Anatomical considerations and surgical technique of porcine cardiac xenotransplantation

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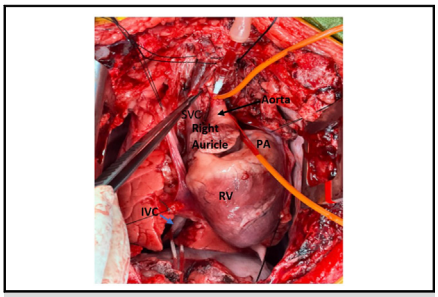
As the result of a perennial shortage of donor hearts, recent research efforts into suitable alternatives have focused on pigs as an ideal species for unlimited supply of organs.[1] Concurrently, there have been major advancements in the ability to modify the pig genome, specifically the α1,3-galactosyltransferase gene knockout, which has led to renewed interest in the potential clinical application of xenotransplantation.[1,2] As we approach the potential for a clinical trial in heart xenotransplantation, there are significant anatomical differences between porcine and human hearts that need thorough understanding by the transplant teams. Here, we describe our surgical technique of donor heart procurement in genetically modified pigs, as well as the alterations required to perform recipient orthotropic heart xenotransplant. As a research purpose, orthotropic heart xenotransplantation was performed in 2 patients declared brain dead, who were observed for 66 hours postoperatively, using standard medications and without additional mechanical circulatory support.[3] Written informed consent was obtained, and the decedent were transferred to the intensive care unit at the New York University Langone Hospital for initial assessment and stabilization. The New York University Research on Decedents Oversight Committee reviewed our xenoheart transplant protocol and provided oversight.[4]



Preparation of donor pig heart for xenotransplantation.

CENTRAL MESSAGE

A thorough understanding of the subtle differences from human heart is essential for donor pig-heart procurement. Size matching especially of the great vessels is crucial for xenotransplantation.

ANATOMICAL DIFFERENCES BETWEEN HUMAN AND PORCINE HEART

The porcine heart has a classic "Valentine heart" shape in contrast to the human heart, which is trapezoidal in silhouette with a markedly eccentric apex as the result of the pig's orthograde posture. The superior and inferior caval veins open into the atrium at right angles to one another, whereas in human hearts the orifices are directly in line (Figure 1).[5] Furthermore, the morphologic right atrium of the pig has a tubular-shaped appendage, as seen in the left atrium in humans. In general, both the right and left atria are more prominent in the pig, with thicker muscle, and this makes enlarging the atria easier for appropriate size-matching to the recipient during biatrial anastomosis. A prominent left azygous vein enters on the left side of the pig heart and drains via the coronary sinus (Figure 2).[5]

The porcine left atrium receives only 2 pulmonary veins, whereas 4 orifices are generally observed in human hearts. With respect to the coronary circulation, the porcine heart is characterized by right-coronary dominance (origin of the posterior descending artery) (Figure 3, *A-C*).[6] The anterior displacement of the porcine aortic trunk compared with that of humans is significant. In addition, the pulmonary trunk is more at right angles with the inlet component of the right ventricle, reflecting the upright stance. In pigs, the orientation of pulmonary valve is more directly aligned with the orientation of the inlet component, an adaptation to the unguligrade stance of the pig. Consequently, the aorta and pulmonary artery are somewhat rotated in orientation and have to be taken care of during xenotransplant procedure.

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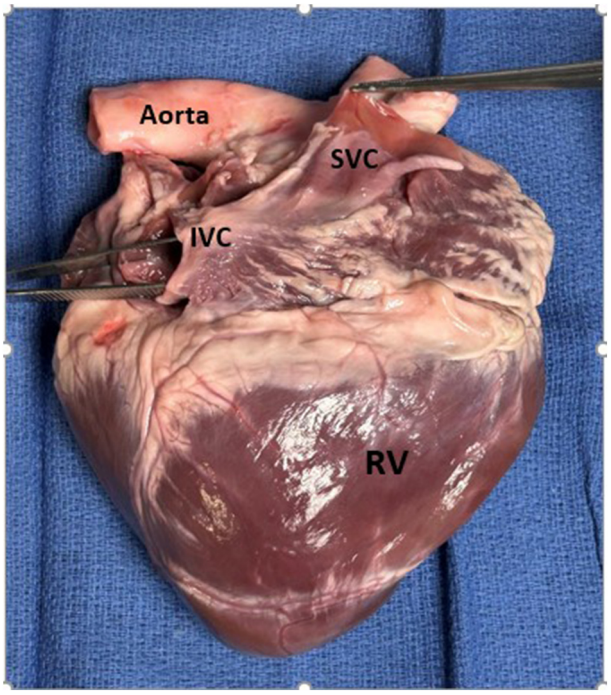

FIGURE 1. Dorsal view of the heart showing the relationship of the superior vena cava (*SVC*) and the inferior vena cava (*IVC*; forceps within its lumen) opening into the right atrium at right angles. *RV*, Right ventricle.

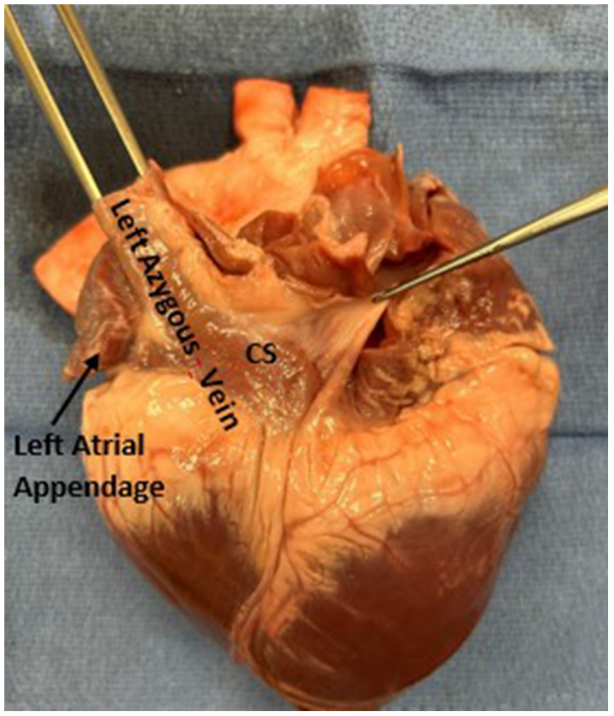

FIGURE 2. Dorsal view of the heart showing large azygous vein on the left side draining into the coronary sinus (*CS*).

DIFFERENCE BETWEEN CONDUCTION SYSTEM OF HUMAN AND PORCINE HEART

In the pig heart, the sinoatrial (SA) node lies at the right side of the terminal crest, at the junction of the cranial vena cava and the right atrial appendage; furthermore, the SA node is relatively lower on the septum than in humans. The SA node appears to be rectangular in its longitudinal direction but has a flattened appearance perpendicularly (Figure 4).[7]

The atrioventricular node resides on the right side of the ventricular septum, an anatomic position similar to that of human hearts, but is more densely innervated (Figure E1).[8]

One of the most prominent features in the right ventricle of the porcine heart is the trabecula septomarginalis, formerly known as moderator band. This muscular strand connects the septal wall of the right ventricle to its free wall and carries Purkinje fibers from the right atrioventricular bundle across the right ventricle's lumen. Compared with human hearts, the trabecula septomarginalis of swine is more prominent and situated more proximal relative to the base of the heart, explaining the faster activation of right ventricle in pig heart (Figure E2). Consequently, there is a variation of the Purkinje fiber network in porcine hearts, which results in differences in ventricular conductivity and contractility.[8] The shorter PR interval and different activation sequence of its ventricles make the swine heart easily excitable and markedly susceptible to ventricular fibrillation. This variability could account for frequent rhythm disturbances observed after xenotransplantation.

SURGICAL TECHNIQUE OF PIG DONOR HEART PROCUREMENT

Our technique of pig heart procurement resembles that of standard human donor heart procurement, with certain important modifications.[9] Essentially, in human heart procurement the superior vena cava (SVC) is ligated, the interatrial groove is vented, and the anterior wall of the inferior vena cava (IVC) is transected. Once the entire cardioplegia is delivered, the IVC posterior wall transection is completed. The left atrium is then excised, ensuring adequate cuff for suturing the anastomosis. The aorta is transected at the level of the innominate artery and the pulmonary artery at the level of bifurcation. Finally, the SVC is transected, confirming its separation from the right pulmonary artery and the entire heart is removed. Pigs to be used for xenotransplant had 10 gene modifications and were placed in barrier containment in a sterile facility where the procurement was performed. The donor pig was brought to the operating suite and placed under anesthesia by trained veterinarians. Familiarity with anatomy of airway of pigs is of utmost importance, as swine have thick tongues, long, narrow oropharyngeal spaces, and elongated soft palate, which can obscure or hide the epiglottis and thereby

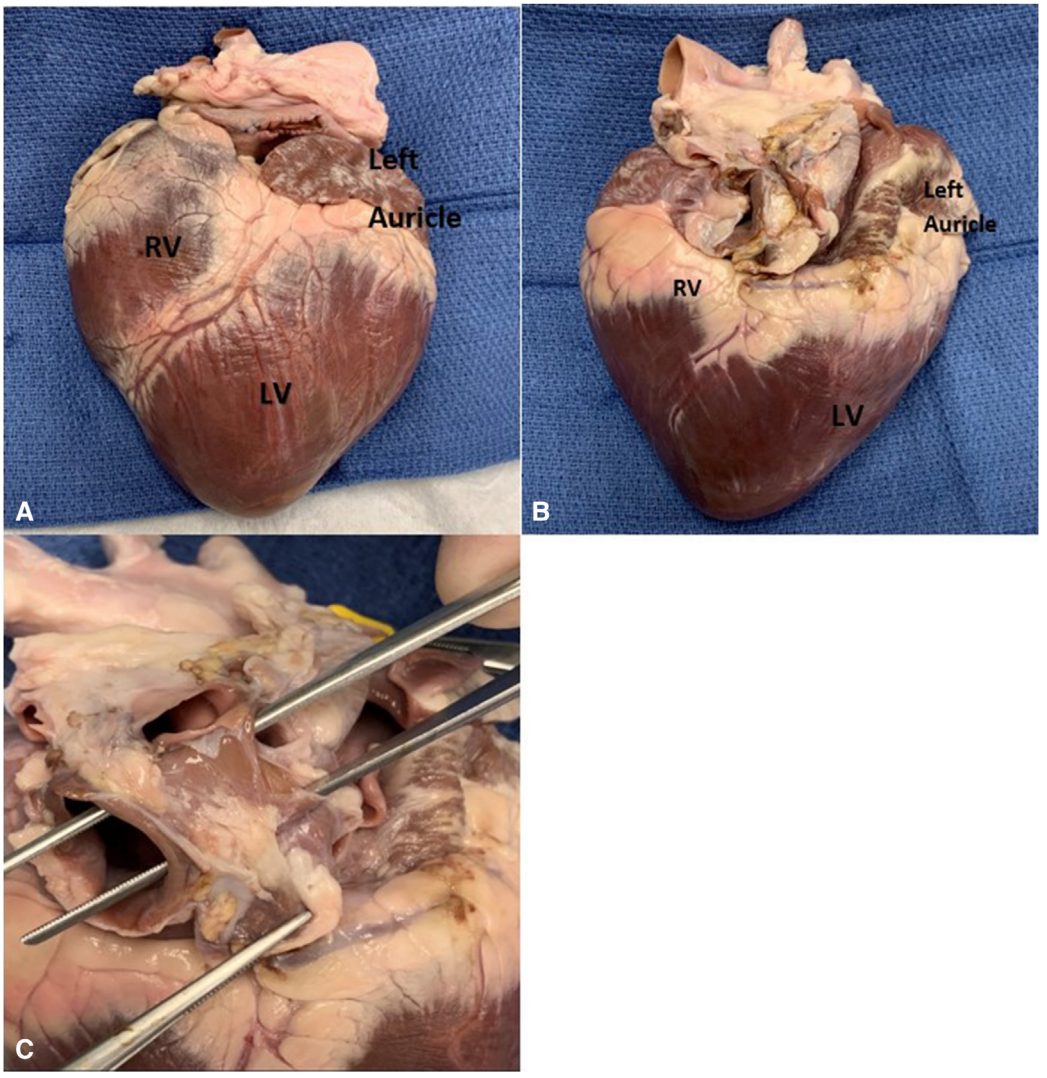

FIGURE 3. A, Anterior aspect of pig heart with a "valentine" shape, blunt left ventricle (*LV*) apex and large triangular left auricle. B, Dorsal surface of the heart showing the LV, right ventricle (*RV*), and the left auricle and (C) pulmonary veins with the forceps passing through it, opening into the left atrium.

complicate endotracheal intubation.[10,11] Pigs are also prone to laryngospasm, especially if lightly anesthetized. Before surgical incision, a femoral arterial line was placed for continuous hemodynamic monitoring. The manubrium in pigs is thicker than in humans. With the innominate vein in its close proximity posteriorly, careful blunt dissection in the superior aspect of manubrium is required to avoid torrential venous bleeding, which can obscure the surgical field. Median sternotomy was performed using a Lebsche knife. A pericardial well was created, and care taken not to "pull-up" the pericardial sutures, given increased susceptibility of pig hearts to ventricular fibrillation and/or hypotension. Every effort should be made to minimize handling the donor heart. In pigs, the aorta and pulmonary artery are rotated as compared with their positions relative to the human heart. In addition, the proximal aorta is short and narrow, thereby making the standard technique of aortic crossclamp challenging (Figures E3 and E4).

In our modified technique, the aortopulmonary window was carefully dissected, separating the 2 great vessels. Using a right-angle clamp, an umbilical tape was passed between the aorta and pulmonary artery. Next, the clamp was passed behind the innominate artery from the left side, and the right-end of the umbilical tape was pulled from behind the innominate artery, thereby encircling the proximal aortic arch. The 2 ends are then passed through a tourniquet, allowing the aortic arch to be "clamped" just beyond the innominate artery. The IVC is relatively long in pigs and can be easily encircled above the diaphragm. The standard dose of intravenous heparin (30,000 units) was administered. To preserve adequate length of the ascending aorta, an 18-Fr cardioplegia catheter was inserted in the proximal innominate

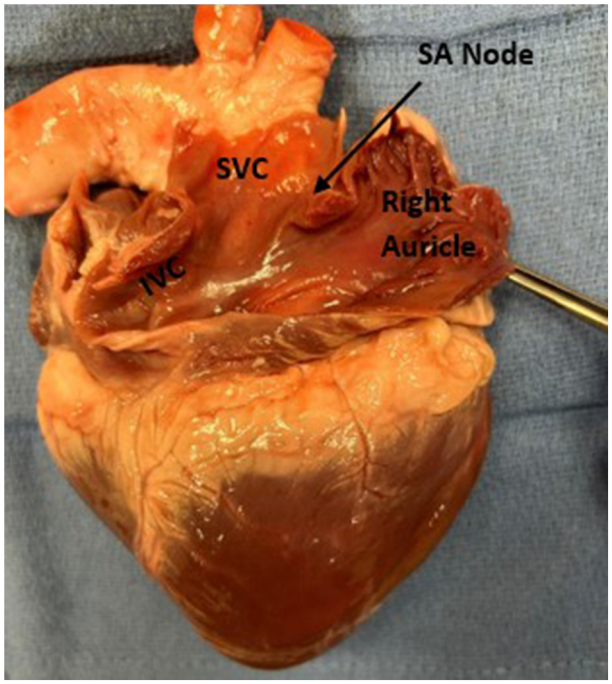

FIGURE 4. Position of the sinoatrial (*SA*) node at the junction of superior vena cava and right atrial appendage. *SVC*, Superior vena cava; *IVC*, inferior vena cava.

artery. Distal control of the innominate artery was obtained. The innominate artery was noted to be small and fragile in the genetically modified pigs. When present, the left azygous vein is small and drains directly into the coronary sinus near the left atrial appendage. Care was taken to identify the opening of the left azygous vein close to the appendage and ligate it using fine silk suture. Once the surgical team was prepared to crossclamp, the innominate artery was tied off distal to the cardioplegia catheter. The SVC was ligated close to the innominate vein, and IVC was divided below the diaphragm, preserving as much length as possible. The left atrium was vented by incising the right pulmonary vein as it entered the heart. The aortic crossclamp was applied by bringing the tourniquet down around the aortic arch and heart preserved by delivering 2 L of cold preservation solution (UW Solution; Organ Recovery Systems, Inc), and the mediastinum packed with ice slush. The aortic root pressure and left ventricular distension was monitored manually. Donor cardiectomy was performed in sequential fashion, beginning with incising the IVC, left atrial cuff then the ascending aorta proximal to the clamp, the pulmonary artery at the level of bifurcation, and finally the SVC below the ligated part. In the first case, the heart-lung block was excised en bloc, and the heart separated from the lungs at the back-table. However, in the second case, isolated heart procurement was performed.

The heart was transported to the recipient center in cold static preservation in University of Wisconsin solution. Cold ischemia time was 3 hours, 11 minutes in the first case and 2 hours 40 minutes in the second case. With isolated heart procurement, cold ischemia time was considerably reduced in the second case.

TECHNICAL CONSIDERATIONS OF PIG-TO-HUMAN HEART IMPLANTATION

For both of our procedures, in the decedent the sternotomy, dissection and cardiectomy were performed per routine as is done in adult orthotropic heart transplantation retaining the right atrial cuff (right atrium is opened from lateral IVC towards the right atrial appendage) for biatrial anastomosis. To summarize in brief, in biatrial technique the left atrial anastomosis is initiated at the base of the left atrial appendage adjacent to the left superior pulmonary vein. The 2 ends of the suture are run inferiorly and superiorly and eventually joined in the middle of interatrial septum. A long 3-0 PROLENE suture (Ethicon) is then used and the suture ends are carried both inferiorly and superiorly to first complete the septal anastomosis, and then they are joined at the lateral wall of the septum. The pulmonary artery and aortic anastomosis are then performed.

There are several important technical aspects to be considered for recipient pig-to-human heart implantation. First, there is inherent size mismatch between the human recipient in chronic heart failure and the standard donor pig heart. Cardiomegaly seen in advanced heart failure patients leads to asymmetric chest expansion and significant enlargement of all chambers and major vessels. With human donor hearts, the bicaval technique has shown to be superior to biatrial in both early and late outcomes.[12] However, at least in our first 2 procedures, the anatomic mismatch between the recipient and donor pig heart necessitated the classic biatrial anastomosis. Given the anatomical differences and the relative orientations of the vena cavae in the pig heart to that of humans, we thought it would be technically easier and less time consuming to perform a biatrial rather than a bicaval anastomosis. In addition, the great vessels of the pig heart can have significant size mismatch relative to human aorta and pulmonary artery. This can be addressed by simple patch augmentation so as to widen the anastomosis. In the first decedent xenoheart procedure, pericardial patch augmentation was necessary on the anterior wall of the pulmonary artery and the aorta. In the second decedent, it was only necessary for the aorta, given the better size matching. It is our understanding that donor pig heart preoperative imaging using a 3-dimensional echocardiogram or computed tomography/magnetic resonance angiogram will be absolutely essential in planning the xenoheart procedure. As our ability to evaluate the size of the pig donor heart and great vessels improves and we have access to a larger inventory of genetically modified pigs, some of the aforementioned alterations may not be necessary. Cardiac xenografts exhibit

growth and diastolic heart failure. Hence, xenografts with growth hormone receptor knockout are used, which show reduced posttransplantation hypertrophy of the heart, thereby eliminating the need for medications such as temsirolimus and afterload-reducing agents in the recipient. The long-term effect of this growth hormone receptor knockout is not known.[13] Enhanced preoperative imaging will remain the cornerstone to achieve better size matching of donor pigs to human recipients.

CONCLUSIONS

Recent advances in genetic modifications of porcine hearts have ushered in a new era in cardiac xenotransplantation. Detailed understanding of the anatomy and conduction pathway of porcine hearts is essential to mastering donor procurement and implantation in humans. Better size matching between the donor heart and recipient may negate some of the modifications that we had to perform to augment the diameter of the great vessels, however, at present, the data on the relative size of the pig heart and great vessels is lacking. It is possible that in the future, with larger availability of pigs, better anatomical characterization will be necessary before heart xenotransplantation. One such approach may involve better characterization of size by 3-dimensional echocardiography or computed tomography/magnetic resonance angiogram.

Conflict of Interest Statement

The authors reported no conflicts of interest.

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

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References

1. Jou S, Mendez SR, Feinman J, et al. Heart transplantation: advances in expanding the donor pool and xenotransplantation. *Nat Rev Cardiol*. 2024;21(1):25-36.
2. Griffith BP, Goerlich CE, Singh AK, et al. Genetically modified porcine-to-human cardiac xenotransplantation. *N Engl J Med*. 2022;387:35-44.
3. Moazami N, Stern JM, Khalil K, et al. Pig-to-human heart xenotransplantation in two recently deceased human recipients. *Nat Med*. 2023;29(8):1989-1997. https://doi.org/10.1038/s41591-023-02471-9
4. Montgomery RA, Stern JM, Lonze BE, et al. Results of two cases of pig-to-human kidney xenotransplantation. *N Engl J Med*. 2022;386(20):1889-1898. https://doi.org/10.1056/NEJMoa2120238
5. Crick SJ, Sheppard MN, Ho SY, Gebstein L, Anderson RH. Anatomy of the pig heart: comparisons with normal human cardiac structure. *J Anat*. 1998;193:105-119.
6. Sahni D, Kaur GD, Jit H, Jit I. Anatomy and distribution of coronary arteries in pig in comparison with human. *Indian J Med Res*. 2008;127:564-570.
7. Lelovas PP, Kostomitsopoulos NG, Xanthos TT. A comparative anatomic and physiologic overview of the porcine heart. *J Am Assoc Lab Anim Sci*. 2014;53:432-438.
8. Laske TG, Shrivastav M, Laizzo PA. The cardiac conduction system. In: Laizzo PA, ed. *Handbook of Cardiac Anatomy, Physiology, and Devices*. 2nd ed. Springer; 2009:159-175.
9. Pasque MK. Standardizing thoracic organ procurement for transplantation. *J Thorac Cardiovasc Surg*. 2010;139:13-17.
10. Hill AJ, Laizzo PA. Comparative cardiac anatomy. In: Laizzo PA, ed. *Handbook of Cardiac Anatomy, Physiology, and Devices*. 2nd ed. Springer; 2009:87-108.
11. Chum H, Pacharinsak C. Endotracheal intubation in swine. *Lab Anim*. 2012;41:309-311.
12. Zijderhand CF, Veen KM, Caliskan K, et al. Biatrial versus bicaval orthotopic heart transplantation: a systematic review and meta-analysis. *Ann Thorac Surg*. 2020;110(2):684-691. https://doi.org/10.1016/j.athoracsur.2019.12.048
13. Goerlich CE, Griffith B, Hanna P, et al. The growth of xenotransplanted hearts can be reduced with growth hormone receptor knockout pig donors. *J Thorac Cardiovasc Surg*. 2023;165(2):e69-e81. https://doi.org/10.1016/j.jtcvs.2021.07.051

Key Words: porcine heart, procurement, cardiac xenotransplantation, genetic modification, immunosuppression

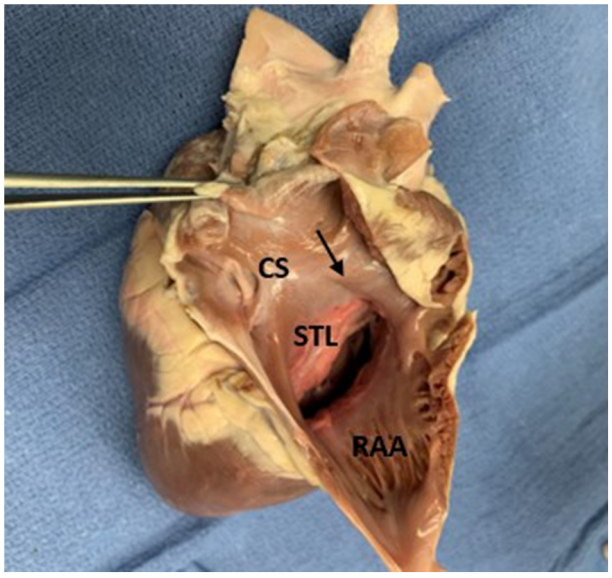

FIGURE E1. The right atrial (*RA*) cavity opened and showing the coronary sinus orifice, the right atrial appendage (*RAA*) with thick pectinate muscle, septal leaflet of tricuspid valve (*STL*) and the location of the membranous septum (*black arrow*). *CS*, Coronary sinus.

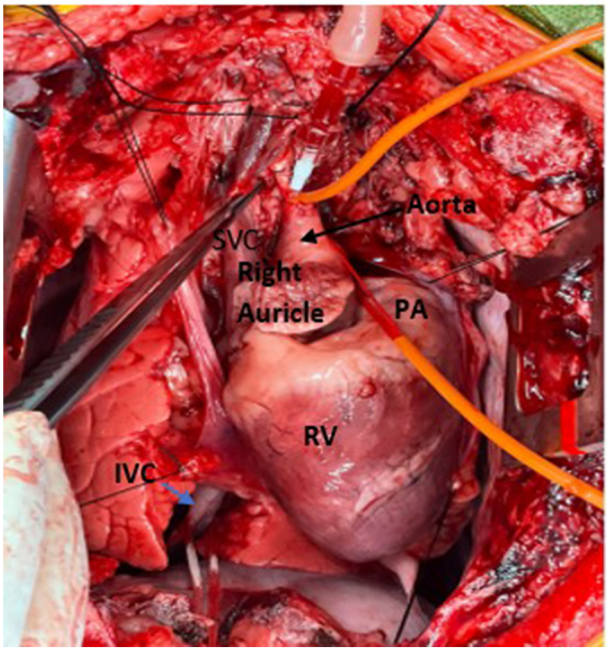

FIGURE E3. Preparation for donor pig procurement showing short and narrow proximal aorta (*black arrow*) with cardioplegia needle in innominate artery, umbilical tape around proximal arch, and inferior vena cava (*blue arrow*) looped with umbilical tape. *SVC*, Superior vena cava; *PA*, pulmonary artery; *IVC*, inferior vena cava; *RV*, right ventricle.

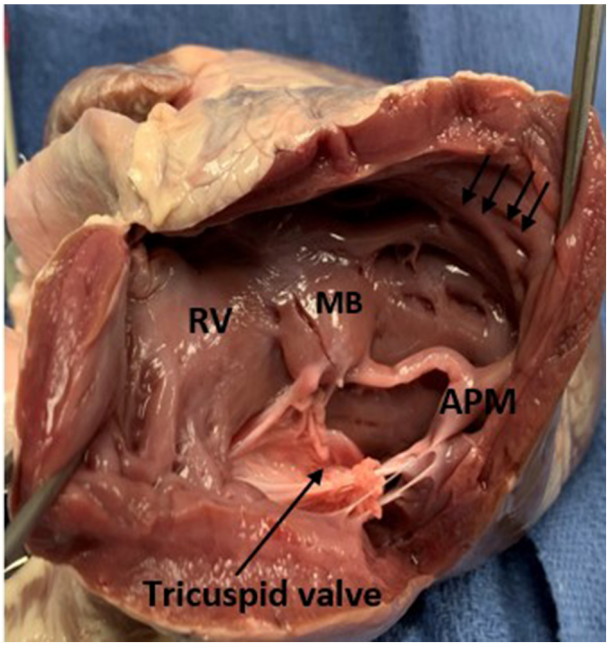

FIGURE E2. The *RV* (right ventricle) cavity opened up showing the moderator band (*MB*), originating higher on the septal wall and attached to the anterior papillary muscle (*APM*). The trabeculae prominent and coarse and toward the apex (*black arrows*).

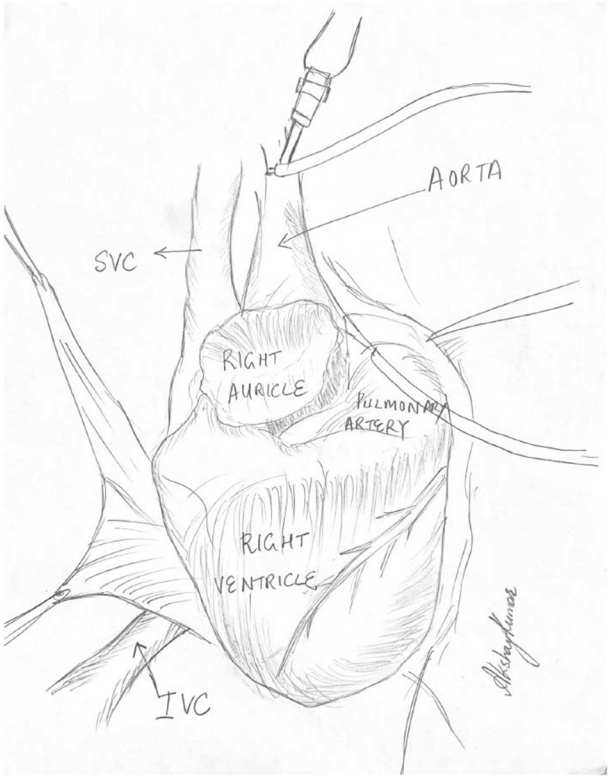

FIGURE E4. Illustration showing the donor pig heart with the great vessels and cardioplegia catheter in distal ascending aorta. *SVC*, Superior vena cava; *IVC*, inferior vena cava.