1. **ESHP software initialization and adjustments**
   1. After initializing the controlling software and de-airing of the ESHP apparatus, donor heart procurement may proceed.
2. **Blood collection and heart procurement**
   1. Perform a median sternotomy.
      1. Open the pericardium with a Metzenbaum Scissor and fix the pericardial edges to the sternum using 1-0 silk suture.
   2. From the two-stage venous cannula placed in the right atrium, collect 750 mL of whole blood from the pig gradually over a period of 15 min, into an autoclaved glass container, and simultaneously replace the volume with 1 L of an isotonic crystalloid solution such as Plasmalyte A.
   3. Add the blood to the perfusion circuit (which has been previously primed with 750 mL Krebs-Henseleit buffer containing 8% albumin) to reach a final volume of 1.5 L of perfusate. The perfusate is a 1:1 combination of Krebs-Henseleit containing 8% albumin solution and whole blood from the donor animal [17](#_ENREF_17).
   4. Place a cardioplegia needle (14-16 F) in the ascending aorta and secure it with a snare.
   5. Connect the cardioplegia cannula to the cardioplegia bag, to add 100 mL of blood to 400 mL of cardioplegia (St. Thomas Hospital Solution No. 2) to reach a final volume of 500 mL blood cardioplegia.
   6. Cross-clamp the ascending aorta with an aortic clamp and deliver the cardioplegic solution into the aortic root.
   7. Excise the heart ensuring all of the aortic arch vessels are procured along with a segment of descending aorta. Preserve up to the pa bifurcation.
3. **Placement of the heart onto the ESHP apparatus and initiation of perfusion**
   1. Place a purse-string suture around the LA orifice using a 3-0 polypropylene suture.
   2. Place the LA cannula into the LA orifice and secure it with a snare (Figure 6).
   3. Suture and close the inferior vena cava with a 3-0 polypropylene suture. Leave the superior vena cava open at the beginning of the perfusion to ensure the right ventricle (RV) remains decompressed until the perfusate warmed and an organized rhythm is achieved.
   4. Gently squeeze the ventricles to de-air the heart. Place the LA cannula over the magnet embedded in the silicon membrane. Ensure the magnet in the silicone and the corresponding metal ring in the LA cannula are properly engaged.
   5. Attach the aorta to the aortic cannula embedded in the silicone membrane. Secure the aorta around the cannula with a silk tie. Trim the aorta to achieve a proper lie without tension or kinking.
   6. Increase the aortic pump speed to 1600 RPM. The remaining air in aortic root will be ejected through the innominate and subclavian branches.
   7. Connect the aortic purge line to the innominate artery. Secure the connection with a silk tie.
   8. Snare the left subclavian artery orifice with a silk tie. Secure the closure with a snare and snap. Through the orifice of the subclavian artery, place an introducer sheath (5f). Ensure that the length of the catheter and its orientation is properly adjusted so that it does not interfere with aortic valve function.
   9. Connect the Ao pressure transducer to the introducer sheath side port.
   10. Read the Ao pressure on the monitor. Adjust the aortic pump speed to reach a mean pressure of 30 mm Hg. At this point (Time 0) the perfusion has started in the non-working mode (Langendorff mode) and appearance of a dark deoxygenated perfusate in the PA line is a reflector of reestablishment of coronary flow. Set a timer to follow duration of the perfusion if needed.
   11. Turn on the heat exchanger and set the temperature to 38 ˚C. The perfusate will warm up to 37-38 ˚C in approximately 10 minutes. For normothermic perfusion of a porcine heart, keep the temperature at 38 ˚C throughout the perfusion.
   12. Maintain the perfusion in non-working mode for the first hour of the perfusion. Adjust the LA pump speed to maintain the LA pressure at 0 mmHg.
   13. Check the dissolved gas status using a blood gas analyzer. Adjust the gas mixture to maintain a pH: 7.35-7.45, arterial partial pressure of carbon dioxide (PaCO2): 35-45 mmHg, arterial partial pressure of oxygen(PaO2): of 100-150 mmHg, and sO2 ≥ 95 %.
   14. Switch to working mode after 1 hour of perfusion in Langendorff mode. For this purpose, enter the desire LA pressure (typically 6-8 mmHg) on the left side of the main page, in the “desired LAP” section of the software, and click on the button to initiate the feedback loop. The activated working mode will appear as a green button, and the LA pump speed will automatically increase and decrease to reach and maintain the desired LA pressure.

Note: The ESHP controlling software automatically calculates and records steady-state hemodynamic and functional indices every ten seconds.

* 1. Assessment of steady state systolic and diastolic function
     1. For assessment and recording of the steady state data, through the introducer sheath placed earlier in the subclavian artery, place a fluid-filled pigtail catheter into the left ventricle (LV) while in working mode.
  2. Assessment of preload recruitable stroke work (PRSW)
     1. Remove the pigtail catheter from the left ventricle, since the catheter may induce arrhythmias during PRSW analysis that will negatively affect the accuracy of the results.
     2. On the main page, in the “Capture PVL” section, adjust the desired rate of drop in LA pump speed during the analysis (typically 100-200 RPM) and desired time during which the analysis will take place (typically 10-12 sec) (Figure 4).
     3. After performing the adjustments mentioned above, click on “Record PVL”. The software will automatically exit working mode and gradually reduce LA pump RPM while simultaneously recording LV stroke work and LA pressure. At the conclusion of data collection, the software will perform linear regression on the newly acquired dataset to yield PRSW. After the ESHP software has completed the analysis, a message will appear on the main page, showing the correlation coefficient of the analysis. Press “OK” if the coefficient (R-value) is desirable (typically > 0.95). The PRSW analysis results will be recorded.

1. **Metabolic assessment of the *ex situ* perfused heart** 
   1. Assess the metabolic state of the heart and the perfusate during ESHP, with the blood gas analysis of the perfusate samples collected from both Ao (arterial), and Pulmonary artery (venous) lines every 1-2 hours.