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TITLE:

Biventricular Assessment of Cardiac Function and Pressure-Volume Loops by Closed-Chest Catheterization in Mice

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SUMMARY:

Presented here is a protocol to assess biventricular heart function in mice by generating pressure-volume (PV) loops from the right and left ventricle in the same animal using closed chest catheterization. The focus is on the technical aspect of surgery and data acquisition.

ABSTRACT:

Assessment of cardiac function is essential to conduct cardiovascular and pulmonary-vascular preclinical research. Pressure-volume loops (PV loops) generated by recording both pressure and volume during cardiac catheterization are vital when assessing both systolic and diastolic cardiac function. Left and right heart function are closely related, reflected in ventricular interdependence. Thus, recording biventricular function in the same animal is important to get a complete assessment of cardiac function. In this protocol, a closed chest approach to cardiac catheterization consistent with the way catheterization is performed in patients is adopted in mice. While challenging, the closed chest strategy is a more physiological approach, because opening the chest results in major changes in preload and afterload that create artifacts, most notably a fall in systemic blood pressure. While high-resolution echocardiography is used to assess rodents, cardiac catheterization is invaluable, particularly when assessing diastolic pressures in both ventricles.

Described here is a procedure to perform invasive, closed chest, sequential left and right ventricular pressure-volume (PV) loops in the same animal. PV loops are acquired using the admittance technology with a mouse pressure-volume catheter and pressure-volume system acquisition. The procedure is described, beginning with the neck dissection, which is required to

access the right jugular vein and the right carotid, to the insertion and positioning of the catheter, and finally the data acquisition. Then, the criteria required to ensure the acquisition of high-quality PV loops are discussed. Finally, the analysis of the left and right ventricular PV loops and the different hemodynamic parameters available to quantify systolic and diastolic ventricular function are briefly described.

INTRODUCTION:

According to the world health organization (WHO), heart disease is the leading cause of death worldwide for both men and women[1–3]. Many studies focus on diagnosing and improving impaired cardiac function[4]; for these applications high-quality and reproducible evaluation of cardiac function is critical. High fidelity and reproducible catheter data are required to assess both etiological and therapeutic responses. For example, the assessment of cardiac function is essential to evaluate the efficacy of drugs and other treatments in preclinical models of myocardial infarction[5]. While many cardiovascular studies focus on left ventricular function, right ventricular function is also a critical determinant of functional capacity and prognosis in patients with pulmonary-vascular disease[6,7]. In patients with advanced heart failure, persistently elevated right-sided and left-sided filling pressures are predictive of the combined risk of death, cardiovascular hospitalization, and heart transplantation[8]. In combined aortic and mitral valve disease, preoperative myocardial function (reflected in parameters such as cardiac index and left ventricular ejection fraction) is the main predictor of long-term survival[9]. Right ventricular function is the major predictor of both morbidity and mortality in pulmonary arterial hypertension[10,11]. Thus, assessment of the right ventricular function is a necessary component of a comprehensive preclinical study using models of pulmonary arterial hypertension[12–14].

Left and right ventricular function are often studied independently. However, because the functions of the left and right ventricles are intimately linked, it is ideal to obtain a biventricular assessment of systolic and diastolic function from a single test[15]. For example, the right ventricle shares oblique fibers in the interventricular septum with the left ventricle, which constitutes one of the mechanical links between the left and right ventricular contractile function[16,17]. This phenomenon, known as systolic ventricular interaction, allows left ventricular contraction to augment the right ventricular contraction. Ventricular interactions during diastole are also important. During diastole, the volume of one ventricle influences the volume of the opposite ventricle, and thereby alters diastolic compliance and preload[18,19]. In pathological conditions, decreased function of one ventricle, or impaired volume loading, can directly or indirectly impair the function of the other ventricle[20]. As a consequence of systolic ventricular interaction, a global decrease in left ventricular function may reduce right ventricular contractile performance[15]. In patients with heart failure due to left ventricular systolic function and increased end diastolic pressure, pulmonary artery pressure is elevated, indirectly increasing right ventricle afterload[21,22]. Conversely, increased right ventricular pressure and volume overload in severe pulmonary hypertension exerts a mechanical compression on the left heart. This D-shaped flattening of the left ventricle, caused by a leftward shift in the interventricular septum, reduces left ventricular volumes and impaired systolic and diastolic function[23–27]. Thus, the assessment of both left and right ventricles is essential to evaluate global cardiac function in preclinical models of human disease.

Cardiac function can also be assessed by noninvasive echocardiography, magnetic resonance imaging (MRI), and invasive catheterization[28–30]. Echocardiography is the most commonly used imaging modality in cardiovascular research because it is relatively inexpensive and accessible[31]. However, echocardiography has several technical limitations, including indirect measurement of filling pressure and limited ability to quantify diastolic function. In addition, the quality of the data obtained by echocardiography is highly operator dependent. Cardiac MRI is a relatively new addition to preclinical imaging armamentarium that has great potential for quantitative assessment of biventricular function. Quantification with cardiac MRI is accurate, as it does not make geometric assumptions of ventricular shape, unlike echocardiography[32]. However, the MRI imaging platform is expensive, and is rarely available. Moreover, the processing of MRI data requires skilled support by a physicist or equivalent scientist, which is lacking in many preclinical laboratories[33]. Similarly, the use of microcomputed tomography (MicroCT) in preclinical and human studies provides quantitative high-resolution three-dimensional (3D) anatomical data that can be obtained noninvasively, allowing longitudinal studies[34]. However, MicroCT imaging requires the injection of contrast agents, which are often expensive. The MicroCT imaging platform, like MRI, is also expensive and also requires a skilled technician.

In contrast, catheterization is an invasive technique that consists of the introduction of a catheter in the right and/or left ventricle to measure pressure and/or volume. The tools required to perform cardiac catheterization are not as expensive as echocardiography, CT, or MRI. Substantial technical proficiency for catheterization and small animal anesthesia is required, however. Catheterization allows direct and accurate assessments of cardiac function[28]. In this protocol, an admittance PV catheter is used to assess cardiac function. This technology, based on the distinct electrical conductance properties of blood and cardiac muscle, allows for the simultaneous recording of pressure and volume within the cardiac cavity and generation of PV loops in real time[5,35]. Briefly, the catheter is comprised of both excitation electrodes and recording electrodes. The excitation electrodes generate an electrical field inside the right or left ventricle. The inner recording electrode measures voltage change, which is proportional to a change in resistance. Deriving ventricular volume is based on Ohm's law (voltage = current x resistance) from which conductance (i.e., the inverse of resistance) is calculated. In this setting, the measured conductance value is a combination of blood conductance and muscle conductance. In the electric field, blood is purely resistive while muscle has both capacitive and resistive properties. The capacitive property of muscle causes a time delay in the measured signal. Tracking this delay, known as the "phase" angle, reports heart tissue intrusion into the field as the heart contracts. This measurement is greatest at systole and lowest at diastole. This property allows the separation of the muscle component of the conductance from that of blood and allows a close approximation of absolute systolic and diastolic volumes. Pressure-volume loops provide a range of hemodynamic parameters that are not readily measurable by other methods, such as simple retrograde catheterization using fluid filled catheters to measure cardiac pressures. Pressure-volume loops measure ventricular pressures but also provide data about contractility, elastance, power, energetics, and efficiency. In addition, PV loops provide robust quantitative measurements[36]. Thus, assessment of cardiac function by PV loops generated by catheterization has emerged as the gold standard in preclinical research[37]. In addition, preclinical

techniques are relevant to human disease where cardiac catheterization, albeit with fluid filled catheters, is common. However, cardiac catheterization in rodents requires impeccable anesthesia and excellent technique to prevent excessive loss of blood, hypoventilation, or changes in body temperature.

In human patients, cardiac catheterization is performed in closed chest configuration and vascular access is achieved via the jugular or subclavian vein for the right ventricle and the radial or femoral artery for the left ventricle. Due to the small size of mice, the closed chest approach is often challenging. Thus, studies conducted in mice commonly adopt an open chested approach. This technique involves opening the thorax, thereby exposing the heart, and facilitating the insertion of the catheter via puncture of the left and/or right ventricular apex[38]. While this approach is technically less challenging and fairly reproducible, its major limitations include hemorrhage and other complications of apical insertion of catheters, and a marked drop in intracardiac pressure resulting from opening the thoracic cavity to atmospheric pressure. Opening the thorax in a ventilated rodent induces a 5–10 mm Hg decrease in left ventricular systolic pressure and 2–5 mm Hg decrease in right ventricular pressure[39]. Therefore, a closed chest approach that is less traumatic for the heart and yields more physiologically relevant measurements that are more easily translated to clinical assessment of cardiac function was developed.

PROTOCOL:

All experiments were performed in accordance with Queen's University biosafety and ethical guidelines (ROMEO/TRAQ#6016826). The procedures followed were performed in accordance with institutional guidelines.

1. Experimental preparation and setup

1.1. Put the catheter in a 10 mL syringe with saline/heparin, at room temperature 30 min before starting the experiment (**Figure 1A**).

1.2. After 30 min, calibrate the catheter (e.g., baseline and acquisition system) according to the manufacturer's recommendations. The acquisition system displays high and low calibration values that are used to calibrate the acquisition system before starting an experiment. Output these values and make sure that they match.

1.2.1. Use the button "**Pressure Balance Control**", "**Coarse +/-** ", or "**Fine +/-**" to set up the baseline pressure value at zero.

1.2.2. Perform a two point calibration for high and low signal.

1.2.2.1. On the control console press "**System Setting**" in the "**Catheter Menu**".

1.2.2.2. Press "**Send Calibration Signal**" in the "**System Setting Menu**" to send the low signal. Ensure that the pressure, volume, phase, and magnitude are at 0 mm Hg, 0 µL, 0˚, and 0 µs

respectively.

1.2.2.3. Press "**Enter**" to send the high signal. Ensure that pressure, volume, phase, and magnitude are at 100 mm Hg, 150 µL, 20˚ and 5,000 µs respectively.

1.2.2.4. Press "**Enter**" to return to the "**System Setting Menu**".

1.2.2.5. Press "**6**" to return to the "**Catheter Menu**". Then press "**Acquire Data**".

1.3. Bend a 30 G needle to approximately 90˚ (**Figure 1B**,**C**). This bent needle will be used to puncture the jugular and carotid veins.

2. Anesthesia and body temperature control (**20–30 min**)

2.1. Place the mouse (28 g, C57BL/6 in this protocol) into an anesthesia chamber containing anesthetic gas (i.e., oxygen 100%, isoflurane 3–4% for induction).

2.2. When the animal is anesthetized, not responding to paw or tail pinch, place the mouse supine on the heating pad set at 37 ˚C.

2.3. Connect the mouse to the respirator through a nose cone providing a mixture of 100% oxygen and 2% of isoflurane. To automatically calculate the recommended ventilation settings, enter the animal's weight into the ventilator's proprietary software using the touch screen. The calculations use the following formula:

Tidal volume = 6.2 x animal mass$^{1.01}$ (kg),

Respiration rate = 53.5 x animal mass$^{-0.26}$ (kg).

2.4. Turn on the anesthetic line from the anesthesia chamber to the nose cone.

2.5. Insert the temperature feedback probe into the rectum, and the pad probe between the pad and the back of the mouse, setting the desired body temperature to 37 °C–37.5 °C. Control the animal's temperature on the monitor screen (**Figure 2A**,**B**).

2.6. Tape down the front paws and one distal paw of the mouse to the heating blanket using surgical tape, leaving one hind paw free to monitor the depth of anesthesia.

3. Intubation

3.1. Perform a 2 cm H-shape ventral midline cervical incision from the manubrium to the level of the hyoid bone.

3.1.1. Pull the skin away from the underlying muscles and cut it off.

3.1.2. Gently move the submandibular gland aside.

3.1.3. Dissect the cervical soft tissue and expose the sternocleidomastoid and the sternohyoid muscle with forceps using the blunt dissection method.

3.1.4. Split the fascia in the middle, overlying the paired sternohyoid. Allow the paired sternohyoid to retract laterally to expose the trachea. Be careful to not damage the carotid arteries and the vagus nerves, which run alongside the trachea.

3.2. Pass forceps underneath the trachea to elevate it. Then, pass a 4.0 surgical silk suture underneath the trachea and make a potential knot in the middle of the suture, which will later be tightened to secure the endotracheal tube (**Figure 3A**).

3.3. Using scissors, make a small cut between the cartilage rings of the trachea below the level of the larynx. Insert the endotracheal tube (**Figure 3B**).

3.4. Connect the tracheostomy tube to the respirator and begin ventilation with 100% oxygen and 2% isoflurane. Tighten the knot around the trachea to secure the endotracheal tube and tape the respirator tubing to the operative table. Ensure that the trachea is not obstructed or collapsed (**Figure 3C**).

4. Right jugular and right carotid isolation

4.1. Right carotid isolation

4.1.1. Using blunt dissection, displace the sternohyoid muscle laterally to expose and isolate the right carotid artery.

4.1.2. Isolate the carotid artery from the vagus nerve by blunt dissection using forceps.

4.1.3. Pass three surgical sutures (4.0) underneath the carotid artery, excluding the vagus nerve.

4.2. Right jugular vein isolation

4.2.1. Displace the submandibular and parotid gland laterally to visualize the right jugular vein. Bluntly dissect and expose the right jugular vein using forceps. Carefully dissect the vein and remove the surrounding fascia.

4.2.2. Pass forceps underneath the jugular vein.

4.2.3. Pass one surgical suture underneath the jugular vein, then tie it at the cranial side of the vein. Apply gentle traction on this suture in the direction of the head using a hemostatic clamp.

4.2.4. Pass two additional sutures underneath the jugular vein. Gently pull the most distal suture in a caudal direction using a hemostatic clamp. Make a loose, potential knot in the middle suture.

4.2.5. Put several drops of warmed, physiological saline on the vessel at the site of anticipated venotomy.

5. Surgical procedures for right ventricular and left ventricular catheterization

5.1. Right ventricular catheterization (**Figure 4 A–D**).

5.1.1. Using the stereomicroscope, identify the jugular vein.

5.1.2. Gently apply superior traction on the vein. Perform a venotomy by inserting a 30 G curved needle between the cranial suture and the middle suture. Insert the needle at a 140˚ angle relative to the vein to ensure it enters in a coaxial manner.

5.1.3. When inserted, dilate the venotomy by moving the needle. Insert the catheter tip in the venotomy, underneath the needle. Then gently tie the middle suture, securing the catheter.

NOTE: Take extreme care not to tie the suture too tightly, because excess force can damage the catheter.

5.1.4. Release the caudal suture, and advance the catheter into the right ventricle, detecting the classical right ventricular pressure waveform on a continuous monitor.

5.1.5. Stabilize the right ventricular pressure. Ensure the correct positioning of the catheter in the right ventricle to generate an optimal PV loop.

5.1.5.1. Stabilize the magnitude, which reflects the blood and muscle, to generate pressure-magnitude loops (i.e., Y axis pressure, X axis magnitude). If required, gently rotate the catheter shaft to achieve optimal placement of the catheter along the axis of the right ventricle.

NOTE: The maximal phase value, which reflects the muscle, should be below 7°.

5.1.6. When the pressure-magnitude loop signal is optimal, press "**Enter**" on the console during the acquisition to perform a baseline scan. Ensure that the heart rate reported on the monitor screen in beats per minute (bpm) is in a physiologic range (i.e., 400–600 bpm).

5.1.7. Generate the PV loops. Change "**Magnitude**" to "**Volume**" as a parameter for the X axis and keep the pressure as the Y axis. When the PV loops signal is optimal, record for 30 s.

5.1.8. Stop the recording. Pull back the catheter and gently wipe with gauze. Put the catheter in heparin/sodium chloride solution and tie the caudal suture to stop bleeding from the jugular vein.

5.2. Left ventricular catheterization (**Figure 5 A–D**).

5.2.1. Gently elevate the right carotid, which was previously isolated (**5A**) by sliding curved forceps underneath the artery.

5.2.2. Tie the previous suture, thereby occluding the artery. Then, gently apply cranially directed traction using a hemostatic clamp.

5.2.3. Pull the most distal suture in a caudal direction using a hemostatic clamp. Make a loose potential knot on the middle suture.

5.2.4. Put several drops of warmed, physiological saline on the vessel at the site of the anticipated arteriotomy. Focus on the cranial section, between the caudal and the middle suture, using the stereotaxic microscope.

5.2.5. Gently apply superior traction on the artery. Perform an arteriotomy, by inserting a 30 G curved needle between the cranial suture and the middle suture. Insert the needle at 140˚ relative to the artery to ensure it enters in a coaxial manner.

5.2.6. Insert the catheter tip into the arteriotomy and then tighten the middle suture to secure the catheter. Simultaneously, release the distal suture and advance the catheter into the aorta to start recording. Ensure that the pressure channel shows a typical aorta trace.

5.2.7. Advance the catheter retrograde across the aortic valve into the left ventricle. Entry into the left ventricle will be evident from the sudden marked drop in diastolic pressure from the aorta.

5.2.8. Stabilize the left ventricular pressure. Ensure the correct positioning of the catheter in the left ventricle to generate an optimal PV loop.

5.2.8.1. Stabilize the magnitude, which reflects the blood and muscle, to generate pressure-magnitude loops (i.e., Y axis pressure, X axis magnitude). If required, gently rotate the catheter shaft to achieve optimal placement of the catheter along the axis of the left ventricle.

NOTE: The maximal phase value, which reflects the muscle, should be below 7°.

5.2.9. Stop the recording. Pull back the catheter and put it in heparin/sodium chloride solution. Then tie the caudal suture.

5.2.10. Clean the catheter with an enzymatic detergent (e.g., endozime).

6. Data analysis (**15–30 min**)

6.1. Perform the PV loop analysis according to established recommendations.

6.1.1. Select the optimal pressure-volume trace (ideally an entire, stable 30 s recording). On the software, click "**Advance**", click "**Loops**", and then click "**Offline Calculation**".

6.1.2. Select volume as a volume channel and pressure as pressure channel.

6.1.3. For consistent results a minimum of 20 loops is necessary.

REPRESENTATIVE RESULTS:

The catheter was placed in a 10 mL syringe containing a solution of heparinized saline at room temperature 30 min before the catheterization (**Figure 1A**). A 30 G needle was bent ~90˚ (**Figure 1B**, **C**), and a 1.45 mm diameter tracheotomy canula was prepared (**Figure 1C**).

Maintenance of physiologic body temperature is critical. The mouse was taped down and connected to the respirator through a nose cone. The feedback probe was placed between the pad and the back of the mouse. A rectal probe was inserted to monitor the animal's body temperature (**Figure 2A**). Body temperature (37.1 ˚C) and pad (40.7 ˚C) temperature were monitored (**Figure 2B**).

Photographs of the critical steps of the intubation procedure are displayed in **Figure 3A**–**C**. Successful and unobstructed intubation resulted in a regular respiratory rate with stable peak pressure (**Figure 2B**).

Pictures of the critical steps of right heart catheterization, from the isolation of the jugular vein (**Figure 4A**–**C**) to the insertion of the catheter in the jugular vein are shown in **Figure 4D**. **Figure 5** shows the critical steps of left heart catheterization, including right carotid artery isolation (**Figure 5 A**,**B**) and catheter insertion (**Figure 5 C**,**D**)

The catheter was introduced into the jugular vein and advanced into the right ventricle. Then the right ventricular pressure was stabilized, and the correct positioning verified. All the catheter's electrodes (6 mm long axis length) needed to be within the right ventricle chambers and not in contact with the ventricle walls. Optimal positioning of the catheter as schematically represented in **Figure 6A** generated optimal PV loops (i.e., triangular, regular). Improper positioning as schematically represented in **Figure 6B** (i.e., contact with the ventricular wall) will result in flawed PV loops (i.e., collapsed and irregular loops).

The catheter was introduced into the carotid, advanced into the aorta, then advanced retrograde across the aortic valve into the left ventricle. The left ventricular pressure was stabilized and right positioning verified. All the catheter's electrodes (6 mm long axis length) should be within the left ventricle chambers and not in contact with the ventricle walls. Optimal positioning of the catheter as schematically represented in **Figure 6C** generated optimal PV loops (i.e., rectangular, regular). Improper positioning as schematically represented in **Figure 6D** (i.e., contact with the ventricular wall) resulted in flawed PV loops (i.e., collapsed, non-rectangular, and irregular loops).

Representative hemodynamics generated by left and right PV loops showed a heart rate of 410 bpm, cardiac output of 9,107 µL/min, and stroke volume of 24.5 µL. Specific right ventricular parameters showed a right ventricular systolic pressure of 21.9 mm Hg, right ventricular end diastolic pressure 1.049 mm Hg, ejection fraction of 56.1%, dp/dt max of 1,469 mm Hg/s, dp/dt max of -1,504 mm Hg/s, end diastolic volume of 38.4 µL, stroke work of 0.068 mJ, pressure-volume area of 0.089 mJ, pulmonary arterial elastance (Ea) of 0.83 mm Hg/µL, and Tau factor of 12.8 ms. Specific left ventricular parameters showed a left ventricular systolic pressure of 77.1 mm Hg, left ventricular end diastolic pressure of 2.33 mm Hg, ejection fraction of 59.1%, dp/dt max of 4,695 mm Hg/s, dp/dt max of -3,553 mm Hg/s, end diastolic volume of 36.9 µL, stroke work of 0.14 mJ, pressure-volume area of 0.22 mJ, arterial elastance (Ea) of 5.37 mm Hg/µL, and Tau factor of 15.1 ms (**Table 1**).

FIGURE AND TABLE LEGENDS:

Table 1: Table of hemodynamic parameters. Left and right ventricular hemodynamic parameter measured in six mice.

Figure 1: Experimental preparation and setup. (**A**) Catheter in a 10 mL syringe of saline/heparin, (**B**), (**C**) 30 G needle bent to approximately 90°, (**D**) tracheotomy canula, 1.45 mm diameter.

Figure 2: Anesthesia, **body temperature control.** (**A**) Mouse with three paws taped, connected to respirator through a nose cone, with feedback and rectal probes inserted. Note that the warming pad is below the surgical blanket. (**B**) Temperature monitor control showing body (rectal) and pad (feedback) temperature and the ventilation parameters: respiratory rate (set RR), mean tidal volume (Meas TV), peak pressure (PeakPress), and the minute ventilation (MinVol).

Figure 3: Intubation procedure. (**A**) The skin was pulled away and cut. The submandibular gland was gently moved aside. The sternocleidomastoid and the sternohyoid muscle were pulled apart and then forceps were passed underneath the trachea, using gentle, blunt dissection. (**B**) Surgical silk (4.0) was passed underneath the trachea and a small cut was made anteriorly between two cartilage rings of the trachea. The tracheostomy was inserted and tied. (**C**) The tracheostomy tube was connected to the ventilator, and the suture was tied around the tubing.

Figure 4: Right ventricular catheterization. (**A**), (**B**), (**C**) The right jugular vein was isolated, then one surgical suture was passed underneath and tied at the cranial side of the vein. Gentle traction was applied on this suture in the direction of the head using a hemostatic clamp. Two additional sutures were passed distally, underneath the jugular vein. The most distal suture was pulled gently in a caudal direction using a hemostatic clamp. A loose, potential knot was made in the middle suture. (**D**) The catheter was inserted in the jugular vein, the middle suture was tied to the catheter. The images in (**C**) and (**D**) are magnified through a stereomicroscope.

Figure 5: Left ventricular catheterization. (**A**), (**B**) The right carotid was isolated, then one surgical suture was passed underneath the jugular vein and tied at the cranial side of the vein. Gentle

traction was applied on this suture in the direction of the head using a hemostatic clamp. Two additional sutures were passed underneath the carotid artery. The most distal suture was gently pulled in a caudal direction using a hemostatic clamp. A loose, potential knot in the middle suture was made. (**C**) The catheter tip was inserted into the carotid artery, and then the middle suture tied to the catheter to secure it. (**D**) The catheter was gently advanced retrograde down the carotid toward the aorta. The images in (**B**), (**C**), (**D**) are magnified through a stereomicroscope.

Figure 6: Schematic representation of catheter positioning and resulting PV loops. (**A**) Optimal catheter positioning in the right ventricle. The tip of the catheter is in the middle of the ventricle, isolated from the ventricle walls. Representative PV loops resulting from an optimal catheter positioning in the right ventricle (i.e., stable, triangular). (**B**) Improper catheter positioning in the right ventricle. The tip of the catheter is in contact with the ventricular walls. Representative PV loops noise resulting from a suboptimal catheter positioning in the right ventricle (i.e., collapsed, irregular). (**C**) Optimal catheter positioning in the left ventricle. The tip of the catheter is in the middle of the ventricle, isolated from the ventricle walls. Representative PV loops resulting from optimal catheter positioning in the left ventricle (i.e., stable, rectangular). (**D**) Improper catheter positioning in the left ventricle. The tip of the catheter is in contact with the ventricular walls. Representative PV loops resulting from a suboptimal catheter positioning in the left ventricle (i.e., collapsed, irregular). A 50 Hz FIR noise filter was applied to generate the PV loops.

DISCUSSION:

Assessment of cardiac function is a critical step for preclinical cardiovascular and pulmonary-vascular research. Presented is a protocol to perform closed chest biventricular assessment of cardiac function in mice. Through this approach, one can generate the right ventricle and left ventricle PV loops in the same mouse. This approach provides a robust and complete assessment of cardiac function, allowing measurement of systolic and diastolic function, as well as stroke volume and cardiac output. Unlike the open chest approach classically used for rodent catheterization, this closed chest technique results in more stable physiology and more physiologically relevant data. While technically more challenging and dependent on operator skills to successfully position the catheter in the right and left ventricle, the closed chest approach limits the trauma and hemorrhage associated with open chest surgery and reduces the drastic pressure changes associated with exposing the lungs to atmospheric pressure. The closed chest approach also better emulates the cardiac catheterization procedure performed in patients, which enhances the relevance of using this technique in preclinical research.

The surgical procedure is the critical step of the protocol. Even when using a surgical microscope for catheter insertion in the jugular vein or carotid artery, which is recommended, this procedure requires practice and technical skill. Careful dissection of the vessels free from surrounding fascia by means of gentle, blunt dissection will increase the success of cannulation while minimizing the risk of hemorrhage. To minimize blood loss, it is crucial to cannulate the carotid in sequential steps: 1) introduce the catheter tip in the carotid artery; 2) gently tie the suture around the portion of the artery that contains the catheter; 3) release the secure suture, allowing catheter movement while maintaining gentle upward traction to minimize bleeding; and 4) advance the catheter to the aorta. Positioning the catheter in the ventricle, as determined by real-time

waveform monitoring, is the most challenging part of this protocol. All the catheter's electrodes should be within the ventricular cavity and none should be touching the wall. Any improper positioning of the catheter will result in irregular PV loops and will adversely affect or preclude data acquisition. Recognizing the characteristic pressure-volume waveform that results from having all electrodes within the ventricle allows one to be confident of an appropriate catheter position. It is critical to obtain a stable ventricular pressure waveform and stable pressure-magnitude loops before shifting to the PV mode and volume acquisition. Proper knowledge of cardiac physiology and anatomy is essential for the success of this procedure. Online reading of the PV traces, from the atrium, the tricuspid valve area, and right ventricle, will show the advancing of the catheter and help achieve proper positioning. It is critical to know the normal heart rate (400–600 bpm), and waveforms and pressures expected (e.g., right ventricular systolic pressure, 18–25 mm Hg, diastolic pressure <5 mm Hg; left ventricular systolic pressure 90–120 mm Hg, diastolic pressure <8 mmHg) in mice to allow the operator to evaluate the veracity of the observed data.

The quality and reproducibility of the data will depend on the speed of the procedure and blood loss or hemorrhage. The procedure from anesthesia to completion of data acquisition takes on average ~30–40 min/mouse. Right heart catheterization from the insertion of the catheter to data acquisition takes 5–10 min, left heart catheterization from the insertion of the catheter to the data acquisition takes another 10–15 min. Publication-quality data is obtained in ~75% of cases. The sequence of steps in the cardiac catheterization should be kept constant between the animals. In this procedure, the mice are intubated first, followed by the right ventricular catheterization, and finally the left ventricular catheterization. The decision to proceed in this order is based on the greater difficulty and bleeding risk of left heart versus right heart catheterization. Old electrical installations could result in a nonspecific 50 Hz noise recording artifact. This noise could be diminished using an FIR filter with a high cutoff at 50 Hz and a low cutoff of 0 on the software. For the volume channel create a new channel/filter/FIR filter. A notch filter of 50 Hz could also be applied during data acquisition to eliminate mains noise and remove any radiofrequency interference.

The faster the catheterization is done, the better the quality of the data. Based on previous experience, it is recommended to acquire the data within 15 min. Increased catheterization time increases the physiologic stress on the animal and increases the risk of arrhythmia due to the presence of the catheter in the cavity. These forces can reduce stroke volume and impair the reproducibility and interpretability of the waveforms. In addition, the tip of the catheter is sharp and can damage or puncture the ventricle. This is particularly important for the right ventricle, which is ~ 1/3rd the thickness of the left ventricle.

Invasive tracheostomy and positive pressure mechanical ventilation result in stable and controlled breathing of the mice and decrease the variability of the PV loops acquisition. However, positive end expiratory pressure (PEEP) is a marked contrast to normal ventilation, which is a negative pressure phenomenon. Together, positive pressure ventilation and PEEP lower cardiac output and reduce right heart pressure. Thus, while required for acquisition of stable data, mechanical ventilation as well as cardiodepressive effects of the anesthesia will

affect the PV loops and should be considered as a limitation. Transiently stopping mechanical ventilation during the brief recording of PV loops is used to eliminate this potential source of artifacts. Note that ventilation efficiency can be confirmed by the capnography monitoring of carbon dioxide.

The technical skills required for the closed-chest approach may be a limitation of this technique. Likewise, it is challenging to obtain proper, stable positioning of the catheter in the ventricle. The odds of success increase with operator experience and with the size and weight of the mice. Catheterization of mice below 20 g is extremely challenging. The unique chamber geometry of the right ventricle might affects volume measurement and should be considered. The anesthetic used, heart rates, temperatures, and animal strain could affect the hemodynamic parameters and should be carefully reported and monitored.

The procedure described in this protocol is a terminal procedure. Due to the invasiveness of the right and left catheterization, the animals should be euthanized immediately after data acquisition. The euthanasia should be performed according to the institution's animal studies guidelines.

In conclusion, in this protocol both right and left ventricular catheterization are performed in the same mouse. Depending on a scientist's specific aims, left or right ventricular catheterization can be performed independently, using the relevant portion of the biventricular procedure. However, the approach presented is optimal for complete assessment of cardiac function.

ACKNOWLEDGMENTS:

The authors would like to acknowledge the help and collaboration of Queen's University animal facility personal. The authors would like to acknowledge the help of Austin Read, TMED MSc candidate.

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DISCLOSURES:

None

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Figure **Figure 1**

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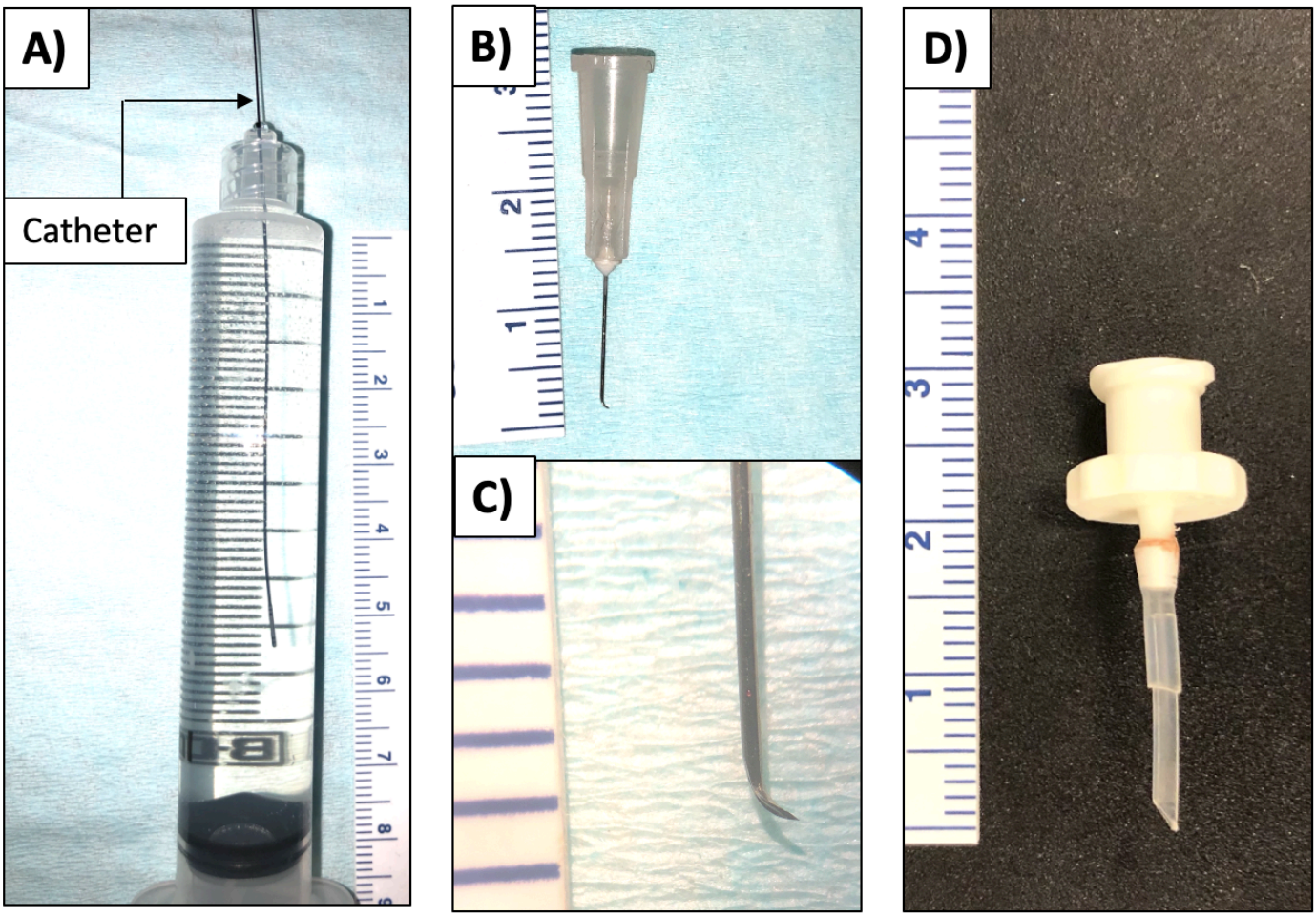


figure 2

Figure 2

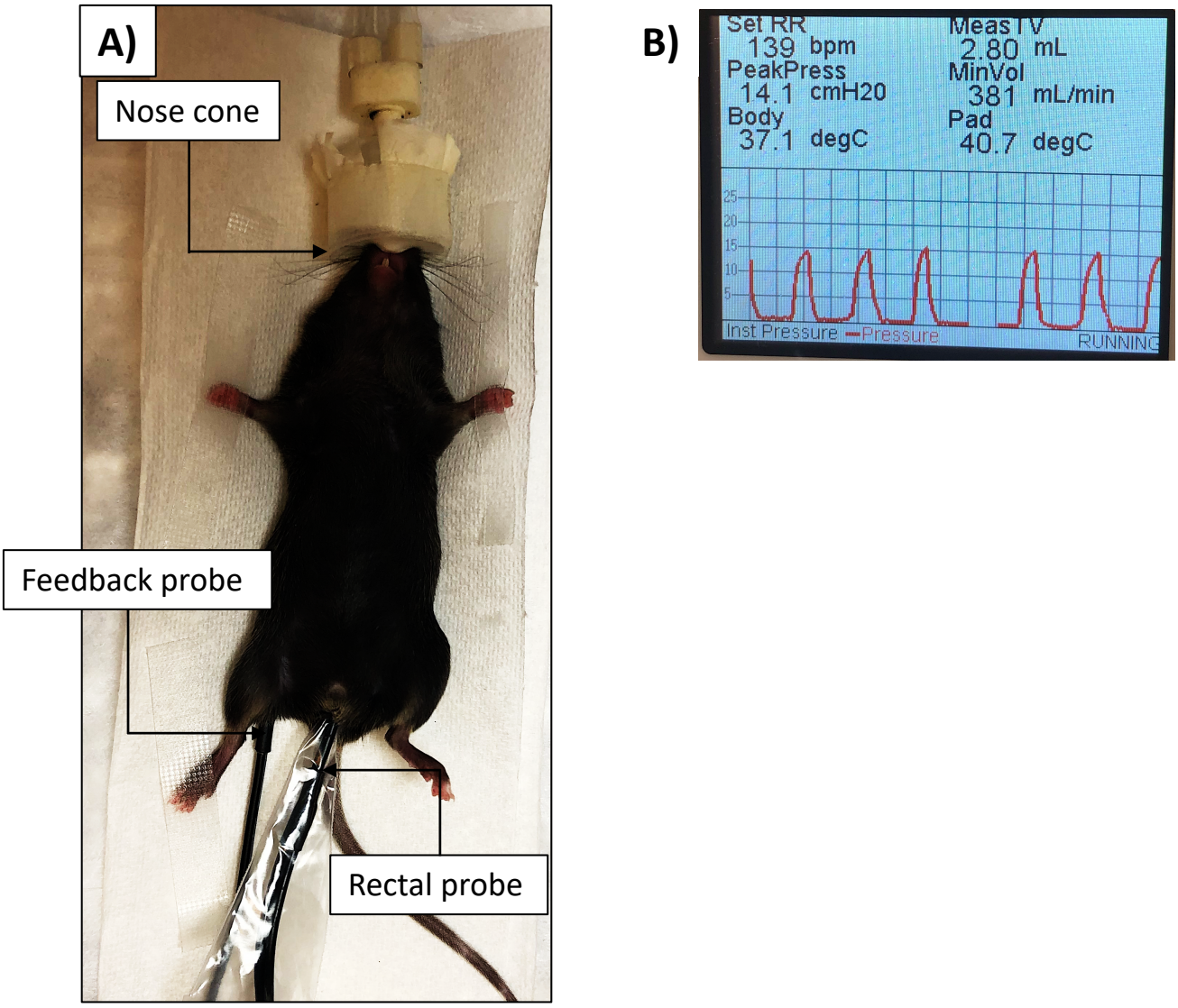
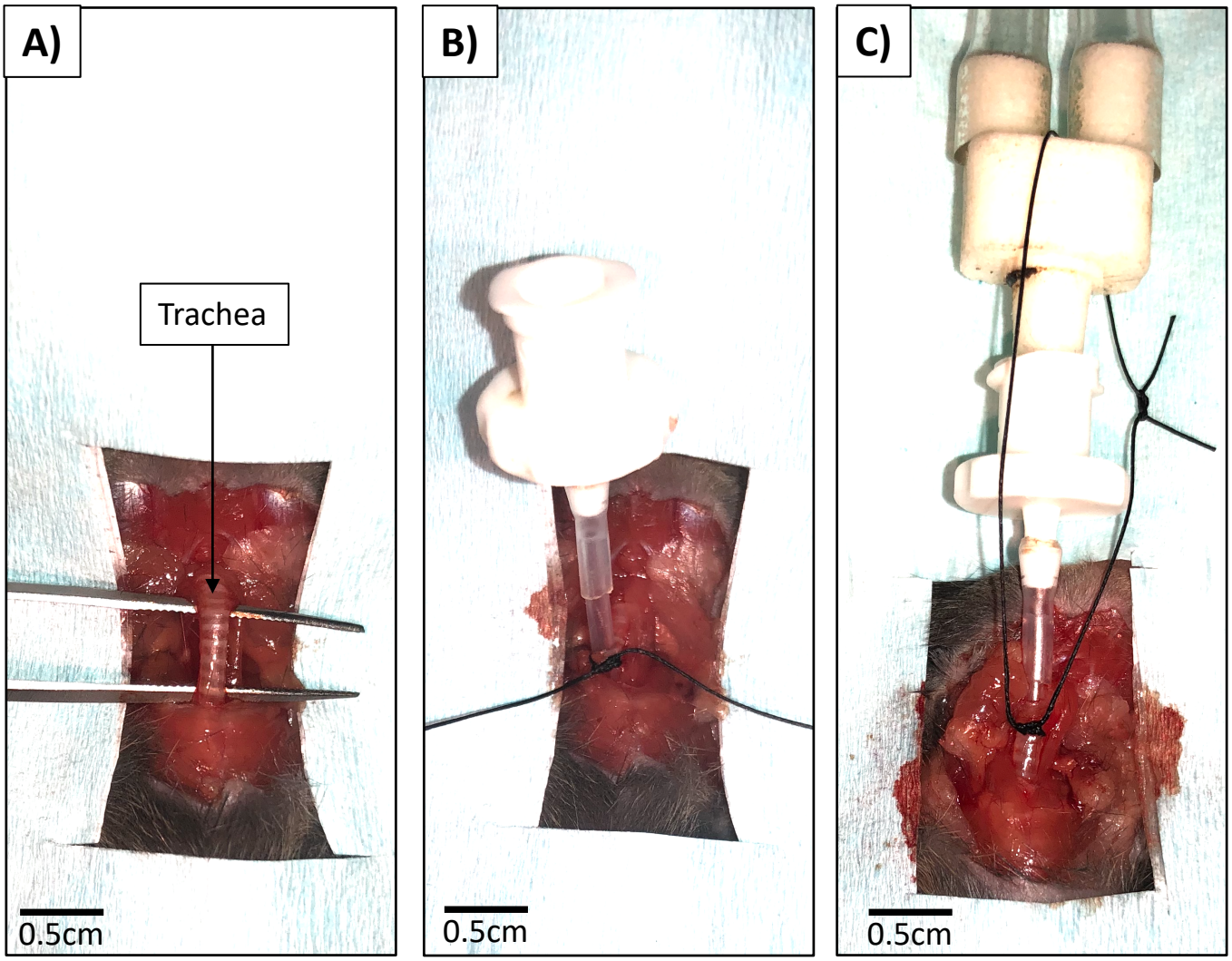


figure 3

Figure 3



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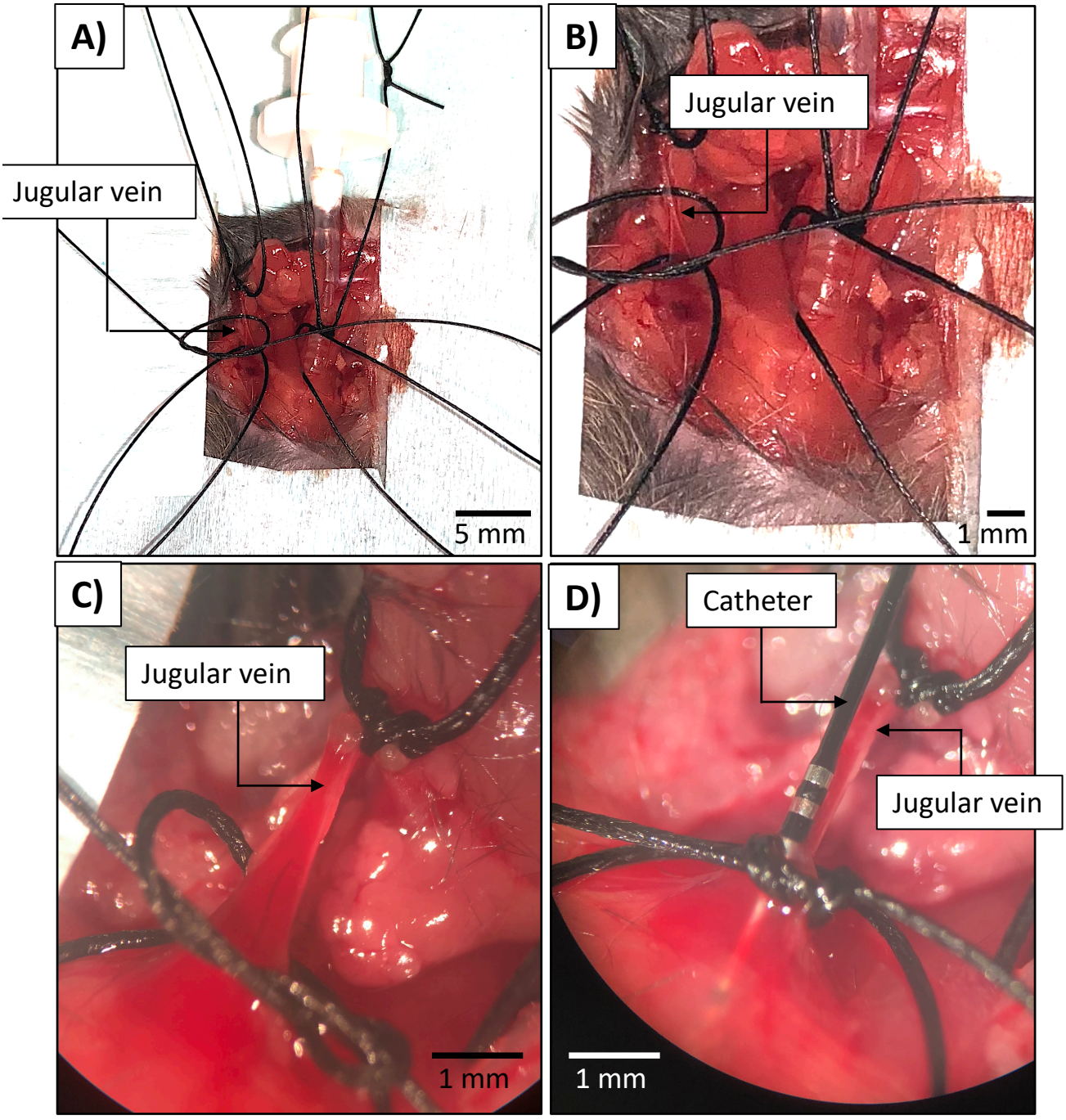


figure 5

Figure 5

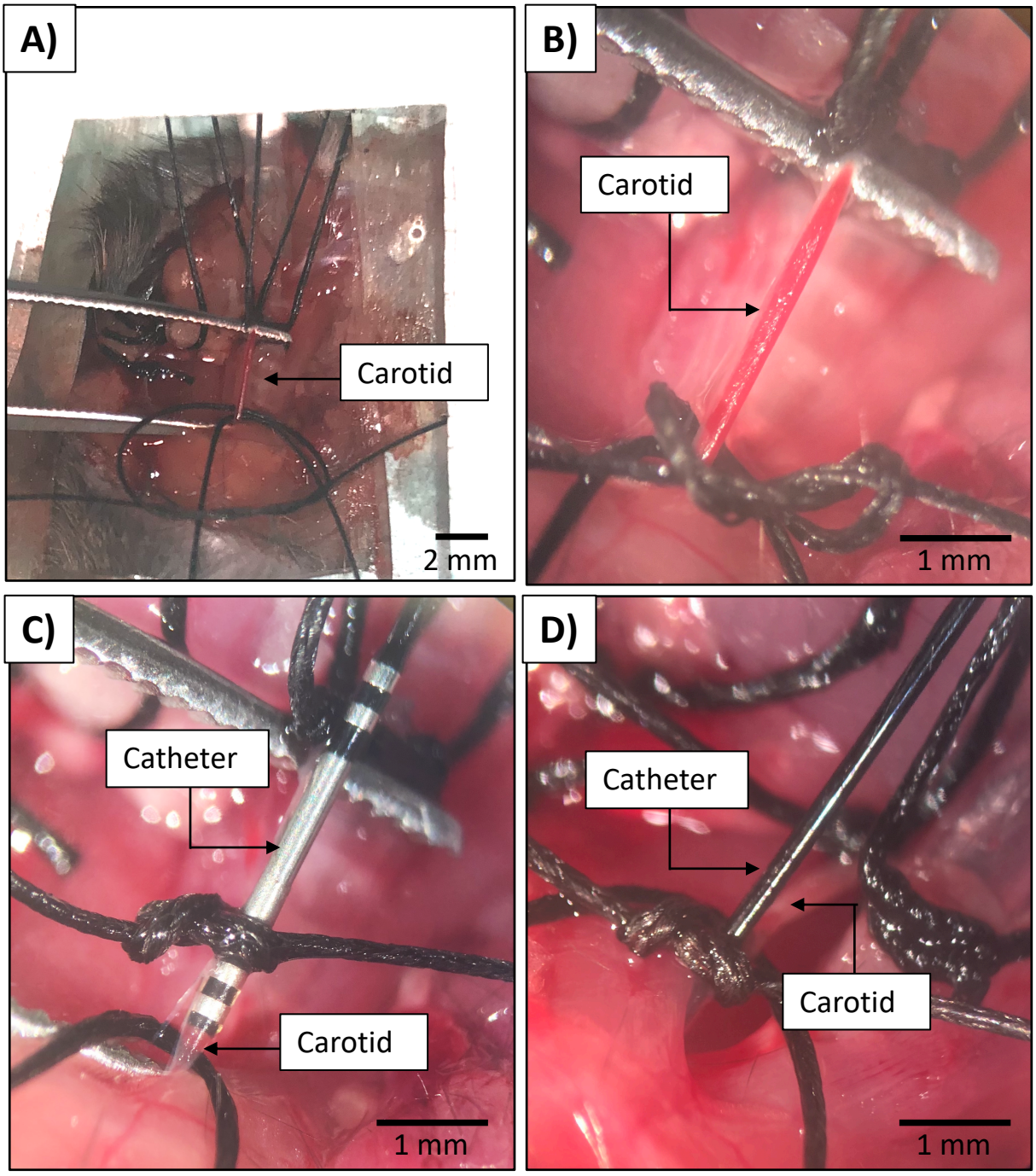
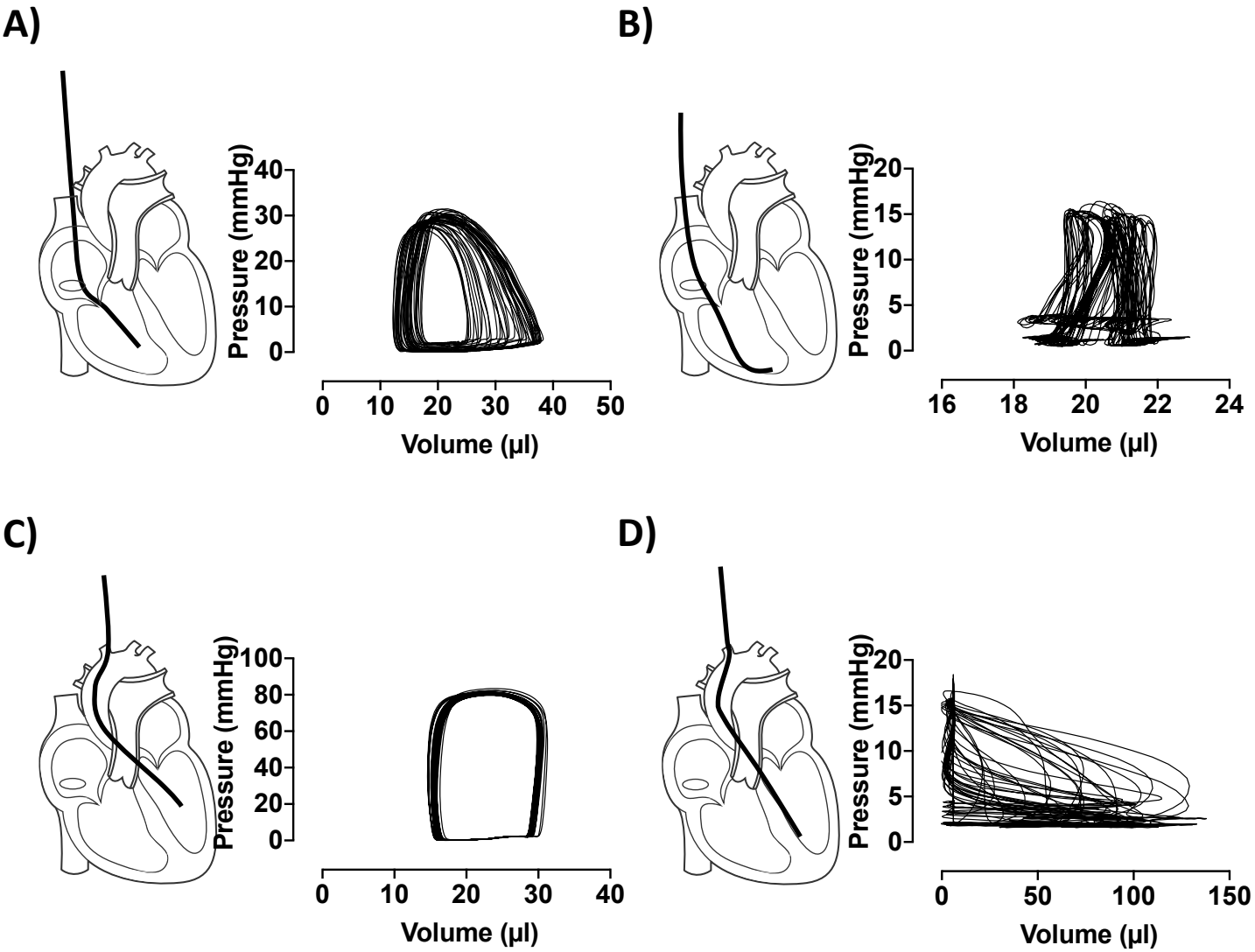


figure 6

Figure 6



Haemodynamic parameters	
HR (BPM)	410.6 ± 23.3
CO (µL/min)	9107 ± 1016
SV (µL)	24.5 ± 2.3
RV function	
RVSP (mmHg)	21.9 ± 2.15
RVEDP (mmHg)	1.042 ± 0.12
EF (%)	56.1 ± 4.4
dP/dt max (mmHg/s)	1469 ± 170
dP/dt max (- mmHg/s)	1504 ± 215
EDV (µL)	38.4 ± 3.7
SW (mJoules)	0.068 ± 0.008
PVA (mJoules)	0.084 ± 0.009
Ea (mmHg/µL)	0.83 ± 0.09
Tau factor (ms)	12.8 ± 0.8
LV function	
LVSP (mmHg)	77.1 ± 2.4
LVEDP (mmHg)	2.33 ± 0.17
EF (%)	59.1 ± 3.6
dP/dt max (mmHg/s)	4695 ± 355
dP/dt max (- mmHg/s)	3553 ± 373
EDV (µL)	36.9 ± 4.8
SW (mJoules)	0.14 ± 0.013
PVA (mJoules)	0.22 ± 0.03
Ea (mmHg/µL)	5.37 ± 0.9
Tau factor (ms)	15.07 ± 1.7

CO, cardiac output; Ea, arterial elastance; EDV, end diastolic volume; HR, heart rate; LVEDP, left ventricular end diastolic volume; LVSP, left ventricular systolic pressure; PVA, pressure volume area; RVEDP, right ventricular end diastolic pressure; RVSP, right ventricular systolic pressure; SV, stroke volume; SW, stroke work; Tau factor, Tau Mirsky. N= 6 mice. Values are expressed ± SEM

Name of Material/Equipment	Company	Catalog Number	Comments/Description
ADVantage Pressure-Volume System (ADV500)	Transonic	FY097B	
Endozime AW triple plus	Ruhof	34521	
Fiber optic dual Gooseneck	Volpi Intralux	# 6000-1	
Forceps	F.S.T	11052-10	
Forceps	F.S.T	11251-20	
Gauze sponges	Dermacea	441400	
Hemostatic clamp	F.S.T	13003-10	
Hemostatic clamp	F.S.T	13018-14	
Heparin sodium	Sandoz	023-3086	100 U/L
High-fidelity admittance catheter	Scisence; Transonic	FTH-1212B-3518	
Isofluorane	Baxter	CA2L9108	
labScribe v4 software	iworx	LS-30PVL	
Needle (30 gauge)	BD	305106	
sodium chloride injection	Baxter	JB1309M	0.9%(wt/vol)
Stereo microscope	Cole-Parmer	OF-48920-10	
Surgical suture	SERAFLEX	ID158000	black braided silk, 4.0
Surgical tape	3M, Transpore	SN770	
Tabletop Single Animal Anesthesia Systems	Harvard apparatus	72-6468	
Tracheotomy canula 1.45 mm diameter	Harvard apparatus	72-1410	
Ventilator, far infrared warming pad for mice and rats PhysioSuite	Kent scientific corporation	# PS-02	

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February 25, 2020

Vineeta Bajaj, PhD
Review Editor, Jove
1 Alewife Center, Suite 200
Cambridge, MA 02140
USA

Re: JoVE61088

Dear Dr. Bajaj,

Encouraged by the reviewers' comments received on January 29th, 2020, we are submitting a revision of our manuscript entitled "**Biventricular assessment of cardiac function and pressure-volume loops by closed-chest catheterization in mice**" We are thankful for the constructive criticisms that we received and we believe that this revision strengthens the manuscript. We provided a detailed response to each of the referees' comments.

According to reviewer comments, we assessed the left and right ventricular function of 6 mice. We included those data as a new table 2 in the revised form of the manuscript.

The work is original and has not been published elsewhere. All authors have read and approved the manuscript in its current format. We have no commercial conflicts of interest to disclose.

We hope you will find this worthy of publication in *Jove*.

Sincerely,

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Blog: http://deptmed.queensu.ca/blog/
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Editorial comments:
Changes to be made by the Author(s):
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.
Thank you for your comment, we carefully proofread our manuscript.

2. Please format the manuscript as: paragraph Indentation: 0 for both left and right and special: none, Line spacings: single. Please include a single line space between each step, substep and note in the protocol section. Please use Calibri 12 points
Done

3. Please expand all abbreviations during the first time use.
Done

4. Please rephrase the Short Abstract/Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words: "Here, we present a protocol to …". Manuscript text is an important component of the submission and video is an associated file which is made after the manuscript acceptance. Please do not use the words "in this video" in the text.
We replaced all the words "in this video" to "in this protocol"

5. Please ensure that the long Abstract is within 150-300-word limit and clearly states the goal of the protocol.
Our abstract is 292 words long. We clearly stated the goal of the protocol e.g. "we will describe the procedure to perform invasive, closed-chest, sequential left and right ventricular pressure-volume loops in the same mice"

6. JoVE policy states that the video narrative is objective and not biased towards a particular product featured in the video. The goal of this policy is to focus on the science rather than to present a technique as an advertisement for a specific item. Please do not use commercial language e.g., Transonic Scisense a mouse Pressure-Volume 42 Catheter, the ADVantage Pressure-Volume System (London, ON) and labScribe v4 software 43 (iworx - 2018; Dover, NH). Please refer to the term using generic language.
We removed the commercial language from our manuscript.

7. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.
We deleted the section material and added a table of materials and reagents in the revised manuscript (below and table 1 in the revised manuscript)

Table 1: Table of materials and reagents

Product	Reference	Company
Isofluorane	CA2L9108	Baxter
0.9%(wt/vol) sodium chloride	B1309M	Baxter
Surgical tape	SN770	3M
Endozime AW triple plus	34521	Ruhof
High-fidelity admittance catheter (Scisense)	FTH-1212B-3518	Transonic
ADVantage Pressure-Volume System (ADV500)	FY097B	Transonic
Tabletop Single Animal Anesthesia Systems	72-6468	Harvard apparatus
Ventilator, far infrared warming pad for mice and rats PhysioSuite	PS-02	Kent scientific corporation
Fiber optic dual Gooseneck	6000#1	Volpi Intralux
Stereo microscope	OF-48920-10	Cole-Parmer
Forceps	11052-10	F.S.T
Forceps	1125120	F.S.T
Hemostatic clamp	13003-10	F.S.T
Hemostatic clamp	13018-14	F.S.T
Surgical suture, black braided silk, 4.0	ID158000	SERAFLEX
Needle (30 gauge)	305106	BD
Gauze sponges	441400	Dermacea
Tracheotomy canula 1.45 mm diameter	72-1410	Harvard apparatus
labScribe v4 software; PV-loops module	LS-30PVL	iworx

8. Please move all the materials from the materials sections to the table of materials instead.

Please see comment above

9. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets, alphabets, or dashes.
Done

10. Please include an ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution.
According to your recommendation we added the statement below:
“All experiments were performed in accordance with Queen’s University biosafety and ethical guidelines (ROMEO/TRAQ#6016826). The procedures followed were performed in accordance with institutional guidelines.”

11. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly.
Done

12. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step.
We simplified individual steps to 2-3 actions.

13. The Protocol should contain only action items that direct the reader to do something.
Done

14. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed?
We believe that our protocol properly answer to the “how” question.

15. Please include all the button clicks in the software, knob turns in the instruments etc.
We included all the button clicks in the software

16. 2b: How is this done?
We extended the point 2b (now 1.2) in the revised form of the manuscript.

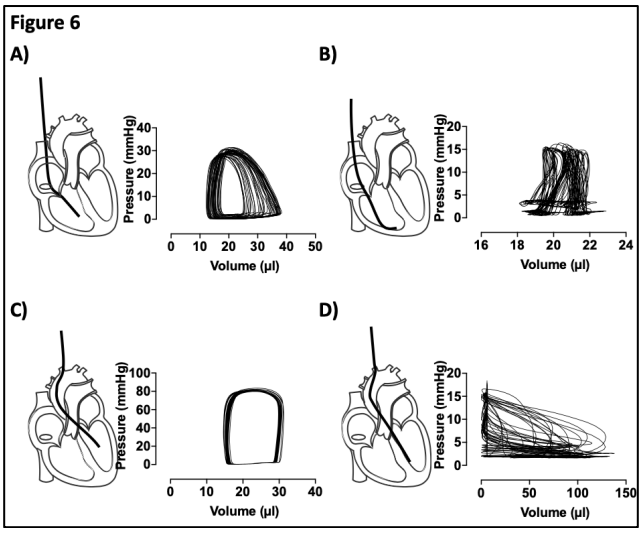
17. 3: How do you maintain sterility during the experiment?
This is a terminal procedure, animals are scarified after the procedure. We do not maintain sterility during the experiment.

18. 7: Please explain how this is done. Please include all the button clicks, knob turns etc.?
We clarified this point. Please note that our protocol focus on the surgical procedure rather than data analysis. Data analysis will depend on the software used and will be different from one distributor to another one (e.g labChart VS iworkx). Since we cannot provide the commercial name of the software in the protocol we believe that is unnecessary to provide more description of the data acquisition. We believe that this section species what constitutes a good practice for cardiac catheterization (e.g. record 20 loops), rather than an exhaustive description of the acquisition procedure.

19. Please move the troubleshooting section to the discussion.
Done

20. There is a 10-page limit for the Protocol, but there is a 2.75-page limit for filmable content. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.
The 2.75 pages was highlighted in yellow according to the author’s instruction.

21. For the catheter placement and pressure volume loops (Figure 6), are these just schematics or real pressure-volume loops. Please provide actual data obtained. Please label both the axis. The representative result should have data from an experiment you performed an experiment, how did it help you to conclude what you wanted to and how is it in line with the title. Data from both successful and sub-optimal experiments can be included.
The results from that manuscript are original and have never been published elsewhere. We described in the legend section how we concluded to a “good” and “bad” pV-loops e.g. “Figure 6 B is a schematic representation of the improper positioning of the catheter in the right ventricle (contact with the ventricular wall) and the resulting flawed PV-loops (collapsed and non-regular loops)”. According to your recommendation we labeled both axis in the revised figures 6 and below.



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Done

Reviewers' comments:
Reviewer #1:
The authors present a method for simultaneous left and right ventricular pressure/volume catheterization in mice to conduct a hemodynamic characterization of whole heart function including interventricular correlations. The methodology is sophisticated, well-described, and represents an interesting approach that may enhance cardiovascular research. There are several questions that arise from the methodology that need to be addressed. Most importantly, the anesthesia appears to be insufficient: The authors do not administer any analgesic therapy before skin incision and surgery. Mice may experience pain. This issue should be evaluated and discussed with an expert for veterinary medicine/laboratory animals. Please find specific comments as depicted below.

1)-Isoflurane is anesthetic, but has not analgesic effect. Even though the experiment is terminal, mice may experience pain during the surgery, especially considering that a stable isoflurane anesthesia is sometimes hard to achieve. The authors should revise the methodology accordingly and add analgesia, e.g. buprenorphine and/or ketamine before skin incision.

Thank you for your comments. We performed our experiments according to the Canadian and our local ethical guideline. Either ketamine or Isoflurane are recommended for terminal procedure. We decided to use isoflurane rather than Ketamine due to the adverse effect of ketamine on central nervous system. Indeed ketamine injection is associated with increased pulmonary blood pressures, heart rate, cardiac output, cardiac work and, myocardial oxygen requirement. We clarified this point and added the sentence below in the discussion section:

"The procedure described in this protocol is a terminal procedure. Due to the invasiveness of the right and left catheterization, the animals should be euthanized immediately after data acquisition. The euthanasia should be performed according to your institution's animal studies guidelines."

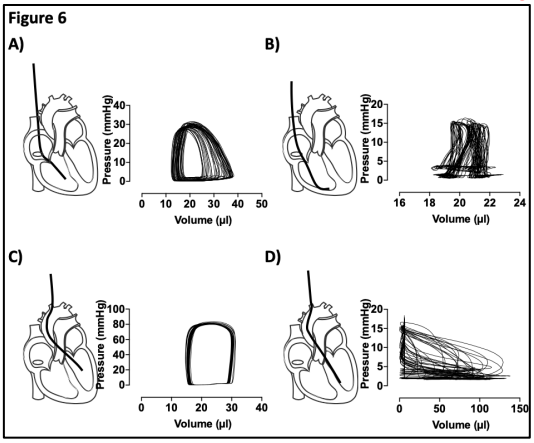
2)-The authors use a nose cone for the primary anesthesia with isoflurane, but then perform endotracheal intubation. The authors should comment on why endotracheal intubation is necessary since this likely influences RV pressure.

Dear reviewer, we decided to perform endotracheal intubation to increase the quality and the reproducibility of the data acquisition. Besides, movement associated with heart catheterization could disconnect the nose cone and result in improper/impaired ventilation. We believe that we addressed the limitation and advantage of endotracheal intubation in the discussion section (and below):

"Invasive tracheostomy and positive pressure mechanical ventilation will result in stable and controlled breathing of the mice and decrease the variability of the pressure-volume loops acquisition. However, positive end expiratory pressure (PEEP) is a marked contrast to normal ventilation (which is a negative pressure phenomenon). Together positive pressure ventilation and PEEP lower cardiac output and reduce right heart pressures. Thus, while required for acquisition of stable data, it must be acknowledged that mechanical ventilation as well as cardio-depressive effect of the anesthesia will affect the pressure volume loops and should be considered as a limitation. Transiently stopping mechanical ventilation during the brief recording of pressure-volume loops is a technique we use to eliminate this potential source of artifact."

3)-The authors should add absolute numbers and units to Fig. 6.

According to your recommendation we added the absolute numbers and units to figure 6 (and below)



4)-The authors state "Ensure that the heart rate reported on the ADV500 monitor screen is in a physiologic range (i.e. >350; <500bpm).", but a physiological heart rate is described as 500-600 BPM in common literature.

Heart rate is variable upon the mouse strain, the mouse weight as well as the type of anesthesia used. For example, the heart rate range for 20-34g mice anesthetized with xylazine is 340-510 min-1 whilst the heart rate range for 20-34g mice anesthetized with isoflurane is 470-620 min-1[1]. In our lab (specific mice strain, different weight, and isoflurane anesthetic) we observed a 350 -500bpm heart rate ranges. We agree that the "physiological" rate range

traditionally reported in the literature is 500-600 BPM. According to your recommendation we changed “>350; <500bpm” to >400; <600bpm.

5)-The authors should comment the units of volume data: Is it measured as "Siemens" for conductance or can this be translated to actual volume, e.g. microliter? With Millar instruments pressure/volume catheters, this can be done by saline calibration and cuvette calibration. Is there a similar approach?
Dear reviewer, unlike Millar instruments, the latest generation of transonic conductance catheter do not require saline calibration and cuvette calibration. The low and high signal calibration is performed before catheterization as described in the manuscript. A second calibration is performed directly in the ventricle of the animal when the pressure and magnitude are optimal.

6)-The authors should add a section on the most important values that are measured, the most important calculations, and the relevance of these values for cardiac physiology/pathophysiology.
According to your comment, we added a new table with hemodynamic data obtained from RV and LV (n= 6 mice) and the description of these values in the “representative results” section. (Table 2 and below)

Table 2: Haemodynamic parameters

Haemodynamic Parameter	
HR (BPM)	410.6 ± 23.3
CO (µl/min)	9107 ± 1016
SV (µl)	24.5 ± 2.3
RV function	
RVSP (mmHg)	21.9 ± 2.15
RVEDP (mmHg)	1.042 ± 0.12
EF (%)	56.1 ± 4.4
dP/dt max (mmHg/sec)	1469 ± 170
dP/dt max (- mmHg/sec)	1504 ± 215
EDV (µl)	38.4 ± 3.7
SW (mJoules)	0.068 ± 0.008
PVA (mJoules)	0.084 ± 0.009
Ea (mmHg/µl)	0.83 ± 0.09
Tau factor (msec)	12.8 ± 0.8
LV function	
LVSP (mmHg)	77.1 ± 2.4
LVEDP (mmHg)	2.33 ± 0.17
EF (%)	59.1 ± 3.6
dP/dt max (mmHg/sec)	4695 ± 355
dP/dt max (- mmHg/sec)	3553 ± 373
EDV (µl)	36.9 ± 4.8
SW (mJoules)	0.14 ± 0.013
PVA (mJoules)	0.22 ± 0.03
Ea (mmHg/µl)	5.37 ± 0.9
Tau factor (msec)	15.07 ± 1.7

CO, cardiac output; Ea, arterial elastance; EDV, end diastolic volume; HR, heart rate; LVEDP, left ventricular end diastolic volume; LVSP, left ventricular systolic pressure; PVA, pressure volume area; RVEDP, right ventricular end diastolic pressure; RVSP, right ventricular systolic pressure; SV, stroke volume; SW, stroke work; Tau factor, Tau Mirsky. N= 6 mice. Values are expressed ± SEM.

7)-The authors should consider to condense figure 3-5 since this is likely redundant to the video. Instead, the authors should provide a figure that shows exemplary hemodynamic data that is obtained from RV and LV. Especially for RV catheterization, this is important. Presenting data with dot plots may help to judge the typical variance between mice.
Thank you for your comment, we added a novel table showing the representative value obtained for RV and LV (Please see comment above). However, we believe that it is important to show endotracheal intubation, RV and LV catheterization in independent figures, we thus decided to leave figure 3-5.

8)-How did the authors validate the different catheter placements displayed in figure 6?.
Dear reviewer the “correct” positioning of the catheter is validated by the generation of the stable pressure-volume loop. It is almost impossible to generate PV-loops without the right positioning of the catheter in the chamber with all the probes in the ventricle. The schemas represent the “correct” and “wrong” positioning of the catheter, according to the transonic troubleshooting guideline. We acknowledge that we did not validate the different catheter placement. We believe that the schematic representation of the catheter’s positioning would be helpful for the reader.

9)-The authors should state what happens with the mouse after the procedure. Was it killed, and how?
After the procedure animals are euthanized by exsanguination (via puncture of the abdominal aorta) and bilateral pneumothorax. According to your recommendation we added the sentence below in the discussion section:
"The procedure described in this protocol is a terminal procedure. Due to the invasiveness of the right and left catheterization, the animals should be euthanized immediately after data acquisition. The euthanasia should be performed according to your institution's animal studies guidelines."

10)-The authors should add a statement on the use of laboratory animals (approval by local facility).
Done. We added the sentence below in the revised manuscript:
"All experiments were performed in accordance with Queen's University biosafety and ethical guidelines (ROMEO/TRAQ#6016826). The procedures followed were performed in accordance with institutional guidelines."

11)-The use of anesthesia that is likely to have a cardiodepressive effect and the mechanical ventilation will influence the obtained results, but are (at least the anesthesia) unavoidable. The authors should list this as an important limitation.
Thank you for your comment, we addressed this limitation with the sentence below
"Thus, while required for acquisition of stable data, it must be acknowledged that mechanical ventilation as well as cardio-depressive effect of the anesthesia will affect the pressure volume loops and should be considered as a limitation"

12)-Please ad a comma to "not as expensive as echocardiography CT or MRI" (page 2, line 109)
Done

Reviewer #2:
Manuscript Summary:
This manuscript concerns the assessment of cardiac function using pressure volume loops. The novelty of this protocol is the assessment of both left and right ventricles in a closed-chest anaesthetized mouse. The topic is introduced well and the rationale for the method is well described. I agree wholeheartedly that the cosed-chest approach is best for this assessment. The authors propose the use of the harvard Tabletop single animal anaesthesia system. This is a particularly high-end system and I commend the authors for using this to control core temperature.

Major Concerns:
I have no major concerns regarding the protocol. The edit and quality of video recording will be key in getting this protocol clear for the viewer. This is particularly close work and as long as the quality of the filming shows in detail the catheterisation procedure and simultaneous PV loops or pressure/volume waves the viewer would be able to understand the procedure better. I trust that the final publication will also include examples of good physiological data as well as data when things are not right.

1) The authors refer to a normal heart rate for mouse hearts under isofurane (2%) to be 350-500 bpm. I would hope that hr could be higher than 500 bpm in a lightly anaesthetised mouse, can the authors confirm this. We routinely get heart rates of 550-580 bpm under isoflurane (1.5% - 2% in oxygen).
Thank you for your concern, please see response to reviewer 1, concern 4.

2) step 6a viii the authors just state to withdraw the catheter and wipe, i would suggest that the authors need to include that at this point you will need to tighten one of the sutures to stop blood flowing out of the jugular vein. Similarly at step 6b iv the user should be careful to tighten the sutures to reduce the chance of blood loss from the artery.
Thank you, we added the following point in the revised manuscript:
"5.1.8.4. Tie the caudal suture to stop blood bleeding from the jugular vein." And "5.2.9.3. Tie the caudal suture."

3) Care should be taken where to tighten the sutures once the catheter has been places. for example if the user was to tighten the suture over the pressure sensor this would damage the catheter.
Dear reviewer, we agree and added the following note to the point 5.1.3.
“Note that extreme care should be taken to not overly tighten the suture since excess force can damage the catheter.”

4) correct filters should be applied on aquisition rather than post hoc as described in section 7. i would recommend a notch filter of 50Hz to eliminate mains 'noise' but also that suitable low pass filters are considered to remove any RF interferrance.

Dear reviewer, in our lab we apply a post hoc filter because we want to collect raw, unmodified, data. However, according to your recommendation, we added the sentence below in the discussion section of the revised manuscript.
“A notch filter of 50Hz could also be applied on acquisition to eliminate mains 'noise' and remove any radio-frequency interference.”

Minor Concerns:
The authors have limited their methodology to certain branded instruments and specific surgical tools. I feel that this is not necessary and the authors should be careful to state that there are alternatives that would not affect the outcome of the experiment. Namely, the data aquisition system is listed as being LabScribe v4. Whist this is certainly very capable software there are multiple alternatives which offer a similar function, it would be worth the authors mentioning that their recommendation is not the only option for this.
Thank you for your concern. Please see the response to the editor’s comments 6, 7 and 8.

In order to confirm adequate ventilation it is worth noting that a capnograph might be useful on the expiration line, this might be worth mentioning in the manuscript and video.
Dear reviewer, we currently don’t use capnograph in our lab. This is a good suggestion and we added the following sentence in the revised manuscript :
“Note that ventilation efficiency can be confirmed by the capnography monitoring of carbon dioxide”

I note that in Figure 1A the catheter appears to be bent at the end, i would strongly advise that bending a catheter will make data acquisition unstable.
Thank you for your concern, whilst we agree that bending catheter could impair data acquisition, we found that it is easier to get proper positioning and subsequent data acquisition with a bent catheter. However, this catheter shaping may reduce longevity of the catheter.

Figure 2A shows a rectal probe inserted with some kind of sheathing, I am unsure what this sheath is, can the authors explain why it is needed?
We use sheathing to protect our probe. It is the procedure that we use in our lab. We agree that is a lab preference, and that the sheathing is not required for probe function.

Reviewer #3:
Manuscript Summary:
The authors have outlined a technique to execute in situ pressure volume recordings of the right and left ventricles in the same mouse. The significance of this approach is well-detailed in the introduction and highlights the interdependence of the left and right ventricles, a critical yet understudied area. Ventricular interdependence becomes especially important in the context of cardiac disease and, perhaps even more during right-sided conditions such as pulmonary hypertension. While this technique requires technical expertise, the authors provide a clear description allowing the reader to re-create the technique especially those with an established expertise. In addition, the authors provide a succinct discussion of the limitations. In general, this technique will be of interest to the scientific community, especially with the aid of visual assistance. The manuscript is clear and well-written, yet some concerns were identified and outlined below.

Major Concerns:
1. The authors provide a brief discussion of how the conductance catheter determines "volume". Considering the RV

has a unique geometry and muscle mass than the left, the authors should provide some discussion or perhaps quantitation of these right-left differences. Furthermore, considering the unique chamber geometry of the RV, there is unique relationship between "wall-tension/stress" and ventricular pressure different from the LV. Please discuss.
Dear reviewer, we agree with this major limitation. The admittance catheter was first designed for LV. The unique chamber geometry of the RV might affect volume measurement and should be considered as a limitation. However, we observed no significant differences in cardiac output when measured in right and left ventricles. This result suggests that volume measurement in RV and LV is reproducible and accurate. According to your recommendation, we added the sentence below in the limitation section of the revised manuscript:
“The unique chamber geometry of the RV might affect volume measurement and should be considered as a limitation.”

2. The authors describe the physiological range of heart rate in mice as 350-500 bpm. As outlined in an early evaluation (Georgakopoulos D, Kass D. J Phys 2001), the force-frequency relationship at heart rates below 500 bpm is non-linear and can lead to confounding issues of "contractility". Please address how this may or may not impact RV function.
Dear reviewer, we agree that according to the work from Georgakopoulos D and colleagues, low heart rates could affect contraction and relaxation function. However, low heart rate associated with anesthesia on 20-34g mice e.g. 340-510 bpm with ketamine + xylazine, 365-550 bpm with pentobarbital sodium is not associated with significant changes in diastolic function in LV (LV end-diastolic pressure of 1-9mmHg and 2-8mmHg respectively)[1]. We agree that among other factors, anesthesia, and low heart rate impact cardiac function. However, the present work is a method paper which aims to focus on the technical (surgical) procedure. According to your comment, we added the sentence below in the limitation section of the revised manuscript.
“The anesthetic used, heart rates, temperatures, the animal strain could affect hemodynamic parameters and should be carefully reported and monitored.”

Minor Concerns:
1. Even though this manuscript is focused on the technique, a large part of the Introduction is devoted to a discussion of ventricular interaction. An example or demonstration of a technique highlighting this property would be appreciated. Perhaps a sudden infusion of volume into the right atrium or left atrium.
Dear reviewer, we agree that ventricular interaction represents a fascinating area that needs to be further investigated. However, it would require extensive experiments that are beyond the topic of this paper. Our method paper aims to focus on the technical (surgical) aspect of left and right heart catheterization.