorta.

3.20) Make a small longitudinal incision in the anterior abdominal aorta with an 11-blade scalpel. If there is ongoing bleeding, troubleshoot as described in 3.10. Secure the heel and toe of the anastomosis with 8-0 prolene suture as described in 3.13.

3.21) In running fashion, complete one half of the suture line and tie to the opposing short suture arm. It is easiest to complete the medial suture line first, as subsequent access would be difficult. Complete the other half of the suture line, but do not tie it down yet.

3.22) Pre-fill the donor heart-lung circuit with fluid (room temperature NS). This is critical because it will a) de-air the graft, and b) maintain the volume status of the recipient after releasing all clamps.

NOTE: The chambers of the heart and the pulmonary vasculature can hold a significant volume of fluid and air.

3.23) Connect the SVC IV catheter to a 20mL syringe of room temperature NS, and infuse its contents slowly over 10-20 seconds, until NS can be seen leaking from the aortic anastomosis.

3.24) Ligate the SVC with a surgical clip, and resect the portion connected to the IV catheter. Gently place a clamp on the ascending aorta to contain the volume within the heart-lung circuit.

3.25) Before finally tying down the aortic anastomosis, release the distal clamp and allow back-bleeding to de-air the aorta and anastomosis. Tie down the aortic anastomosis.

3.26) Check for bleeding at the suture lines, and if needed, repair sites of bleeding as described in 3.16. Release the proximal clamp and ensure hemostasis.

3.27) With clamps released, the donor heart should resume beating within minutes.

3.28) Pay close attention to the recipient's vital signs and administer volume resuscitation as needed. After a reperfusion period of 20-30 minutes, the donor IVC may be unclamped.

3.29) Depending on the experimental endpoints, either close the animal's abdomen and let it recover from anesthesia or prepare animal for heart-lung assessment for acute experiments,.

NOTE: Examples of graft assessment include *in vivo* measures of load-dependent and load-independent hemodynamics, *in vitro* measures of function in Langendorff and working heart modes, and (in survival surgery) echocardiographic or MRI investigations.

3.30) At the end of acute terminal experiments, animals are euthanized by exsanguination.

REPRESENTATIVE RESULTS:

The HAHLT technique described above is highly technical and requires close attention to detail. **Table 1** highlights some of the key factors associated with successful versus unsuccessful procedures, and can be used as a guide for troubleshooting technical difficulties.

After the recipient aorta is unclamped, the graft coronary arteries should be seen to fill with oxygenated blood. Accordingly, the myocardium should become pink and well perfused. In technically successful experiments, the heart will begin to beat shortly after graft reperfusion. The graft should be left in an unloaded state (with donor IVC clamped) for a period of at least 20-30 minutes to allow for functional recovery. Following that, the graft's loading conditions

may be altered to suit experimental aims. More quantitative measures of graft function (and a successful outcome) can be employed as desired. As noted in the protocol, *in vivo* and *in vitro* functional studies, as well as echocardiographic and MRI investigations can provide such information. **Figures 1** and **2** are examples of *in vitro* baseline and preload-occlusion pressure-volume data that can be derived acutely with this methodology. Hemodynamic data from these studies can provide investigators with cardiac output, stroke volume, end-systolic and end-diastolic volumes, heart rate, ejection fraction, end-systolic elastance, and preload recruitable stroke work.

Figure 1: Baseline pressure-volume data.
Figure 1 shows baseline pressure-volume data from an *in vitro* working heart assessment of cardiac function.

Figure 2: Preload-occlusion pressure-volume data.
Figure 2 shows preload-occlusion pressure-volume data from an *in vitro* working heart assessment of cardiac function.

Table 1: Characteristics of successful versus unsuccessful HAHLT procedures.
Table 1 provides examples of factors associated with successful and unsuccessful donor, graft, and recipient operations.

DISCUSSION:
Success with the technique described here will be predicated on several factors. Key among them will be ensuring stability of both donor and recipient animals, adopting meticulous operative technique that is safe and associated with minimal blood loss, ensuring complete cardioplegic arrest with uniform graft cooling, minimizing total ischemic time, and adequately de-airing the graft. As acknowledged above, the technique's technical complexity is its chief limitation.

We have advanced previous HAHLT techniques in several respects. The modifications described in donor and recipient operations provide a means of performing necessary operative steps in a controlled and efficient manner. The myocardial protection protocol described, coupled with diligent topical myocardial cooling is an ideal means of minimizing injury during the cold ischemic period. The end result is a HAHLT graft in which geometry has been optimized, injury has been minimized, and intrinsic graft function has been preserved.

The technique described above yields a HAHLT graft with near physiologic ventricular-loading conditions. Once the technique has been mastered, the transplant configuration permits complete *in vivo* characterization of graft function. As noted, the preload and afterload conditions can be altered acutely or chronically, and donor lung may be acutely re-ventilated. Investigators can readily and broadly apply this model to the study of many medical conditions, while retaining the ability to study graft structure and function.

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DISCLOSURES:
The authors have nothing to disclose.

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Table 1
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Table 1. Characteristics of Successful Versus Unsuccessful HAHLT Procedures.

	Successful Procedure
Donor	
Donor Stability	Stable
Preparatory Dissection	Efficient, Limited
Blood Loss	Minimal
Time to Explant	Minimized
Cardioplegic Arrest	Rapid
Graft	
Total Ischemic Time	< 60 Minutes
Cardioplegic Arrest	Maintained Periodically
Graft Cooling	Maintained Uniformly
Recipient	
Recipient Stability	Stable
Preparatory Dissection	Efficient
Clamp Times	< 20 Minutes
Anastomosis Time	< 40 Minutes
Blood Loss	Minimal
Graft De-airing	Complete
Volume Resuscitation	Adequate
Successful Reperfusion	Yes
Return of Stable Cardiac Function	Yes

Unsuccessful Procedure

Unstable

Inefficient, Excessive

Excessive

Prolonged

Delayed

> 60 Minutes

Incomplete

Inadequate

Unstable

Inefficient

> 20 Minutes

> 40 Minutes

Excessive

Incomplete

Inadequate

No

No

Figure 1

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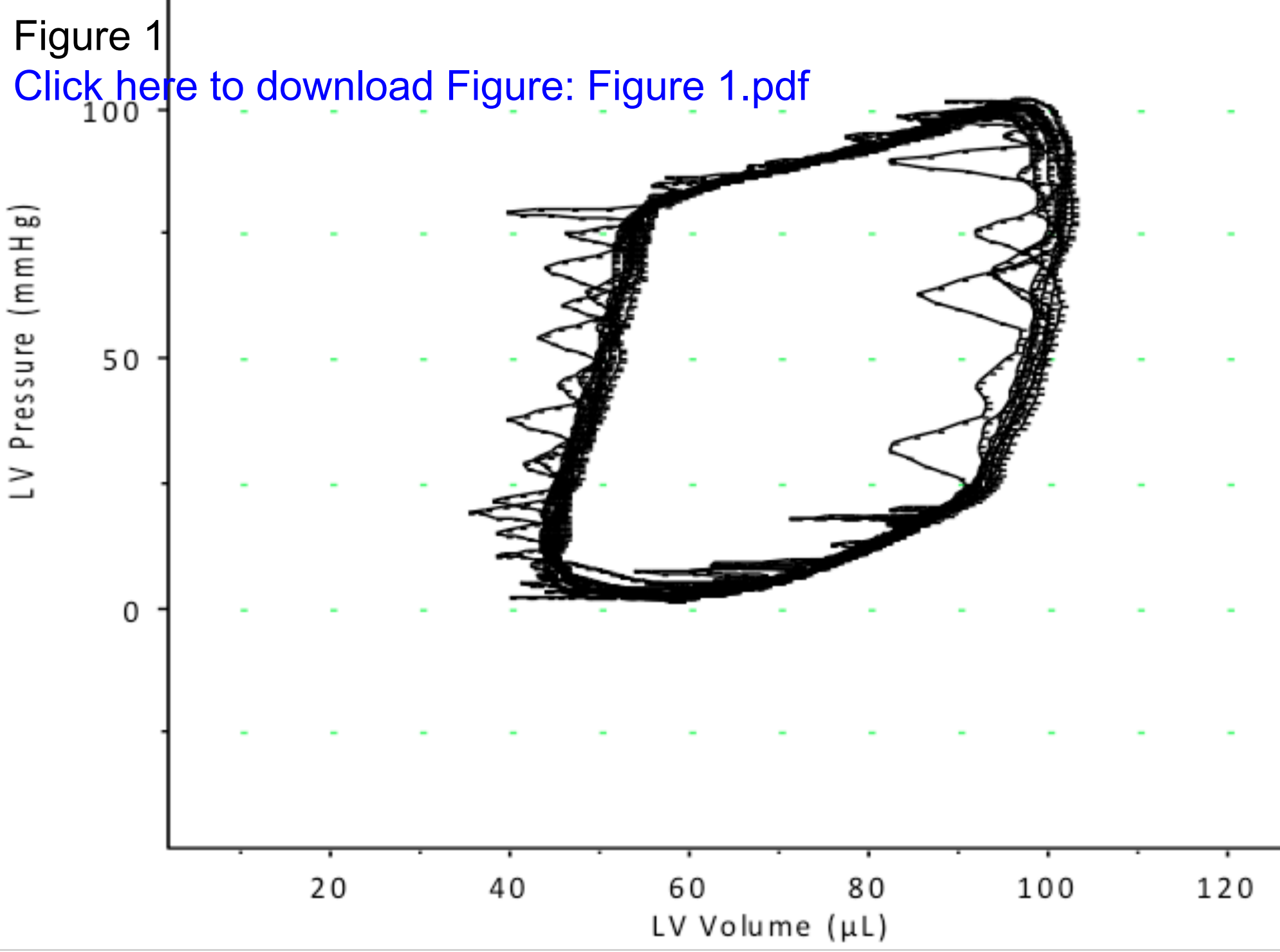

Figure 2

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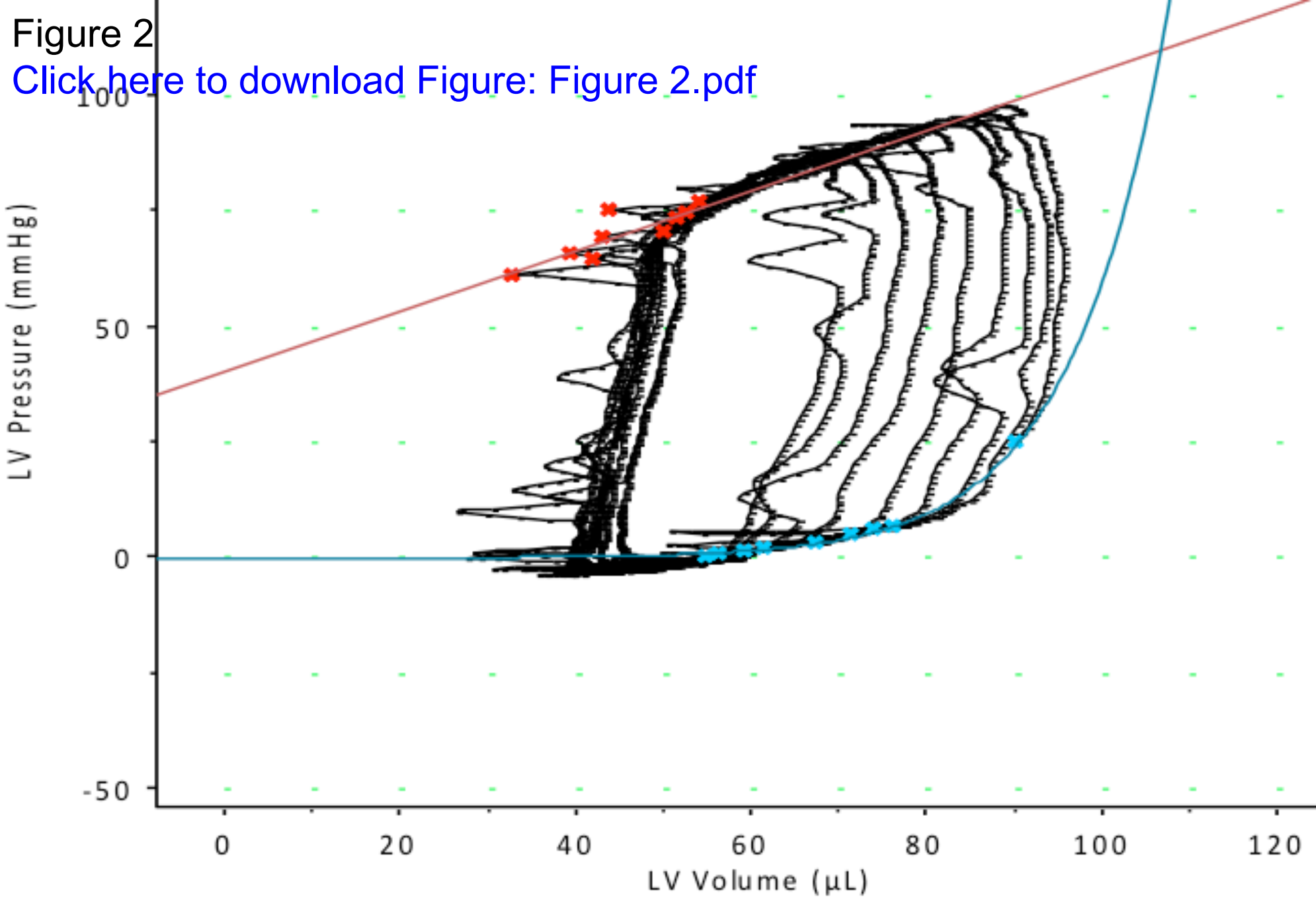

Excel Spreadsheet- Table of Materials/Equipment
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Table of Reagents/Equipment

Material/Equipment	Company	Catalog Number
Celsior Cardioplegic Soution	Genzyme	
Rodent Ventilator	Harvard Apparatus	Model 683
Vital Sign Monitor	Nonin	Model 9847V
IV Cannulae	Jelco	3063
IV Tubing	CareFusion	MP9259-C
Surgical Clips	Teleflex Medical	001204
Sutures	Ethicon	LA54G, EPM8738
Surgical Instruments	Not Applicable	Not Applicable

Comments
The solution is kept on ice throughout the procedure. We prepare our own solution, with slight modifications.

Displays SpO_2 and heart rate.

24-26G x 3/4" cannulae.

Short-length connector tubing (18cm).

Horizon titanium ligating clips.

3-0 silk reel, and 8-0 prolene suture (double-armed, x needle).

The instruments used are generic, and can be purchased from any surgical supply company.

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Title of Article: RAT HETEROTOPIC ABDOMINAL HEART/SINGLE-LUNG TRANSPLANTATION IN A VOLUME-LOADED CONFIGURATION

Author(s): MARK J. KEARNS, YINGJIN WANG, JOHN H. BOYD

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List of Changes for Jove Submission JoVE52418

"Rat Heterotopic Abdominal Heart/Single-Lung Transplantation in a Volume-Loaded Configuration"

- The following text modifications were made in track-changes mode.

1. Added "by pedal pinch" in the text. It is now found in section 1.8.

2. Now a sub-section of 1.8. Changed to 1.8.n, n+1, etc….

3. Wording changed as requested. Found in section 1.10.

4. Changed to imperative tense (now in section 1.17).

5. Changed. Now section 2.4.

6. Changed to include confirmation of anesthesia in sections 1.8 (donor operation) and 3.1 (recipient operation).

7. Added to steps 1.5 and 3.1. I also included new sections 3.2 and 3.3 to describe recipient positioning, prepping, and ventilator approach.

8. Itemized responses:

    a. An ethics statement is present at the beginning of the protocol.
    b. Euthanasia method (exsanguination) was explicitly noted in sections 1.16 and 3.30.
    c. Anesthesia and confirmation of anesthesia are included in the text.
    d. Use of vet ointment is noted in the protocol.
    e. Not applicable.

9. Small steps in the protocol were combined to contain 2-3 actions per step.

10. In addition to condensing the protocol (as per step 9 above), I have highlighted its most important sections. In total, the highlighted text occupies no more than 2.75 pages, as requested. If you're unable to view the highlighted text in the submitted manuscript, please let me know and I'd be happy to indicate the critical protocol points in bold text.

11. Now found in section 3.29. The expanded protocol includes examples of heart-lung graft assessments, which are described in greater detail in the results section.

12. Expanded representative results, and included suggestions on how to analyze the outcome.

13. We have adhered to this format, as requested by JoVE.

14. The discussion section addresses points a-e, as requested by JoVE.

List of Changes for Jove Submission JoVE52418—Post-Peer Review

"Rat Heterotopic Abdominal Heart/Single-Lung Transplantation in a Volume-Loaded Configuration"

The following text modifications were made in track-changes mode.

EDITORIAL COMMENTS

1. 3.4 and 3.22 changed. 3.29 left unchanged, as the instruction depends on the investigator's endpoint.
2. Done.
3. They are original figures, not previously published.

REVIEWER COMMENTS

I would like to sincerely thank each of the reviewers for their feedback and thoughtful critique of our methods manuscript. I hope that you find the following responses satisfactory.

Reviewer #1

Major Concerns

1. N/A

Minor Concerns

1. That's a great suggestion. At this point we haven't confirmed antegrade aortic flow by angiography or echocardiography. We would like the focus of this manuscript to be on the methodology itself, while grouping confirmatory findings of antegrade flow (along with other endpoints) into a future 'results' oriented manuscript.
2. Because we are not pursuing recovery surgery at this point in time (certainly this will be a future aim), we find it quicker and more practical to perform tracheotomy. The tracheal cannula is also less likely to fall out compared with rodent orotracheal cannulae.
3. Thank you! We have corrected the 2nd typo, and kept the correct spelling of 'loupes'.
4. I do use 8-0 prolene for both venous and arterial anastomoses. It is more common to use 10-0 for micro-vascular anastomoses (even at our animal facility), but my feeling is that 8-0 does a great job for both. I've experimented with 7-0, which is certainly bulkier and less flexible, and would not recommend that. We use a BV 130-5 needle with the 8-0 prolene.
5. At this point in time we haven't conducted chronic experiments, so I can't report any experience with thromboembolic complications in that setting. I can report that we have had a thrombotic complication at the site of IVC anastomosis acutely, likely related to under-dosing of heparin. The paper you

cited contained a very nice description of the authors' learning curve and results with a cohort of 90 rats. In terms of learning curves, we are somewhere in the mid-point of our curve, and I don't feel such an analysis would be appropriate for us with the current manuscript.

6. Good point. This is a recent, high-quality publication in a relevant area. I've added the recommended reference to our manuscript!

Reviewer #2

Major Concerns

1. Thank you for suggesting this. I agree with your points, and have expanded the introduction. I added a brief discussion about the volume-loaded configurations that have been described by other groups. Further, I highlight that our circulation is unique in 2 respects. First, its exact configuration is unique, and despite not being utilized in rodent transplant research, it is likely the most versatile circulation for transplant investigators. Second, our circulation lends itself to direct functional assessment *in vivo*, and we describe how this may be achieved.
2. This is a good point. Since we have not conducted experiments confirming that the transplanted lung tissue is capable of providing "full" systemic oxygenation, we have reworded the sentence in a more general way.
3. I agree, and will work with the JOVE editors to provide updated *in vivo* figures prior to publication.
4. We currently have no chronic time-points. The methodology reported in this manuscript was developed to serve our broader research interests, which in the short-term only involve acute time-points. In the future, it is our interest to fully explore the benefits that this transplant model has to offer, and report our results in a separate manuscript. For now, however, it would be remiss if we didn't report on our model's broad range of investigative applications.

Minor Concerns

1. Thank you! Corrected.
2. Modified to indicate that a cannula, of at least 16 Gauge size, should be used.
3. I have specified that I use 3.5x loupes, and an operating microscope with 3.4-21.3x magnification.
4. Changed to indicate that we use the right hindlimb for monitoring purposes.
5. Corrected.
6. Modified to reflect our use of a 14 Gauge tracheal cannula.
7. Modified to indicate that it usually requires 10-15cc of NS over 10-20 seconds. The flush is continued until the aortic run-off appears dilute and the heart arrests.
8. Clarified that we use the 16 Gauge cannula attached to the 10mL cardioplegia syringe.
9. Clarified the volume, rate, and frequency of cardioplegia administration.
10. Revised.

11. Modified to indicate that we retract the bowels superiorly and to the left.
12. Modified to indicate that we use either bulldog or Yasargil clamps.
13. Modified sections 3.10 and 3.20 to indicate that we make a longitudinal incision on the anterior aspect of both IVC and abdominal aorta.
14. Thank you for picking this up! We intend to adhere to "heel and toe" terminology. The relevant manuscript sections were modified accordingly.
15. Modified to indicate that the donor IVC clamp should be left in place.
16. Modified to indicate that we are referring to the ascending aorta. I have also specified that the aorta should be free of peri-aortic fat, and adequately separated from the pulmonary artery in order to prepare it for anastomosis.
17. Modified, as indicated above.
18. Modified.

Reviewer #3

Major Concerns

1. I agree with you. If the JOVE editors have access to an illustrator/art department, I'd be happy to prepare a rough sketch, and guide the illustration of an appropriate figure.

Minor Concerns

1. N/A