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TITLE:

The Isolated Large Mammal Heart: A Blood-Perfused Langendorff Platform for Arrhythmia Inducibility Testing.

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SUMMARY:

Studying the mechanisms underlying life-threatening arrhythmias is challenging because coronary perfusion and cardiac pump function deteriorate during ventricular arrhythmic episodes, which often cause irreversible tissue damage or death. Conventional cannulation of the aorta and selective cannulation of the coronary arteries in isolated Langendorff-perfused heart models maintain continuous coronary flow during arrhythmias, enabling comprehensive electrophysiological mapping while preserving myocardial viability.

ABSTRACT:

Studying the mechanisms underlying life-threatening arrhythmias is challenging because cardiac pump function decreases significantly during episodes, which can be fatal for patients or experimental animals. Although arrhythmias can often be converted to sinus rhythm using electrical defibrillation, this approach can cause cardiac damage and result in the premature termination of experiments. In addition, inadequate myocardial perfusion during arrhythmic episodes can result in irreversible tissue damage.

The isolated Langendorff-perfused heart is a superior experimental model for studying the onset and maintenance of arrhythmia. Despite limitations including the absence of extracardiac autonomic nervous system input and altered mechanical properties due to unloaded contraction, both conventional and advanced Langendorff perfusion techniques provide continuous coronary flow. In conventional Langendorff perfusion, the aorta is cannulated to provide retrograde perfusion through the aorta, ensuring adequate oxygen and nutrient delivery to the myocardium, even during ventricular arrhythmias. Our advanced Langendorff perfusion involves directly cannulating the coronary arteries, which makes myocardial perfusion independent of cardiac performance and enables the heart to eject blood through the aorta under physiological conditions. Both techniques facilitate comprehensive electrophysiological mapping of the endocardial and epicardial surfaces using multi-electrode grids, as well as transmural needle electrodes.

In this article, we outline conventional and advanced Langendorff perfusion methods for large mammalian hearts, focusing particularly on blood perfusion. This approach enables the extended investigation of arrhythmia mechanisms while maintaining myocardial viability and allowing detailed electrophysiological characterization.

INTRODUCTION:

The heart is essential for maintaining systemic homeostasis, functioning as a highly dynamic organ that continuously interacts with other physiological parameters. Investigations of cardiac function in vivo therefore have high translational value, as they characterize the behavior of the entire organism as an integrated system. However, it can be difficult to isolate intrinsic cardiac mechanisms in whole-body studies, and interventions at the cardiac level may compromise systemic stability in turn. This limitation is particularly critical in research on the initiation and maintenance of ventricular tachyarrhythmias. Sustained arrhythmias can quickly lead to impaired pump function, decreased arterial pressure, global hypoperfusion, and a significant increase in myocardial metabolic demand. In this field, therefore, experimental models that provide controlled and stable perfusion, ensuring adequate oxygen and nutrient delivery, and avoiding reflex mechanisms, are essential.

One such central model is the coronary perfused isolated heart preparation, also known as Langendorff perfusion. This approach was first experimented with by Carl Ludwig and Elias Cyon and was subsequently optimized by Oscar Langendorff in the late 19th century¹. This pioneering work was a major step forward in cardiac physiology, as it enabled the controlled investigation of mammalian hearts, independent of systemic influences. Using this approach, Langendorff and his contemporaries demonstrated several key phenomena, including post-rest potentiation. In the following decades, the model played a pivotal role in groundbreaking discoveries that now constitute the foundation of cardiovascular physiology, including the Frank-Starling mechanism, coronary flow autoregulation, the functional and anatomical underpinnings of reentrant arrhythmias, and the activation sequence of the human heart²⁻⁴. The continuing relevance of the Langendorff model is evident in contemporary studies published in this journal that employ it for optical mapping of membrane potentials and calcium dynamics, myocyte isolation, preservation of donor organs, and extended configurations, such as the working-heart preparation⁵⁻⁸. Each application has yielded specific modifications tailored to the scientific questions addressed.

Here, we present a blood-perfused, isolated heart model for large mammals. This approach offers several unique advantages. Firstly, blood perfusion rather than a crystalloid solution enables an extended experimental duration due to improved osmolar and ionic stability, as well as a more physiological oxygen-carrying capacity^{9,10}. Secondly, complex arrhythmias arise more readily in large mammalian hearts; in contrast, inducing them in small mammals often requires substantial, non-physiological interventions¹¹. Consequently, large animal models are better suited for studying ventricular and atrial arrhythmias. Furthermore, arrhythmogenesis is strongly influenced by dynamic changes in electrophysiological properties and spatiotemporal heterogeneity, both of which critically depend on cardiac loading conditions^{12,13}. Ideally, a more advanced evolution of the Langendorff setup—the working-heart preparation—would allow precise control of loading

conditions and enable investigations in the presence of electromechanical coupling. However, in working-heart mode, coronary perfusion remains dependent on left ventricular pump function, which can constrain the ability to reliably assess the onset and stabilization of ventricular arrhythmias.

To address this issue, we have developed an advanced version of the Langendorff preparation that can be quickly reconfigured between an unloaded retrograde perfusion mode and a working-heart mode with stable coronary perfusion as well as adjustable preload and afterload. This allows for a systematic comparison of cardiac performance and arrhythmia dynamics under varying load conditions within the same experiment. Here, we present the design, implementation and methodological considerations of this advanced blood-perfused large-mammal heart model.

PROTOCOL:

Ethics statement

No animals were sacrificed for the purposes of this manuscript. The hearts used in this study were obtained from animals involved in separate investigations. Sheep hearts (n=5) were collected from animals that had undergone caesarean sections to deliver near-term fetuses, while pig hearts (n=3) were obtained from animals used in a different study. Both investigations were conducted in accordance with national and institutional guidelines and in compliance with the European Commission Directive, with approval from the local Animal Experiments Committee (sheep: approval number AVD10700202216526; pig: approval number WP 2022-006-001). Some data were derived from hearts that have been used in other publications ^{14,15}.

1. Blood Collection and Recirculating Perfusate Preparation

- 1.1. To prevent coagulation, administer heparin intravenously (25,000 IU for large animals, adjusted proportionally for smaller animals) and allow 5–10 minutes for systemic heparinization.
- 1.2. Collect approximately 600–1500 mL of whole blood from the femoral artery or superior vena cava using sterile technique. Adjust the volume based on animal size and expected heart weight to ensure a sufficient volume of perfusate for recirculation. It is recommended to monitor mean arterial pressure and end-tidal CO₂ to ensure they remain above 60 mmHg and 3.0%, respectively; however, values this low are rarely encountered. Store the collected blood at room temperature, maintaining gentle agitation to prevent settling.
- 1.3. Prepare the recirculating perfusate by mixing the collected blood with modified Tyrode's solution at a 1:1 ratio.
- 1.4. Establish a recirculating perfusion system with a total perfusate volume of 1,400–1,500 mL within the Langendorff setup.
- 1.5. Oxygenate the perfusate continuously with carbogen (95% O₂ / 5% CO₂) using a gas dispersion system. Oxygenators designed for cardiopulmonary bypass procedures are ideal

for this purpose.

- 1.6. Warm the perfusate to the body core temperature of the mammal under investigation using a temperature-controlled water bath and heat exchanger coil system. Heating should be applied using a countercurrent system, and the water bath temperature should be calibrated by circulating the perfusate alone prior to use. Continuously monitor the perfusate temperature with a thermometer probe throughout the procedure to maintain physiological conditions.

2. Heart Explantation

- 2.1. Perform a median or left lateral sternotomy to expose the thoracic cavity.
- 2.2. Rapidly excise the heart by transecting the great vessels, taking care to preserve sufficient vessel length for subsequent cannulation. Ideally, the aorta should be cut distal to the brachiocephalic trunk, leaving at least 3–5 cm of vessel length. The brachiocephalic artery may also be used for placement of a hemostatic valve to facilitate potential left ventricular catheterization. [Figure 1](#) provides a schematic representation of possible aortic cannulation sites, and [Supplemental Figure 1](#) shows photographic documentation.
 - 2.2.1. Prior to excision, induction of ventricular fibrillation by applying a brief direct current (e.g., contact with a 9 V battery to the right ventricular myocardium or a single DC shock) may prevent air embolism in the coronary vasculature during explantation.
- 2.3. At the posterior aspect of the heart, carefully—but rapidly—dissect the lung tissue to preserve the pulmonary veins and posterior atrial wall, maintaining both the structural integrity required for potential cannulation and the electrical continuity of the left atrium.
- 2.4. Immediately submerge the explanted heart in ice-cold modified Tyrode's solution (composition: NaCl 128.0 mmol/L, KCl 4.7 mmol/L, CaCl₂ 1.5 mmol/L, MgCl₂ 0.7 mmol/L, NaHCO₃ 28.0 mmol/L, NaH₂PO₄ 0.5 mmol/L, glucose 11.0 mmol/L) and gently squeeze to remove residual blood.
 - 2.4.1. If prolonged heart preservation is required, alternative methods include cardioplegic arrest induced by high-potassium solutions¹⁶ or continuous perfusion with oxygenated preservation solutions¹⁷.
- 2.5. Transfer the heart to the experimental laboratory within 5 minutes of explantation to minimize ischemic time and ensure optimal tissue viability for perfusion experiments.

3. *Reconfigurable Langendorff Perfusion Setup*

Conventional retrograde aortic perfusion is suitable when experiments require the heart to remain mechanically unloaded, as it follows the standard Langendorff approach ([Figure 1A](#)). In contrast, direct coronary perfusion ([Figure 1B](#)) offers several functional advantages. It enables controlled loading of the left atrium and ventricle and maintains antegrade flow while ensuring coronary perfusion. This configuration also supports the application of a defined afterload for transitions toward working-heart conditions, facilitates induction of ventricular tachyarrhythmias, and allows independent perfusion of the right and left coronary circulations with distinct solutions or pharmacological compositions.

3.1. Conventional retrograde perfusion - preparation of the heart.

- 176 3.1.1. Perform all procedures in a bath containing ice-cold modified Tyrode's solution.
- 177 3.1.2. Cannulate the aorta with an appropriately sized cannula and secure with suture to
- 178 prevent leakage ([Movie 1](#)). Perform the "Coronel test" to make sure the cannulas is
- 179 properly fixed in the aorta ([Movie 2](#)).
- 180 3.1.3. Ensure that the left ventricular cavity can be vented; either by making a small incision
- 181 at the left ventricular apex or by inserting a catheter through the left atrium and
- 182 traversing the mitral valve. Venting is essential, as the cavity will otherwise fill with
- 183 blood from the Thebesian veins, which cannot be ejected during retrograde perfusion.
- 184 3.1.4. The majority of the perfusate effluent will drain through the coronary sinus. The
- 185 drainage can either be directed to the reservoir directly or collected via right ventricular
- 186 pumping. In the latter case, cannulation of the pulmonary artery is recommended to
- 187 prevent blood from flowing over the ventricular surface, which may affect signal quality
- 188 in subsequent experimental recordings. See [Supplemental figure 1](#) and [2](#) for
- 189 photographic documentation.
- 190 3.1.5. Connect the aortic cannula to the retrograde perfusion apparatus. ([Movie 3](#))
- 191

192 **3.2. Advanced Langendorff System by direct coronary perfusion.**

- 193 3.2.1. During all procedures the heart should, at least partly, be submerged in a bath
- 194 containing ice-cold modified Tyrode's solution.
- 195 3.2.2. The proximal segments of the right coronary artery and left main coronary artery
- 196 should be carefully isolated over a length of approximately 3–5 mm ([Movie 4](#)). Place 0
- 197 silk sutures beneath each vessel to allow later fixation of the coronary cannulas (see
- 198 [Supplemental Figure 1, panels B and D](#)) ([Movie 5](#)).
- 199 3.2.3. Determine the optimal aortic entry site through the aortic wall into the coronary ostia
- 200 based on the cannula shape ([Figure 1, left upper inset](#)). Place two purse-string sutures
- 201 around each entry site to prevent leakage and reduce the risk of aortic wall rupture.
- 202 ([Movie 6](#))
- 203 3.2.4. Using a scalpel (blade 11), create an incision of sufficient size to introduce the cannula.
- 204 Insert the cannula into the coronary artery, positioning it as proximally as possible, and
- 205 secure it with the previously placed sutures ([Movie 7](#)).
- 206 3.2.5. Ensure complete filling of both the aorta and cannula with Tyrode's solution to prevent
- 207 air embolism. Close the purse-string sutures securely.
- 208 3.2.6. Perfusate effluent will primarily drain via the coronary sinus, which can be directed
- 209 either to the reservoir directly or via right ventricular pumping (as mentioned above).
- 210 3.2.7. Cannulate the ascending aorta with an appropriately sized cannula and secure with
- 211 sutures to prevent leakage ([Supplemental Figures 1A and E](#)) ([Movie 8](#)). Perform the
- 212 "Coronel test" to make sure the cannula is properly fixed in the aorta.
- 213 3.2.8. Optionally, a hemostatic valve may be attached at the brachiocephalic artery to allow
- 214 for subsequent catheter placement ([Supplemental Figures 1A and 2B](#)). ([Movie 9](#)).
- 215 3.2.9. For experiments under loading conditions, left atrial cannulation is required to provide
- 216 blood supply and allow control of preload ([Figure 2](#)). Anatomical variations in the left
- 217 atrial posterior wall between species may influence cannula size and the choice of

vessels for ligation. The left pulmonary vein is the preferred site due to its relatively large ostium ([Supplemental Figures 2](#)). ([Movie 10](#))

3.2.10. Finally, connect the aortic cannula to the perfusion apparatus to complete the setup.

4. Physiological Monitoring and Electrophysiological Mapping

- 4.1. Begin coronary perfusion and allow an equilibration period of 10–15 minutes to enable the heart to transition from a quiescent to a spontaneously beating state ([Video 11](#)).
- 4.2. For rhythm monitoring, place a bipolar ventricular electrogram.
- 4.3. If necessary, restore organized electrical activity by defibrillating the heart with a single direct current shock of 20 J applied externally. Confirm successful defibrillation through electrocardiographic monitoring or visual inspection of coordinated contractions. In case of defibrillation, allow a stabilization period of 20–30 minutes before initiating experimental protocols.
- 4.4. Adjust the perfusion flow rate to maintain a mean coronary perfusion pressure of 60–70 mmHg, continuously monitored via an in-line pressure transducer. NB Perfusion rates are typically 2–3 mL/min/g of heart tissue, which can be further monitored using inline flow probes.
- 4.5. Blood electrolyte concentrations (Na^+ , K^+ ,) can be measured using the i-STAT point-of-care blood analyzer (Abbott Point of Care Inc., Princeton, NJ, USA). Throughout the experiment, monitor perfusate pH, electrolyte concentrations, and oxygen saturation at regular intervals (every 30–60 minutes) and make adjustments as necessary to maintain physiological conditions.
 - 4.5.1. Measurements should be performed according to the manufacturer's instructions.
- 4.6. For unipolar electrogram recordings, place a reference electrode at the aortic root and secure it in position.
- 4.7. [Figure 2](#) illustrates an example experiment with physiological monitoring, including left atrial and ventricular pressures, ventricular electrograms, and coronary flow and pressure recordings, demonstrating the integration of functional and perfusion measurements.
- 4.8. Position multi-electrode arrays on the epicardial or endocardial surfaces of interest (e.g., the right ventricular outflow tract), ensuring that the array dimensions and electrode density are appropriate for the experimental objectives. Secure the arrays using sutures and connect them to a multichannel data acquisition system.
- 4.9. Record unipolar electrograms at a sampling rate of 2–16 kHz, depending on the resolution required for the specific experimental protocol.

5. Experimental electrophysiological studies

5.1. Pacing & thresholding

- 5.1.1. Connect a current-controlled stimulator to the experimental setup. Use a bipolar stimulation electrode with two electrode poles connected to the negative and positive outputs of the stimulator. Bipolar stimulation is preferred as it produces localized myocardial activation.
- 5.1.2. Determine the stimulation threshold using the following procedure:

- 260 5.1.2.1. Set the initial output to approximately 1 mA, which should reliably capture
261 the myocardium in the isolated heart. This confirms correct stimulator connection
262 and adequate electrode-tissue contact. Capture can be easily detected through
263 electrocardiographic monitoring or visual inspection of contractions.
- 264 5.1.2.2. Reduce the output to zero, then gradually increase in 50 μ A increments until
265 myocardial capture occurs.
- 266 5.1.2.3. Refine the threshold by decreasing and increasing the output in steps of 10
267 μ A and 5 μ A to determine the precise stimulation threshold.
- 268 5.1.2.4. Set the stimulation intensity for subsequent protocols at 1.5 times the
269 determined threshold.
- 270 **5.2. Determine the effective refractory period using an S1-S2 protocol:**
- 271 5.2.1. Select a basic cycle length (S1) of 450-800 ms depending on heart size and species.
- 272 5.2.2. Deliver at least 8 S1 stimuli before introducing a premature stimulus (S2) to avoid
273 short-term cardiac memory effects.
- 274 5.2.3. Progressively shorten the S1-S2 interval until the myocardium fails to capture. The first
275 S1-S2 interval that fails to capture is the effective refractory period.
- 276 5.2.4. Alternative approach: First decrease the S1-S2 interval until capture fails, then
277 incrementally increase the S1-S2 interval in small steps until capture resumes. This
278 method provides more precise determination of the refractory period, though values
279 will be slightly longer than with the first approach due to cardiac memory.
- 280 **5.3. Induce arrhythmias using a programmed electrical stimulation protocol with**
281 **multiple premature stimuli (S1-S2-S3-S4-S5):**
- 282 5.3.1. Begin by determining the effective refractory period as described in step 5.2.
- 283 5.3.2. Set the S1-S2 interval to 10-20 ms longer than the determined refractory period.
- 284 5.3.3. Introduce a second premature stimulus (S3) at 10-20 ms longer than the determined
285 refractory period and progressively decrease the S2-S3 interval until capture fails. Set
286 the S2-S3 interval to 10-20 ms longer?
- 287 5.3.4. Repeat this process with additional premature stimuli (S4, S5) as needed until an
288 arrhythmia is induced.
- 289 5.3.5. Typically, up to 4 premature stimuli should be sufficient for arrhythmia induction in
290 most preparations.
- 291 **5.3.6. *Optional refinement for optimal timing:***
- 292 5.3.6.1. Position a recording electrode adjacent to the stimulation site to fine-tune
293 premature stimulus intervals. The T-wave of the unipolar electrogram recorded
294 near the stimulus electrode will display a positive deflection. Local repolarization
295 ends at the steepest portion of the rising phase of the local T-wave.
- 296 5.3.6.2. Time the delivery of S2 to coincide precisely with the rising phase of the local
297 T-wave following S1. Apply the same principle for subsequent premature stimuli
298 (S3, S4, S5), timing each stimulus to the rising phase of the T-wave generated by
299 the preceding stimulus.
- 300

301 6. **Data Analysis**

- 302 6.1. Export recorded electrograms to analysis software.

- 303 6.2. Determine local activation time by identifying the maximal negative derivative ($-dV/dt$) of
304 the unipolar electrogram at each electrode site.
- 305 6.3. Measure activation timing relative to the onset of the earliest ventricular deflection
306 (Purkinje or working myocardium) or pacing spike detected within the electrode array.
- 307 6.4. Generate activation maps and conduction velocity calculations using custom or
308 commercially available electrophysiology analysis software.
- 309 6.5. Perform statistical analysis of experimental outcomes according to the study design.
- 310

311 **7. Termination of Experiment**

- 312 7.1. At the conclusion of experimental procedures, stop perfusion and disconnect the heart from
313 the apparatus.
- 314 7.2. In case tissue samples are needed, rapidly excise regions of interest and process according
315 to the experimental protocol (e.g., fixation for histology, snap-freezing for molecular
316 analysis).
- 317 7.3. Dispose of biological waste according to institutional biosafety protocols.
- 318 7.4. Flush heating coils and tubing with distilled water. Drain all lines completely.
- 319 7.5. Clean and sterilize all reusable equipment according to standard laboratory procedures.

320
321

REPRESENTATIVE RESULTS:

Oxygenation and Maintenance of Blood-Tyrode's Perfusion Mixture

To maintain physiological conditions during ex vivo perfusion experiments, it is necessary to adequately oxygenate the blood-Tyrode's mixture. To achieve this, we use extracorporeal membrane oxygenators integrated into the perfusion circuit (see [Figure 2 & 3A](#)). To demonstrate adequate perfusion, we assessed the physiological state of the myocardium by determining the refractory periods of the ventricles in five conventional Langendorff-perfused sheep hearts ([Figure 3B](#)). These were found to be 283 ± 19 ms in the right ventricle and 296 ± 13 ms in the left ventricle at a pacing cycle length of 450 ms. These results are comparable to those observed in vivo by others¹⁸. Next, we investigated whether the composition of the blood-Tyrode's mixture remained stable throughout the experiment. During the 3-hour perfusion period, the pH, pCO₂, bicarbonate and potassium concentrations remained within physiological ranges and did not change ([Figure 3C](#)). However, the sodium concentration did increase slightly during the experiment (156 ± 2 vs. 169 ± 4 mmol/L, $p = 0.01$).

Electrophysiological Characterization Using Advanced Langendorff-perfusion

[Figure 4A](#) shows the advanced Langendorff configuration, which was used to measure ventricular pressure and ventricular unipolar electrograms simultaneously. As described above, we inserted a catheter to measure left ventricular pressure via the brachiocephalic artery ([Supplementary Figure 1](#)) and guided it through the aortic valve. We cannulated one of the pulmonary veins, which enabled us to fill the left atrium and ventricle and increase preload. Without preload, we observed a systolic pressure of ± 40 mmHg in the left ventricular cavity, which increased to ± 85 mmHg after when filled. When increased preload was applied, the shape of the T-wave in the unipolar electrogram, measured from the epicardium of the left ventricle, changed, indicating altered repolarization. This demonstrates the capacity of direct perfusion of the coronary arteries to allow measuring both mechanical and electrical function.

Electrophysiological Mapping in Conventional Langendorff Preparations

An advantage of the isolated heart is that it provides access to anatomical structures that are difficult to reach in vivo. [Figure 4B](#) shows epicardial mapping of Bachmann's bundle in an isolated pig heart. A high-density multielectrode grid (13×16 electrodes) was used to enable detailed spatial electrophysiological characterization of cardiac activation. Pacing from either the left or right atrium produced distinct propagation sequences, which are visible in the activation maps. Note that the electrogram at location b shows a single deflection during right atrial pacing whereas the deflection becomes fractionated when pacing from the left atrium.

Transmural activation is also difficult to measure in vivo. Although needle electrodes can be used in open-chest experiments ¹⁹, the isolated heart allows placement of needles in both the anterior and posterior walls and the ventricular septum. [Figure 4C](#) illustrates transmural activation of the ventricular walls and septum during pacing from either side of the ventricular septum. We inserted needles into the ventricular septum, accessing it by navigating through the tricuspid valves. Each needle contained four recording sites at different depths of tissue. Sequential pacing from opposite sides of the septum produced activation patterns that propagated transmurally, as visualized in the four-chamber reconstruction of electrode positioning and activation timing.

Arrhythmia Induction Protocol

Another advantage of Langendorff perfusion is the possibility of studying the origin and maintenance of arrhythmias. Programmed electrical stimulation is a reliable method of initiating arrhythmias in isolated hearts ²⁰. Using a decremental pacing protocol (S1-S2-S3-S4-S5), we successfully triggered sustained ventricular arrhythmia in an isolated pig heart ([Figure 4D](#)). The electrogram trace shows the transition from paced rhythm to arrhythmic activity, and the corresponding activation maps reveal the disorganized electrical propagation that is characteristic of ventricular tachyarrhythmia. This demonstrates the reproducibility of arrhythmia induction using standardized stimulation protocols in Langendorff-perfused preparations.

FIGURE AND TABLE LEGENDS:

Figure 1. Retrograde perfusion as a method for ex vivo perfusion of the isolated heart.

(A) A schematic drawing showing the insertion of a cannula into the aorta. Note that the cannula does not pass the entrance of either the left or right coronary artery, which is essential for enabling retrograde coronary perfusion. This is the conventional Langendorff-perfusion method (B) A novel approach to enabling selective perfusion of the coronary system by directly cannulating the coronary arteries. We refer to this approach as advanced Langendorff perfusion. The top left panel shows the cannulas to be inserted into the coronary arteries, and the bottom panel shows a schematic drawing of this process. The advanced Langendorff approach enables cannulation of the aorta to facilitate left ventricular ejection. The schematic drawing on the right demonstrates that inserting the aorta cannula superior to the first brachiocephalic artery enables non-invasive access to the ventricle for pressure catheter placement.

Figure 2. A schematic representation of advanced Langendorff perfusion with the parameters that can be investigated.

(A) The graphs at the left show the total coronary flow and the simultaneously recorded pressures in the left atrium, left ventricle, and the aorta. The trace at the bottom is a bipolar electrogram recorded simultaneously from the ventricle. All signals are recorded in a sheep heart. Coronary artery pressure is decoupled from native left ventricular systolic function, yet it retains a pulsatile pattern corresponding to the roller pump's operating frequency. (B) A schematic drawing showing the components required for advanced Langendorff perfusion.

Figure 3. Oxygenation of the Blood-Tyrod's perfusion mixture.

(A) A photograph of an oxygenator commonly used for Extracorporeal Membrane Oxygenation in a heart-lung machine. The use of an oxygenator is essential during blood-perfused Langendorff perfusion. (B) The graph showing the refractory period during blood perfusion indicates the physiological state of the right and left ventricular myocardium. (C) The graphs show pH, pCO₂, HCO₃⁻ Na⁺ and K⁺ concentrations before and after a ±3-hour experiment (n=5).

Figure 4. A variety of examples of electrophysiological experiments that can be performed during both conventional and advanced Langendorff perfusion.

(A) An example showing an advanced Langendorff perfusion sheep heart. The graph at the right indicates the interventricular pressure of the left ventricle before and after applying an electrical pacing protocol. Below are unipolar ventricular electrograms recorded before and after applying preload to the ventricle. Note the changes in the local T-wave. (B) The photographs show a multi-electrode array (13x16) on the Bachmann bundle of a

conventional Langendorff perfused pig heart. The activation maps show the direction of activation during either left or right atrial pacing. At the right, unipolar electrograms during right and left atrial pacing are shown from the locations indicated in the activation maps. (C) The photographs show a conventional Langendorff perfused pig heart with transmural needle electrodes, each containing 4 electrode terminals. The photo in the middle shows the needle electrodes that were placed into the ventricular septum from the right side. The schematic representation at the bottom shows a 4-chamber view illustrating transmural activation sequences during either pacing from the left or right side of the septum. (D) Data derived from a conventional Langendorff-perfused pig heart. The unipolar electrogram shows induction of ventricular arrhythmia using programmed stimulation (S1-S2-S3-S4). The activation maps are made from the beats indicated in the trace. LA: left atrium; RA: right atrium; LV: left ventricle; RV: right ventricle; VPB: ventricular premature beat.

Supplemental Figure 1

Cannulations for establishing direct coronary perfusion in the isolated heart. **(A)** Overview of the freshly excised sheep heart with major cannulation sites identified. The aortic arch is cannulated for an aortic outflow cannula (AoC), and a hemostatic valve (AOhv) is positioned in the brachiocephalic artery to allow non-invasive left ventricular access. The pulmonary artery cannula (PAC) is secured for outflow drainage. Anatomical landmarks include the right ventricular anterior wall (RVAW), left ventricular anterior wall (LVAW), and left atrial free wall (LAFW). **(B)** Exposure of the left coronary ostium with a rubber strap placed below the left coronary artery catheter (LCc) for visualization. The left atrial free wall (LAFW) is shown for orientation. **(C)** Placement of the right coronary artery catheter (RCc) after isolation of the right coronary ostium. Both aortic catheters (AoC, AOhv) and the pulmonary artery cannula (PAC) are visible. **(D)** Close-up view of the right coronary artery (RCA) with a rubber strap in place, illustrating positioning required for selective perfusion. **(E)** Larger view of the final left coronary cannulation configuration with the left coronary artery catheter (LCc). The left ventricular anterior wall (LVAW) and left atrial free wall (LAFW) are shown for spatial orientation. See legend to supplemental figure 2 for abbreviation list.

Supplemental Figure 2

Views of the heart mounted on the perfusion apparatus.

A) Posterior view of the heart. The left atrial cannula, clamped to prevent emptying of atrial volume, is oriented toward the viewer. A hemostatic valve and a second cannula in the left atrial veins are also visible. **(B)** Right anterior oblique view, slightly rotated anteriorly, showing the pulmonary artery and aortic hemostatic valve. **(C)** Cranial view of the heart, providing a clear view of atrial and pulmonary vein cannulation. **(D)** Left anterior view of the heart. **(E)** Anterior view of the heart.

Abbreviations (applied to both figures where relevant): AO, aorta; AOhv, aortic

460 hemostatic valve; PA, pulmonary artery; AoC, aortic outflow cannula; PAC, pulmonary artery
461 cannula; RVAW, right ventricular anterior free wall; LVAW, left ventricular anterior wall;
462 LVLW, left ventricular lateral free wall; LVPW, left ventricular posterior wall; IVGr,
463 interventricular groove; RAFW, right atrial free wall; LAFW, left atrial free wall; LPVc, left
464 pulmonary vein cannula; RSPV, right superior pulmonary vein; RIPV, right inferior
465 pulmonary vein; RIPVhv, right inferior pulmonary vein hemostatic valve; LCc, left coronary
466 artery catheter; RCc, right coronary artery catheter.

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DISCUSSION

We demonstrate that both conventional and advanced Langendorff approaches offer a flexible experimental environment for studying cardiac arrhythmias in a controlled setting. Blood-perfused Langendorff perfusion maintains stable physiological parameters over extended periods, enabling comprehensive electrophysiological characterization and a variety of recording methods, such as single electrode measurements, high-density mapping arrays, and transmural needle electrodes. Crucially, we demonstrate that programmed electrical stimulation reliably induces arrhythmias in these preparations, thereby validating ex vivo perfused hearts as models for studying arrhythmogenic mechanisms. Advanced Langendorff perfusion further enhances experimental capabilities by enabling the simultaneous assessment of ventricular pressure dynamics and electrophysiology. Together, these findings establish perfused hearts as an invaluable tool for studying arrhythmia mechanisms, offering precise experimental control while maintaining physiological relevance.

The isolated Langendorff-perfused heart model has been in use for over a century, playing a key role in landmark discoveries in electrophysiology. For example, Durrer and colleagues used it to determine the activation sequence of the human heart in 1970^{1,2,4,9}. During the same period, Neely and his team adapted Langendorff's classic heart model to create a dynamic 'working heart' setup²¹. This setup allowed the heart to pump blood antegradely through the aorta to maintain its own coronary perfusion. This working heart system has been adopted by many laboratories for studying cardiac mechanics and its relationship with electrophysiology^{10,22,23}. This has shown that intraventricular pressure and wall stretch may affect triggers and substrates for arrhythmias. However, studying arrhythmias in the working heart setup is difficult as cardiac output and coronary flow fall during an arrhythmia. Although quick defibrillation can convert the arrhythmia, in our experience it takes time for contractile force to normalize and to restore coronary flow in the isolated heart setting. For this reason, we believe that making coronary perfusion independent of ventricular contraction, as we effectuate here, provides a more stable platform for studying the relationship between cardiac mechanics and the mechanisms of arrhythmias.

The crystalloid perfusion fluid composition presented by Tyrode in 1910 has become the main fluid used for Langendorff perfusion to date^{3,24}. For large mammal hearts, however, perfusion with blood is preferred for of several reasons. The main advantages of perfusion with blood or red blood cells over a crystalloid solution, such as Krebs-Henseleit (KH) solution or Tyrode's solution, lie in the significantly improved physiological relevance and stability of the heart preparation, particularly oxygen supply¹⁰. Despite being aerated with high oxygen concentrations (e.g. 95% O₂), crystalloid perfusates have a supraphysiological oxygen pressure (~450 mmHg) but a very low oxygen-carrying capacity. This results in

approximately 1.5 ml O₂ per 100 ml, which makes working hearts highly susceptible to hypoxia. The inclusion of red blood cells in the perfusate significantly increases the oxygen-carrying capacity (e.g. to 11 ml O₂ per 100 ml at 25% hematocrit) and increases myoglobin oxygen saturation in the myocardium, creating an oxygen reserve that is crucial in the event of increased oxygen demand or a drop in arterial pO₂. Furthermore, blood perfusion helps to achieve more physiologically relevant hemodynamics, as the supraphysiological coronary flow rates required in experiments with KH-perfused hearts are reduced to within the *in vivo* range with the addition of red blood cells ²⁵. Functionally, blood- or red blood cells -perfused hearts suffer much less from oedema than hearts perfused with crystalloid fluids and maintain ATP levels higher than those in KH-perfused hearts during longer perfusion periods ²⁶. This results in high functional stability, with left ventricular developed pressure deteriorating by less than 5% per hour, while also reducing the incidence of ventricular fibrillation after programmed stimulation ²⁷. Finally, it has been observed that the conduction velocity in isolated Purkinje fibers is often faster in perfusion with blood than with Tyrode's solution, suggesting that electrophysiological experiments performed with blood perfusion more accurately reflect the *in vivo* situation ²⁸.

Our perfusion system needs a minimum of 1500 ml, which is more than the volume of blood we can collect from the animal, especially pigs younger than 6 months. Therefore, we use a 1:1 mixture of Tyrode's solution and blood. Our data showed that the pH of the blood-Tyrode mixture was 7.5±0.03, while stable throughout experiments, is more alkaline than what we expect based on human blood gas analysis. pH measurements in *in vivo* sheep, however, have shown that in sheep the pH is more alkaline ²⁹. Concentrations of HCO₃⁻ and K⁺ and pCO₂ were as expected and did not change throughout the experiment. Sodium, on the other hand, increased over time course of the experiment. This has been observed before in isolated heart perfusion ³⁰. As the intracellular sodium concentration in cardiomyocytes and blood cells is <10 mM it is unlikely that the sodium increase comes from intracellular sources ³¹. It is more likely that movement of water from the perfusion fluid (±100 ml) into the tissue has concentrated the sodium in the perfusate ³².

When performed by an experienced team, conventional or advanced Langendorff perfusion can have a high success rate. However, there are several key steps, which we will briefly discuss here. After excision, it is critically important to put the heart directly on ice. Once on cooled, there is sufficient time (up to an hour) to prepare the aorta and the right and left coronary arteries. When placing the coronary cannulas for advanced Langendorff perfusion, this should be done below the fluid surface, with the cannulas filled with Tyrode's solution in order to avoid trapping of air bubbles. The tip of the cannula should also be positioned as close to the coronary ostium as possible to avoid occluding early arterial branches. In particular, the sinoatrial nodal artery is at risk of inadvertent closure by the right coronary cannula. For placing the aorta cannula in the conventional Langendorff setup, it is important to ensure that the cannula and the aorta are filled with Tyrode's solution when connecting

the cannula to the setup.

We prefer controlling perfusion based on pressure because it resembles native coronary regulation more closely than constant-flow perfusion. Under pressure-controlled conditions, coronary resistance can adjust dynamically, allowing flow to vary in a physiologically meaningful way. We therefore maintain a target perfusion pressure of 60–70 mmHg, as this range is recommended to reduce the risk of pressure-induced oedema³³. Flow rate is continuously monitored to verify adequate global perfusion, which typically reaches 2–3 ml/min/g of heart weight under physiological conditions. During the experiment, it is important to monitor the minimum level of perfusion fluid in the main reservoir. If the level gets too low, the pump will draw in air, which, when it reaches the heart, will cause occlusion of small branches of the coronary arteries and thus result in ischemia. The oxygenator also functions as a bubble trap, although its capacity is naturally limited. Maintaining the temperature is important during the experiment, as temperature has a significant impact on ion channel kinetics, and consequently on conduction and repolarization.

Determining the desired preload level in advance when measurements are performed under working-heart (loaded) conditions is advisable. The height of the preload chamber determines the preload pressure, with an approximate hydrostatic relationship of 1.36 cm H₂O per mmHg. It is also essential to ensure that the tubing connecting the preload chamber to the left atrium has a sufficiently large diameter to permit passive flow greater than the expected cardiac output. If the resistance in this line is too high, a strongly contracting ventricle may create a suction effect in the left atrium, resulting in non-physiological filling. On the outflow side, the Windkessel functions as a compliant arterial reservoir, analogous to the native elastic aorta. The adjustable clamp positioned downstream of the Windkessel chamber mimics the resistance of the arterial system. This resistance should be adjusted to achieve the desired end-systolic pressure and establish a physiological afterload for the preparation.

Limitations

A limitation of the isolated heart preparation is that it is disconnected from the autonomic nervous system, with significant consequences for studying arrhythmias. For example, when studying arrhythmias, the absence of sympathetic input results in much smaller repolarization gradients^{19,34}. These gradients form the basis of the functional substrate required for the initiation of re-entry and, consequently, ventricular fibrillation. It also impacts mechanics, as the contractility of the ventricular myocardium and, consequently, its force of contraction are reduced, meaning its effect on electrophysiological parameters is also reduced. Despite this limitation, the isolated heart model has formed the basis of our understanding of ventricular fibrillation, providing insights that have led to the anti-arrhythmic strategies currently used in clinical practice³⁵.

Conclusion

In conclusion, both conventional and advanced Langendorff approaches offer versatile experimental platforms for the study of cardiac arrhythmias in a controlled environment. The ability to maintain stable physiological parameters while assessing both ventricular pressure dynamics and electrophysiology simultaneously establishes perfused hearts as valuable tools for studying arrhythmogenic mechanisms with precise experimental control and physiological relevance.

ACKNOWLEDGMENTS:

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DISCLOSURES:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES:

1. Langendorff, O. Untersuchungen am überlebenden Säugethierherzen. *Pflüg. Arch. Für Gesamte Physiol. Menschen Thiere* **61**, 291–332 (1895).
2. Bell, R. M., Mocanu, M. M. & Yellon, D. M. Retrograde heart perfusion: The Langendorff technique of isolated heart perfusion. *J. Mol. Cell. Cardiol.* **50**, 940–950 (2011).
3. King, D. R., Hardin, K. M., Hoeker, G. S. & Poelzing, S. Reevaluating methods reporting practices to improve reproducibility: an analysis of methodological rigor for the Langendorff whole heart technique. *Am. J. Physiol. Heart Circ. Physiol.* **323**, H363–H377 (2022).
4. Durrer, D. *et al.* Total Excitation of the Isolated Human Heart. *Circulation* **41**, 899–912 (1970).
5. Schechter, M. A. *et al.* An isolated working heart system for large animal models. *J. Vis. Exp. JoVE* 51671 (2014) doi:10.3791/51671.

- 623 6. DeWitt, E. S., Black, K. J. & Kheir, J. N. Rodent Working Heart Model for the Study of
624 Myocardial Performance and Oxygen Consumption. *J. Vis. Exp. JoVE* 54149 (2016)
625 doi:10.3791/54149.
- 626 7. Lang, D., Sulkin, M., Lou, Q. & Efimov, I. R. Optical Mapping of Action Potentials and Calcium
627 Transients in the Mouse Heart. *J. Vis. Exp.* 3275 (2011) doi:10.3791/3275.
- 628 8. Hatami, S. *et al.* Normothermic Ex Situ Heart Perfusion in Working Mode: Assessment of
629 Cardiac Function and Metabolism. *J. Vis. Exp. JoVE* <https://doi.org/10.3791/58430>
630 (2019) doi:10.3791/58430.
- 631 9. Sutherland, F. J. & Hearse, D. J. The isolated blood and perfusion fluid perfused heart.
632 *Pharmacol. Res.* **41**, 613–627 (2000).
- 633 10. Kuzmiak-Glancy, S., Jaimes, R., Wengrowski, A. M. & Kay, M. W. Oxygen demand of
634 perfused heart preparations: how electromechanical function and inadequate
635 oxygenation affect physiology and optical measurements. *Exp. Physiol.* **100**, 603–616
636 (2015).
- 637 11. Boukens, B. J., Rivaud, M. R., Rentschler, S. & Coronel, R. Misinterpretation of the mouse
638 ECG: ‘musing the waves of *Mus musculus*’: Analysing the mouse electrocardiogram. *J.*
639 *Physiol.* **592**, 4613–4626 (2014).
- 640 12. Coronel, R., Wilms-Schopman, F. J. G., Opthof, T. & Janse, M. J. Dispersion of repolarization
641 and arrhythmogenesis. *Heart Rhythm* **6**, 537–543 (2009).
- 642 13. Opthof, T., Meijborg, V. M. F., Belterman, C. N. W. & Coronel, R. Synchronization of
643 repolarization by mechano-electrical coupling in the porcine heart. *Cardiovasc. Res.* **108**,
644 181–187 (2015).
- 645 14. Blok, M. *et al.* Functional conduction system mapping in sheep reveals Purkinje spikes in

the free wall of the right ventricular outflow tract. *Front. Physiol.* **16**, 1631426 (2025).

15. Hoogendijk, M. G. *et al.* Hexagonal Multielectrode Design for Recording Local Electrograms With Superior Characteristics. *JACC Clin. Electrophysiol.* **11**, 1606–1612 (2025).

16. Kaffka Genaamd Dengler, S. E. *et al.* Validation of the slaughterhouse porcine heart model for ex-situ heart perfusion studies. *Perfusion* **39**, 555–563 (2024).

17. Längin, M. *et al.* Cold non-ischemic heart preservation with continuous perfusion prevents early graft failure in orthotopic pig-to-baboon xenotransplantation. *Xenotransplantation* **28**, e12636 (2021).

18. Ma, L. & Wang, L. Effect of acute subendocardial ischemia on ventricular refractory periods. *Exp. Clin. Cardiol.* **12**, 63–66 (2007).

19. Meijborg, V. M. F. *et al.* Stellate ganglion stimulation causes spatiotemporal changes in ventricular repolarization in pig. *Heart Rhythm* **17**, 795–803 (2020).

20. Wellens, H. J. & Durrer, D. Electrical Stimulation of the Heart in Patients with Ventricular Tachycardia. (1972).

21. Neely, J. R., Liebermeister, H., Battersby, E. J. & Morgan, H. E. Effect of pressure development on oxygen consumption by isolated rat heart. *Am. J. Physiol.* **212**, 804–814 (1967).

22. Vaillant, F. *et al.* Magnetic resonance-compatible model of isolated working heart from large animal for multimodal assessment of cardiac function, electrophysiology, and metabolism. *Am. J. Physiol. Heart Circ. Physiol.* **310**, H1371-1380 (2016).

23. Vaillant, F. *et al.* Influence of pericardium on ventricular mechanical interdependence in an isolated biventricular working pig heart model. *J. Physiol.* **603**, 285–300 (2025).

- 669 24. Tyrode, M. V. The mode of action of some purgative salts. *Arch. Int. Pharmacodyn.*
670 *Thérapie* **20**, 205–223 (1910).
- 671 25. Mouren, S., Vicaut, E., Lamhaut, L., Riou, B. & Ouattara, A. Crystalloid versus red blood
672 cell-containing medium in the Langendorff-perfused isolated heart preparation. *Eur. J.*
673 *Anaesthesiol.* **27**, 780–787 (2010).
- 674 26. Bergmann, S. R., Clark, R. E. & Sobel, B. E. An improved isolated heart preparation for
675 external assessment of myocardial metabolism. *Am. J. Physiol.* **236**, H644–661 (1979).
- 676 27. Gillis, A. M., Kulisz, E. & Mathison, H. J. Cardiac electrophysiological variables in blood-
677 perfused and buffer-perfused, isolated, working rabbit heart. *Am. J. Physiol.* **271**, H784-
678 789 (1996).
- 679 28. Rosen, M. R., Gelband, H. & Hoffman, B. F. Effects of blood perfusion on
680 electrophysiological properties of isolated canine Purkinje fibers. *Circ. Res.* **30**, 575–587
681 (1972).
- 682 29. Mitchell, B. & Williams, J. T. Normal blood-gas values in lambs during neonatal
683 development and in adult sheep. *Res. Vet. Sci.* **19**, 335–336 (1975).
- 684 30. Kappler, B. *et al.* Investigating the physiology of normothermic ex vivo heart perfusion in
685 an isolated slaughterhouse porcine model used for device testing and training. *BMC*
686 *Cardiovasc. Disord.* **19**, 254 (2019).
- 687 31. Guthe, C. C., Harris, A. L., Thio, B., Moreland, R. S. & Bohr, D. F. Red blood cell sodium in
688 the DOCA hypertensive pig. *Hypertens. Dallas Tex* 1979 **5**, V105–109 (1983).
- 689 32. Woo-Ming, M., Wu, N. M., Lester, J. L. & Malinin, T. I. Water content of hearts perfused
690 with crystalloid and nutrient solutions. *J. Mol. Cell. Cardiol.* **12**, 371–386 (1980).
- 691 33. Skrzypiec, D. M., Pollintine, P., Przybyla, A., Dolan, P. & Adams, M. A. The internal

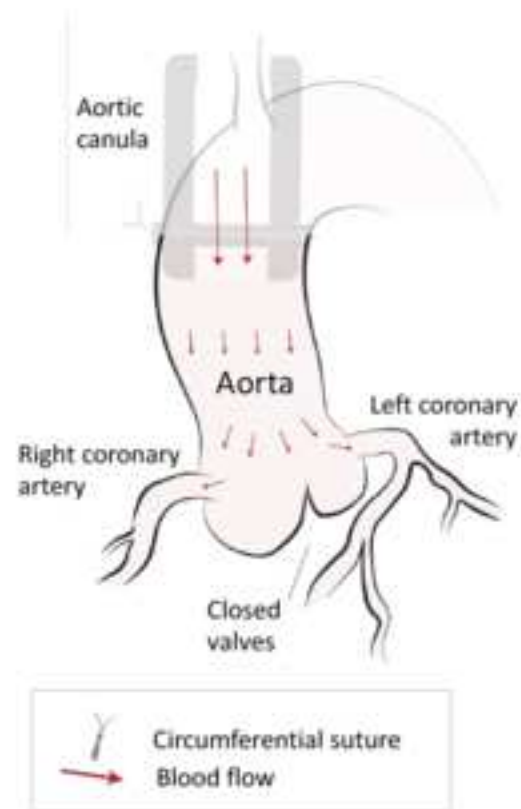
mechanical properties of cervical intervertebral discs as revealed by stress profilometry.
Eur. Spine J. Off. Publ. Eur. Spine Soc. Eur. Spinal Deform. Soc. Eur. Sect. Cerv. Spine Res. Soc.
16, 1701–1709 (2007).

34. Vaseghi, M. *et al.* Sympathetic innervation of the anterior left ventricular wall by the right
and left stellate ganglia. *Heart Rhythm* **9**, 1303–1309 (2012).

35. Boukens, B. J. & Janse, M. J. Brief history of arrhythmia in the WPW syndrome - the
contribution of George Ralph Mines: Brief history of arrhythmia in the WPW syndrome.
J. Physiol. **591**, 4067–4071 (2013).

Figure 1.

A Conventional Langendorff
Retrograde perfusion



B Advanced Langendorff perfusion
Direct cannulation of the coronary arteries

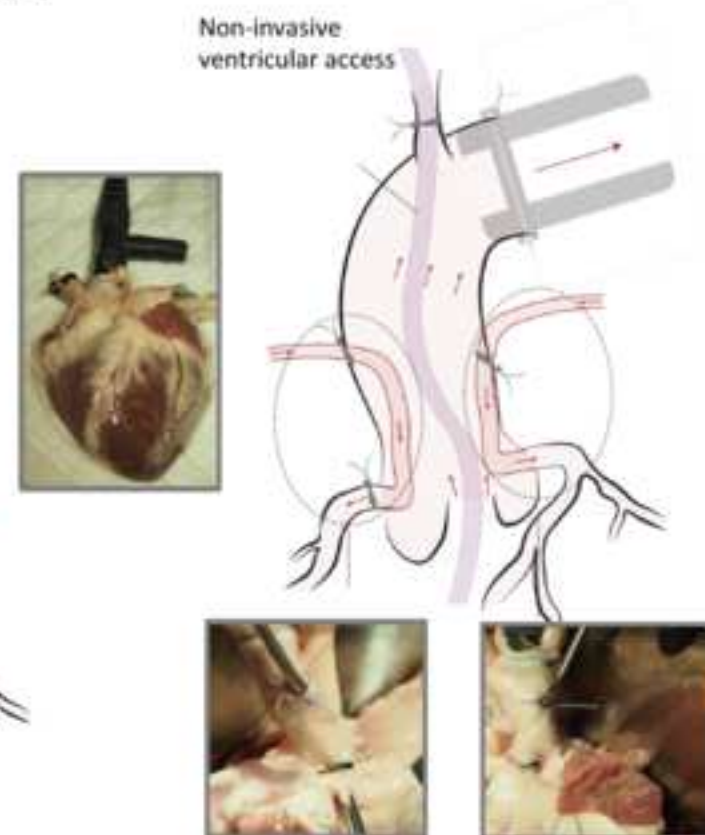
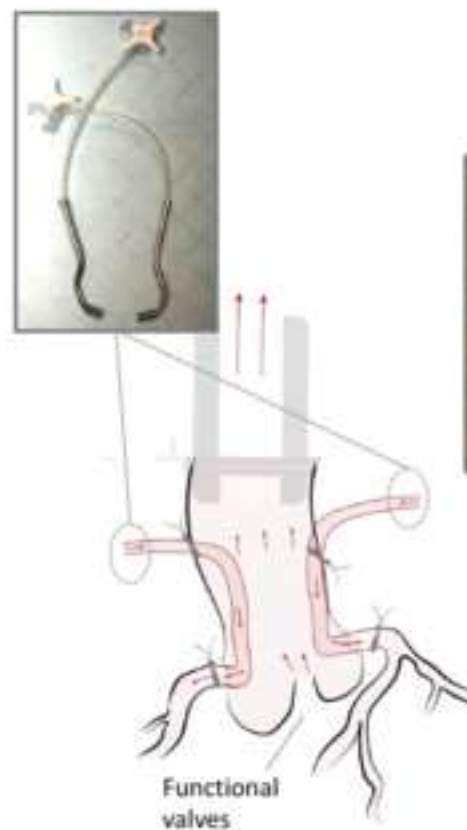


Figure 2.

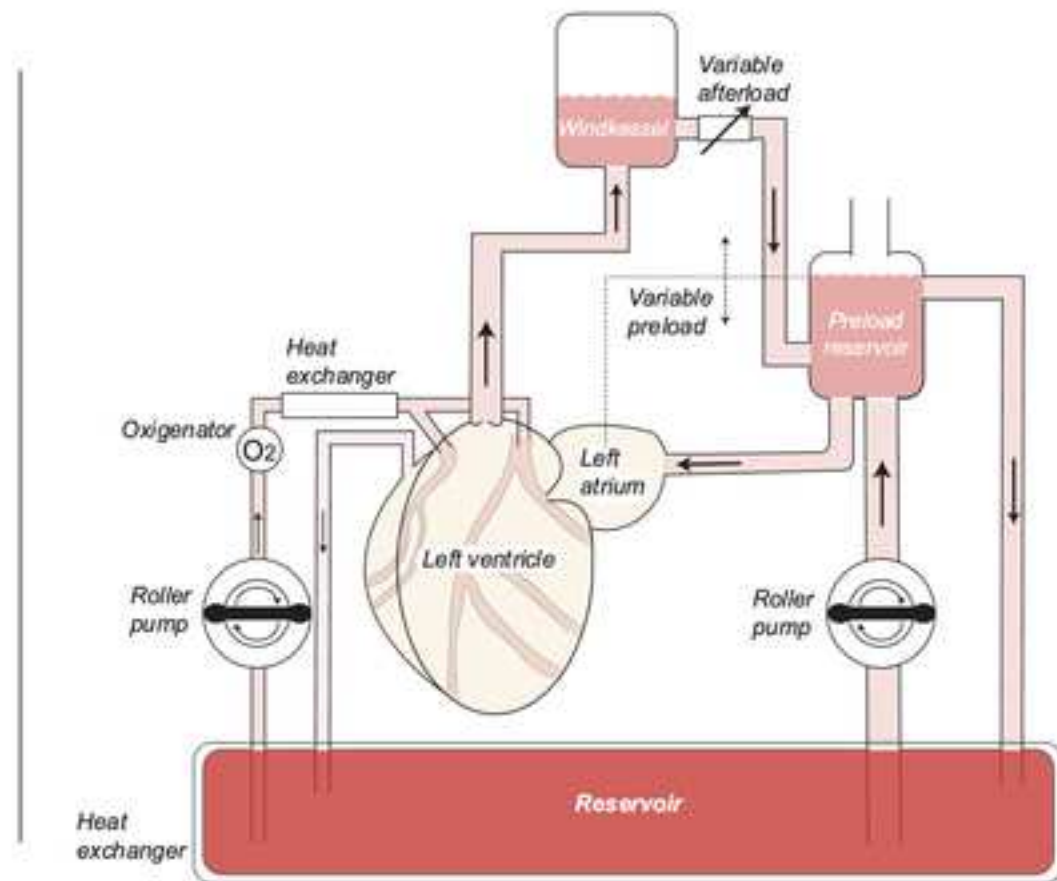
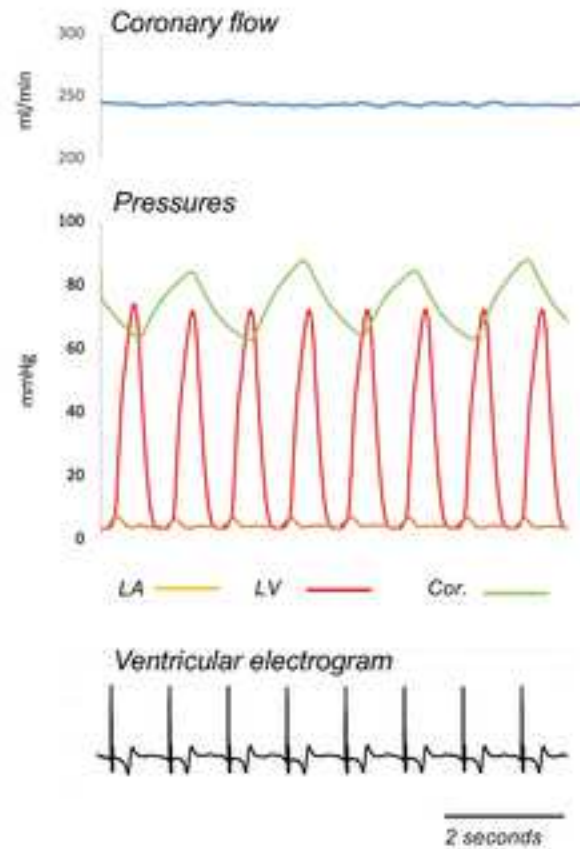


Figure 3.

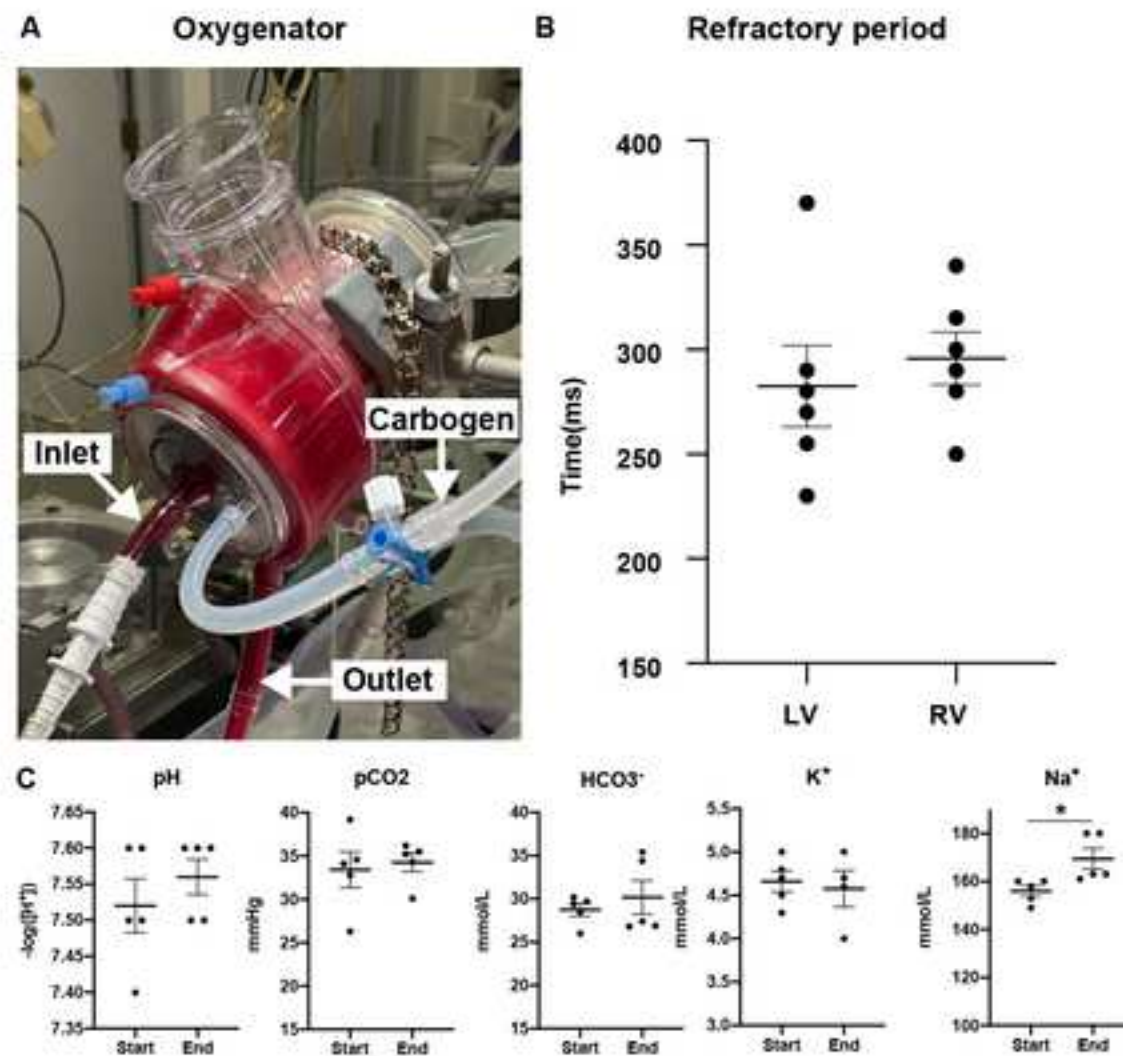
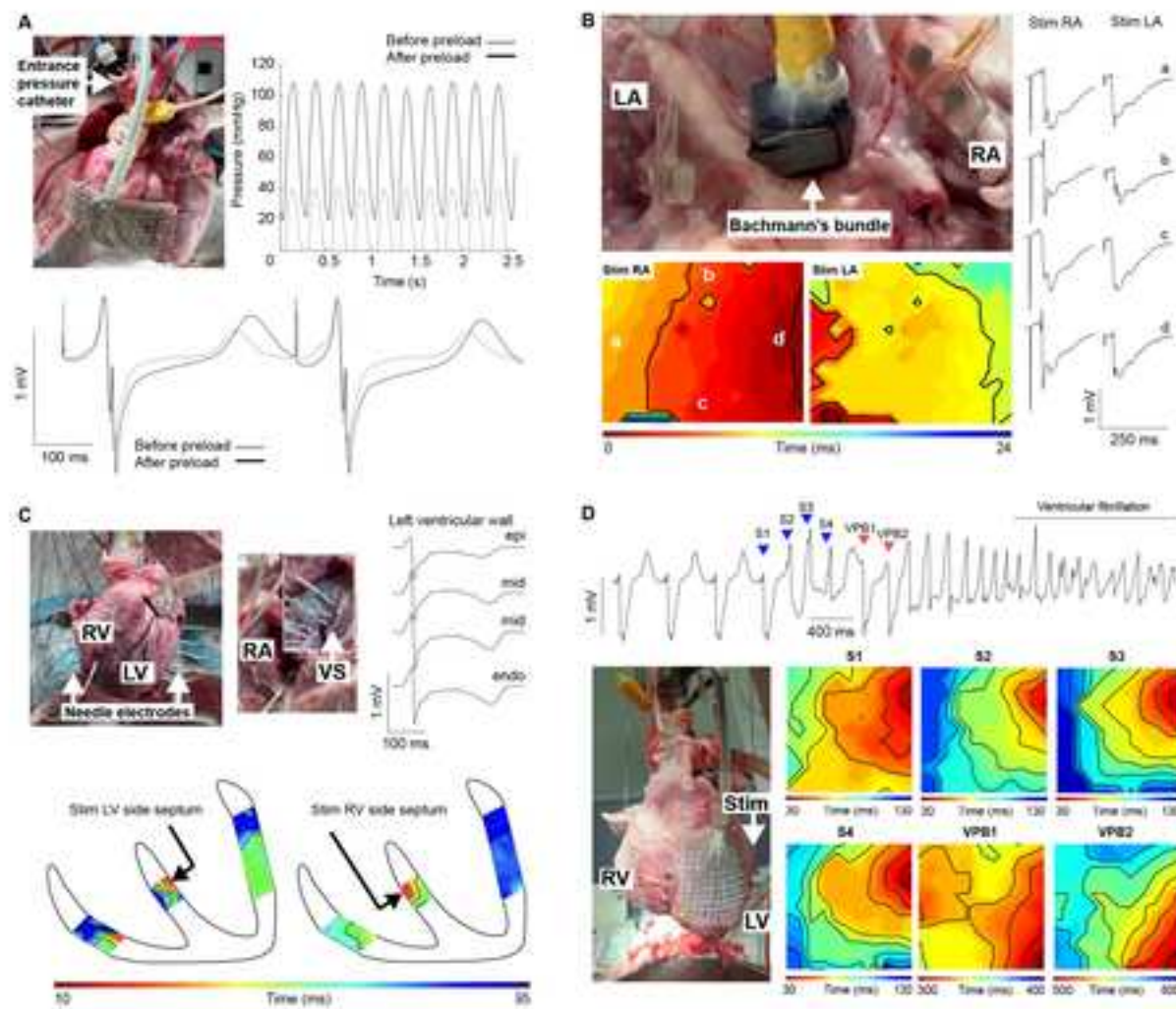


Figure 4.

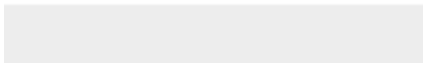
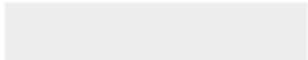


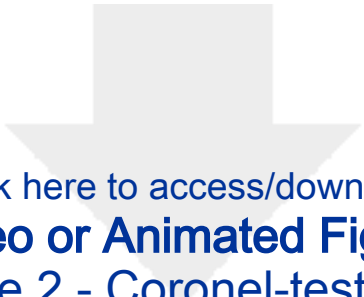


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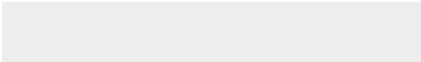
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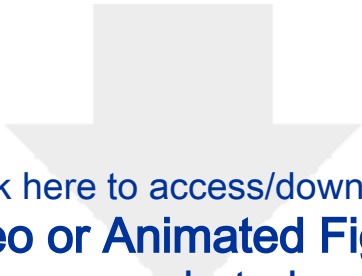
Movie 1 - Placing cannula aorta outflow II.MOV





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Movie 2 - Coronel-test.mov

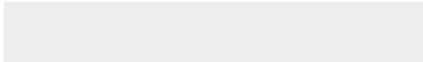


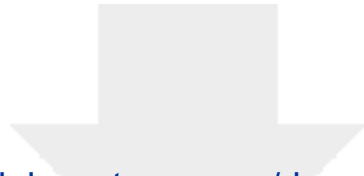


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Video or Animated Figure

Movie 3 - Connecting cannula to langendorff setup.MOV

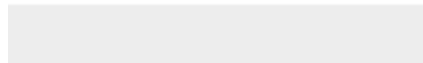




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Movie 4 - Exposing RCA.MOV

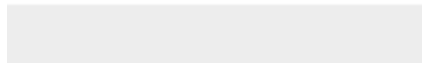


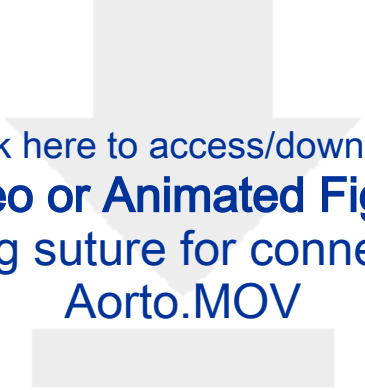


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Movie 5 - Placing suture around RCA.MOV

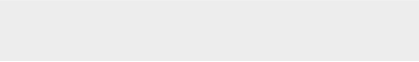
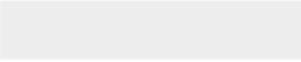


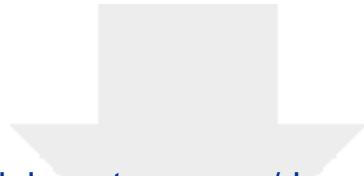


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Movie 6 - Placing suture for connecting cannule in
Aorto.MOV

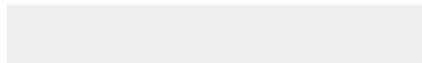


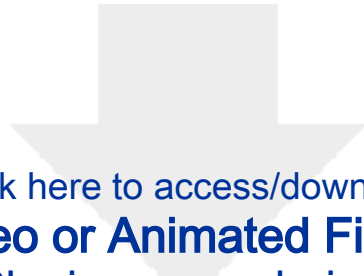


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Movie 7 - Placing RCA cannula.MOV

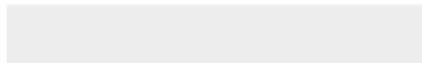


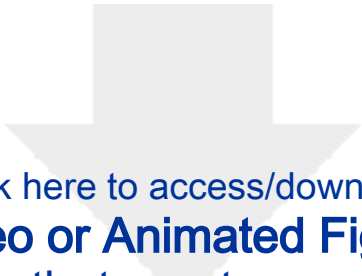


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Movie 8 - Placing cannule in Aorta.MOV

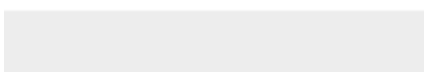
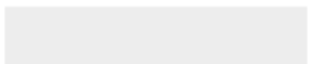


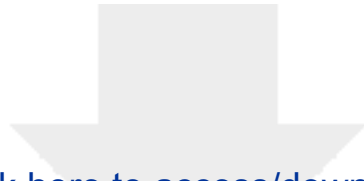


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[Movie 9 - Placing catheter entrance in subclavian.MOV](#)

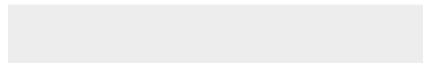


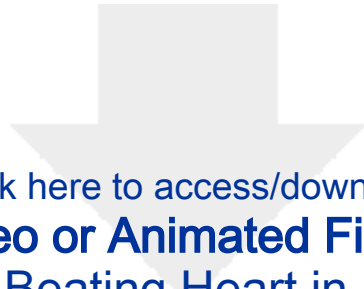


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Movie 10 - Placing LA inflow.MOV

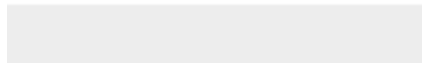


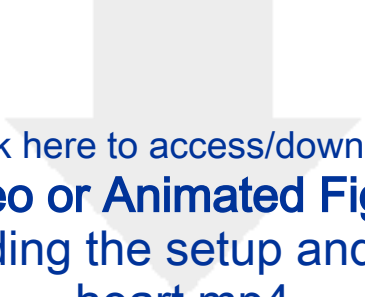


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Video or Animated Figure

Movie 11 - Beating Heart in Setup.MOV





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Video or Animated Figure

Movie 12 - Building the setup and connecting the
heart.mp4

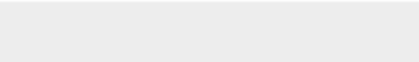
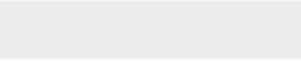


Table of materials

Table 1. Perfusion System Components

Component	Description / Notes
Perfusion tubing	Appropriate diameters for all circuit segments
Quick-connect tubing connectors	For rapid, leak-free circuit assembly
Tubing clamps	General-purpose clamps for securing or isolating tubing
Windkessel compliance chamber	With inlet and outlet
Preload reservoir	Inlet/outlet and open air vent to avoid negative pressure
Adjustable pinch clamp	For afterload control
Heating pump (reservoir)	Maintains reservoir temperature
Heating pump (heating coil)	Drives heating-coil circulation
Heating coil	For fluid temperature conditioning
Heating-circuit tubing	For heating-water circulation
Roller pump (≤ 800 mL/min)	Used for coronary perfusion
Roller pump (> 5 L/min)	Used for the working-heart circulation
Carbogen supply	Gas mixture source
Oxygenator	For oxygenating perfusate and bubble trap
Stainless steel coronary cannulas	For right and left coronary ostia
3D-printed aortic cannula + counterpiece	Connection interface to perfusion system
3D-printed left atrial cannula	For LA connection
8F hemostatic valve	For brachiocephalic cannulation
5F hemostatic valve	For pulmonary vein cannulation
Millar catheters (2×, 5–8F)	For LA and LV pressure recordings
Baxter in-line pressure transducer	For coronary pressure monitoring
Bridge amplifiers (3×, 5-V excitation)	For pressure sensors
Optional: Transonic in-line flow probes	For coronary flow monitoring
Optional: Transonic flowmeter console	For flow-probe readout
Bipolar electrodes (2×)	~10-mm interelectrode spacing
Differential amplifier	For electrogram measurements

Component	Description / Notes
Data acquisition system	For multi-channel recording
Pacing unit	For electrical stimulation

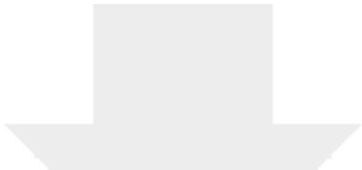
Table 2. Tyrode Solution Composition (for ~2 L)

Component	Amount
MQ water	1.5 L
NaCl	300 g
KCl	14 g
CaCl ₂ stock solution (1300 mM)	44.6 mL
MgCl ₂ stock solution (25%)	19.6 mL
NaHCO ₃	91 g
NaH ₂ PO ₄	2.42 g
Glucose	2 g

Table 3. Heart Explantation and Preparation Materials

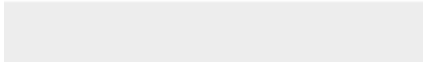
Component	Description / Sizes
Mechanical ventilator	For controlled ventilation
Endotracheal tubes	7–9 mm
Anesthesia pumps	For maintenance
Anesthetics	Per institutional protocol (e.g., pentobarbital, propofol, sufentanil)
Heparin	5000 IU/mL
Syringes	2–60 mL
Needles	18–22 G
Forceps	Various sizes
Scissors	Various sizes
Uterine scissors	Large, for cardiac explantation
Scalpel handles	Size 3 and 4
Scalpel blades	#11 and #23
Needle holder	—
Rib retractor	—

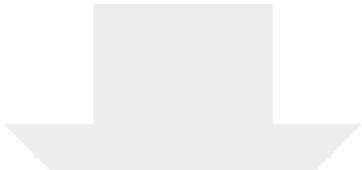
Component	Description / Sizes
Sutures	0, 2-0, and 4-0 (needle sizes 22–30 mm)



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