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Sterile pericarditis in Aachener minipigs as a model for atrial myopathy and atrial fibrillation

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TITLE:

Sterile Pericarditis in Aachener Minipigs as a Model for Atrial Myopathy and Atrial Fibrillation

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SUMMARY:

We describe a sterile pericarditis model in minipigs to study atrial myopathy and atrial fibrillation (AF). We present surgical and anesthetic techniques, strategies for vascular access, and a protocol to study the inducibility of AF.

ABSTRACT:

Atrial fibrillation (AF) is the most common arrhythmia caused by structural remodeling of the atria, also called atrial myopathy. Current therapies only target the electrical abnormalities and not the underlying atrial myopathy. For the development of novel therapies, a reproducible large animal model of atrial myopathy is necessary. This paper presents a model of sterile pericarditis-induced atrial myopathy in Aachener minipigs. Sterile pericarditis was induced by spraying sterile talcum and leaving a layer of sterile gauze over the atrial epicardial surface. This led to

inflammation and fibrosis, two crucial components of the pathophysiology of atrial myopathy, making the atria susceptible to the induction of AF. Two pacemaker electrodes were positioned epicardially on each atrium and connected to two pacemakers from different manufacturers. This strategy allowed for repeated non-invasive atrial programmed stimulation to determine the inducibility of AF at specified time points after surgery. Different protocols to test AF inducibility were used. The advantages of this model are its clinical relevance, with AF inducibility and the rapid induction of inflammation and fibrosis—both present in atrial myopathy—and its reproducibility. The model will be useful in the development of novel therapies targeting atrial myopathy and AF.

INTRODUCTION:

Atrial fibrillation (AF) is the most prevalent cardiac arrhythmia, leading to significant morbidity, mortality, and healthcare expenses[1]. In many cases, AF is merely the electrical symptom of the underlying atrial myopathy, which is defined by structural, electrical, autonomic, and contractile remodeling of the atria. This atrial myopathy can lead to AF and stroke[2,3]. Most therapies only target the electrical remodeling but do not target the underlying structural changes in the atria (inflammation and fibrosis)[4-7]. This is probably one of the reasons why current therapies are only marginally effective, especially in more advanced atrial myopathy[8].

A reproducible animal model is crucial to target the inflammation and fibrosis present in atrial myopathy. Atrial tachypacing models have been developed in several large animal species[9-12]. In these models, the atrial tissue is paced continuously for long periods to induce electrical and eventually structural changes. The major disadvantages of tachypacing models are the long duration before structural signs of atrial myopathy appear and their relevance only for clinical syndromes in which electrical abnormalities develop before the atrial myopathy. A theoretical risk is pacing-lead failure due to fibrosis during long follow-up[9].

In models of sterile pericarditis, sterile talcum is sprayed over the epicardial surface of the atria to induce an acute inflammatory and fibrotic reaction, resulting in atrial myopathy[13,14]. Pigs have cardiac anatomy and physiology similar to that of humans, and therefore, porcine models have high translational relevance. The advantages of using minipigs are that they are easier to handle due to their smaller size than conventional pig strains and can be maintained for a long period without any significant increase in body weight[10]. All these reasons make sterile pericarditis in minipigs an excellent model for the investigation of atrial myopathy and fibrillation. This protocol and video aim to facilitate the setup of this model in different research facilities and standardize protocols to study the inducibility of AF.

PROTOCOL:

This protocol has been approved by the University of Antwerp Ethical Committee for Animal Testing (case number 2019-29) and follows the animal care guidelines of the University of Antwerp. Seventeen 6-month-old Aachener minipigs (male, castrated) weighing ~20 kg were selected for this study.

1. Medication and anesthesia

1.1. Premedication

1.1.1. Ensure that the pigs are fasted for 12 h, but with unlimited access to water.

1.1.2. For sedation, administer the following in one intramuscular injection: atropine 0.05 mg/kg, ketamine 10 mg/kg, midazolam 0.5 mg/kg.

1.1.3. Determine the exact weight of the pig after it has lost consciousness (approximately 10 min post dose). Transport the pig to the operating theater.

1.1.4. Place the pig on a heating pad.

1.1.5. Apply ECG monitoring, pulse oximeter, and perform an initial thermometry.

1.1.6. Insert an over-the-needle catheter (22 G) into the marginal ear vein or the external saphenous vein.

1.2. Anesthesia

1.2.1. For the induction of anesthesia, administer a bolus of propofol (1–4 mg/kg IV) before starting intubation. If superficial anesthesia is noted, administer an extra bolus of midazolam 0.2 mg/kg IV, and proceed to the intubation after ~5 min.

1.2.2. Intubation

1.2.2.1. Place the pig in prone position.

1.2.2.2. Ask an assistant to hold the mouth of the animal open using two slings of gauze and/or a mouth spreader. Spray 1 mL (10 mg) of lidocaine in the larynx with a 2 mL needleless syringe, wait for 30–60 s to desensitize the larynx, and then continue.

1.2.2.3. Place an endotracheal tube (ETT) with an internal diameter of 6.5 mm using a laryngoscope. Use a laryngoscope to visualize, displace the epiglottis from the soft palate, and place a stylet into the ETT for better manipulation.

NOTE: The pig's mouth cannot be opened widely, and the distance from the nose tip to the larynx is long. Therefore, visualization of the *rima glottis* is limited. Hence, the ETT and the stylet help visualization.

1.2.3. When connecting the ventilator, give supplementary medication if needed: midazolam 0.5 mg/kg IV and/or alfentanil 30 µg/kg IV.

1.2.4. Use the following ventilator settings: volume control ventilation (VCV) with a pre-set tidal volume of 10 mL/kg, leading towards a peak inspiratory pressure (PIP) of 11–15 cmH_20, a positive end-expiratory pressure PEEP of 2–5 cmH_20; respiratory rate: 12–16 Brpm to maintain end-tidal CO2 (ETCO2) between 35–45 mmHg; FiO_2: 50% (to be reduced when saturation is 100%); sevoflurane 2.5%.

1.2.5. For analgesia, use alfentanil 0.5–1 $\mu g \cdot (kg \cdot min)^{-1}$ CRI.

1.2.6. Administer a bolus of 10 mL/kg of plasmalyte 3–5 $mL \cdot (kg \cdot h)^{-1}$ over 10–20 min to correct hypotension due to hypovolemia.

1.2.7. Administer 1 g of cefazoline IV. For every 2 h of surgery, administer an extra 500 mg of cefazoline IV.

NOTE: For an overview of the emergency medication to have at hand in the operating theater, see **Table 1**. Urinary bladder catheterization is difficult in male pigs and, in general, not necessary for this procedure.

1.2.8. Shave the thoracic and neck region of the animal.

1.2.9. Apply vet ointment to the eyes to prevent dryness and eye irritation during anesthesia.

1.2.10. Continuously monitor the vital parameters. Check the depth of anesthesia at least every 10 min by assessing whether the jaw tonus is relaxed, the palpebral reflex is absent, the eyes are rotated, and there are no behavioral signs of excitation. Check the color of the mucosa and capillary refill time to evaluate tissue perfusion. Record all data, together with all administered medication, in an individual anesthetic chart.

1.2.11. Arterial line placement

1.2.11.1. Prepare the pressure conducting system. Add 5000 IU of heparin to an IV bag of 500 mL of 0.9% NaCl.

1.2.11.2. Return the animal to supine position. Extend the leg and locate the femoral artery using ultrasound with the vascular probe in carotid setting. Disinfect the inguinal zone with chlorhexidine.

1.2.11.3. Puncture the femoral artery using ultrasound guidance. Insert a 3 Fr sheath using the Seldinger technique.

NOTE: Because of the small diameter of the femoral artery, it can be helpful to let an assistant insert the guide wire through the needle. Just the action of lifting the ultrasound probe may dislocate the needle tip.

1.2.11.4. Fixate the sheath with a suture. Connect the sheath to the transducer and flush. Monitor the arterial blood pressure in real time.

2. Surgery

2.1. Preparation

2.1.1. Ensure that the animal is supine in a stable position. For extra stability, place prewarmed IV bags in a paraspinal position to support the animal.

2.1.2. Place the earthing plate of the electrocautery underneath the animal. Use a small amount of ultrasound gel to ensure proper contact with the skin.

2.1.3. Disinfect the skin of the animal using 2% iodine. Ensure that the neck, thorax, upper limbs, and upper half of the abdomen are covered.

2.1.4. Place sterile drapes. Wrap the claws of the animal in sterile sheets or gloves as well. Use sterile gauze to retract them.

2.1.5. To ensure sterile conditions, drape the surgical area with sterile surgical covers, use sterile instruments, and work under sterile conditions until skin closure.

NOTE: Throughout the procedure, surgeons must wear a hair cap, a mouth mask, a surgical gown, and sterile gloves.

2.2. Surgical placement of a permanent central venous catheter (CVC)

2.2.1. Make a 5 cm incision in the groove at the medial border of the sternocleidomastoid muscle. Bluntly dissect until the internal jugular vein is reached.

2.2.2. Remove fibrous tissue around the vein and place a squared suture with Prolene 6-0 around the desired catheterization site to gain vessel control.

2.2.3. Cannulate the internal jugular vein with a 3 French triple-lumen CVC using the Seldinger technique. Tighten the Prolene 6-0 suture around the catheter.

2.2.4. Fixate the handle of the catheter to the sternocleidomastoid muscle.

2.2.5. Tunnel the three catheter lumina separately and attach the ends firmly to the skin. Put on the needle-free injection port.

2.2.6. Close the incision site in two layers.

2.3. Sternotomy

2.3.1. Make a median incision from the manubrium of the sternum to 3 cm below the xiphoid process until the sternum becomes apparent.

2.3.2. Bluntly dissect caudally from the xiphoid process. Put a finger on the visceral side of the sternum and remove connective tissue as far as possible following the visceral sternal surface.

NOTE: The connective tissue is removed to prevent myocardial injury whilst performing sternotomy.

2.3.3. Use the sternum saw to cleave the sternum. Control all bleeding sites. Use the sternum spreader to enlarge the access to the thoracic cavity. Avoid damaging the pleura.

2.3.4. Open the pericardium carefully and use suspension sutures to keep it out of the surgical field.

2.4. Pacemaker lead placement (see **Figure 1**)

2.4.1. Place a pacemaker lead on the left atrium.

2.4.1.1. Test the extension and retraction mechanism of the lead's fixation screw. Then, put the tip on a (curved) forceps and curve the stylet by 60° if necessary.

2.4.1.2. Put a compress on the left ventricle and gently pull it aside to have a view of the left atrium.

NOTE: Pressure on the ventricle will quickly cause hypotension. Make sure the anesthesiologist anticipates this with low-dose norepinephrine through the CVC. **Release the ventricle when the mean blood pressure drops below 40 mmHg for >20 s.** Only proceed when the blood pressure of the animal has normalized.

2.4.1.3. Upon visualization of the left atrium, firmly put the lead tip on the left atrial free wall, as close as possible to the pulmonary veins and as far as possible from the ventricle. Screw it in by extending the helix into the atrial tissue, preferably with a slight inclination. Do this as fast as possible and release the pressure on the left ventricle immediately.

2.4.1.4. Measure the sensing and pacing threshold and impedance of the lead using a programmable electrical stimulator or pacemaker programmer. Ensure that there is no ventricular overcapture (broad QRS on ECG) when pacing at high voltages (10 V). If not satisfied, retract the helix of the lead and start over from step 2.4.1.1.

NOTE: Normal pacing threshold should be <1 V with a pulse width of 0.5 ms (typically ~0.5 V @0.5 ms).

2.4.2. Place a pacemaker lead on the right atrium, completely analogous to the placement of the left atrial lead.

2.4.3. Ensure that both leads leave the thorax at the midline; the left atrial lead must be tunneled through the abdominal subcutaneous fat from the xiphoid process to the left flank, the right atrial lead to the right flank.

2.4.4. Make a pacemaker pocket in the subcutaneous fat at the left and right flank of the pig. Connect the pacemakers to the leads and place them inside the pockets. Connect a pacemaker capable of performing (50 Hz) burst pacing with the left atrial lead (to allow pacing) and a pacemaker from a different manufacturer to the right atrial lead (to allow sensing).

2.5. Induction of sterile pericarditis

2.5.1. Expose the atria again by gently pulling aside the ventricles. Cover up the ventricles with gauze (and take the gauze away afterward).

2.5.2. Spray sterile talcum over the epicardial surface of both atria using the dispenser that is included in the pack. As bradycardia and hypotension will follow this manipulation, give the heart enough time to recover.

2.5.3. Leave one layer of sterile gauze on the epicardial surface of the atria.

2.5.4. Check the position of the pacemaker leads one last time before starting closure.

2.6. Closing the chest

2.6.1. Leave a drain in the mediastinum and tunnel it to the skin surface.

2.6.2. Close the pericardium with Prolene 6-0.

2.6.3. Close the sternum using a classical cerclage technique with stainless steel wire.

2.6.4. Close the subcutis in two layers with resorbable thread.

2.6.5. Perform a sternal block by infiltrating 5 mL of 0.5% bupivacaine into the skin; ensure bone contact with the sternum to infiltrate the periosteum.

2.6.6. Close the skin with a continuous intradermal suture using resorbable thread.

3. Postoperative care

3.1. Progressively, turn off all sedatives while closing the skin of the animal.

3.2. Keep the animal in the surgery room with close monitoring of body temperature, ventilation and airway patency, oxygenation, and hemodynamic parameters.

3.3. Due to a substantial drop in body temperature that frequently occurs during the procedure, keep the animal warm using blankets, heating pad, and hot packs. Provide oxygen during recovery, especially when shivering is noted.

3.4. Apply a fentanyl patch of 50 µg/h for postoperative analgesia. Because there is a delay of 6–8 h before the fentanyl patch becomes effective, administer 0.05–0.1 mg/kg of morphine subcutaneously to bridge this period.

3.5. When the animal is stable, is showing an increase in body temperature; can lift its head; is swallowing; shows normal ocular reflexes; and is breathing spontaneously, freely, and deeply without an ETT in place, without signs of upper airway obstruction; it can be transported back to the pen. Provide means of heating during the recovery phase (e.g., infrared lamp, heating mat, blankets).

NOTE: **Avoid putting the animal back in the pen too soon** as respiratory arrest is possible, even hours after the cessation of narcotics.

3.6. Perform a check-up on the animal: every 15 min during the first hour postoperatively, then hourly for the first 4–6 h or more frequently if the animal is uncomfortable. When the animal shows signs of pain, administer supplementary morphine subcutaneously 0.025–0.05 mg/kg every 2 h until it is comfortable. Administer 1 g of cefazoline 8 and 16 h after surgery.

NOTE: Pain assessment consists of subjective elements such as attitude, behavior (standing, eating, drinking), and grimace. Objective signs of pain are elevated heart rate, elevated respiratory rate, and superficial respiration. The animal will return to its normal status and behavior within 24 h. Remove the fentanyl patch on day 3 post operation.

4. Atrial tachypacing for induction of AF

4.1. Inject ketamine 10 mg/kg and midazolam 0.5 mg/kg intramuscularly (without atropine) and wait until a sufficient level of sedation is reached.

4.2. Weigh the pig again for follow-up. Place the animal in a restraining sling and bring it to the operating theater.

4.3. Attach ECG and saturation monitoring and place the programmer heads over their corresponding pacemakers. Interrogate the pacemakers.

4.4. Check the pacemaker settings for the occurrence of spontaneous AF. Look for a ventricular lead warning when using a dual-chamber pacemaker.

4.5. Determine impedance and sensing and pacing thresholds. When performing electrophysiology (EP) studies, always pace at twice the threshold voltage and watch for an increase in voltage threshold during the experiment.

4.6. Determine the atrial effective refractory period (AERP) approximated by the shortest cycle length at which 1:1 capture is maintained during burst pacing.

NOTE: This method is different from clinical AERP determination but more relevant to this protocol.

4.7. Determine the conduction time between left and right atrial leads by measuring the time between the initiation of the pacing spike and the atrial depolarization on the right atrial lead.

4.8. For the first protocol, apply a burst pace for 20 s with a cycle length of AERP + 30 ms. After the cessation of the pacing, check for the presence of AF and measure how long the episode lasts. Pause for at least 5 s between each pacing session and wait until the sinus rhythm heart rate has recovered to baseline. Repeat this ≥10 times; note the display of the AF inducibility as a percentage—the proportion of "successful" attempts to the total amount of attempts to induce AF.

NOTE: Only episodes > 5 s are considered relevant.

4.9. For the second protocol, apply a burst pace for 20 s, starting with a cycle length of AERP + 20 m. During the following burst, decrease the cycle length until the minimal cycle length with 1:1 capture. Repeat this at least 10 times. Note the AF duration and AF inducibility.

4.10. For the third protocol, apply a burst pace for 5 s at 50 Hz. Repeat this at least 10 times. Note the AF duration and AF inducibility.

4.11. Let the animal awaken or continue with other procedures (e.g., echocardiography, treatment, blood draw)

5. Euthanasia

5.1. After the experiment, the animals are euthanized with an overdose of IV pentobarbital (50 mg/kg, IV).

6. Sham surgery

6.1. Perform the same protocol without spraying talcum over the atrial epicardium or leaving a layer of sterile gauze.

REPRESENTATIVE RESULTS:

Morbidity and mortality:

When we started developing this model of sterile pericarditis in Aachener minipigs, we noticed perioperative mortality of 4 out of 17 pigs (23.5%): 3 out of 4 deaths occurred in the first 6 surgeries because of a "learning curve effect." The etiologies were the following: 2 pigs died because of postoperative respiratory arrest; this problem was solved by reducing the dose of alfentanil. One pig died because of ventricular fibrillation during the first pacing session and one during the testing of the pacing lead: this was due to ventricular overcapture because the left atrial lead was placed too close to the ventricle. During the follow-up period, all animals survived until sacrifice. Further, signs of discomfort disappeared 24 h postoperatively. If any signs of discomfort persist after this time, the investigator should be suspicious of complications.

Pacing properties:

A gradual increase in the voltage threshold and impedance of the left atrial lead were observed during the experiment (**Figure 2A**). However, this varied among animals and never led to non-capture. AF inducibility began to increase two weeks after surgery up to ~25% on average. The "AERP + 30 ms" protocol was the least effective, showing AF inducibility ~10%. Decremental pacing and 50 Hz burst pacing increased AF inducibility to ~40% (**Figure 2B**).

Histology:

Figure 3 shows higher levels of interstitial/perivascular fibrosis in the sterile pericarditis animals compared to shams.

FIGURE AND TABLE LEGENDS:

Figure 1: Experimental setup of the pacing leads. A pacemaker for atrial tachypacing is connected to a lead screwed into the left atrium. Similarly, a pacemaker for sensing the right atrial electromyogram is connected to a lead screwed into the right atrium of the pig. Abbreviation: EGM = electrogram.

Figure 2: Evolution of electrophysiology parameters over time. (**A**) Lead impedance increases over time, indicating increased fibrosis (n = 6). Error bars indicate standard deviation. (**B**) Decremental pacing and 50 Hz burst pacing protocols are more successful than the AERP + 30 ms pacing protocol; AF inducibility (**B**) and AF duration (**C**) increase over 2 weeks after surgery (n=4). (**D**) Example of atrial electrograms of the left atrial pacemaker. Upper: induction of an episode of atrial fibrillation after 5 s of 50 Hz burst pacing. Lower: AF was not induced after 50 Hz burst pacing. Abbreviations: AF = atrial fibrillation; AERP = atrial effective refractory period.

Figure 3: Interstitial/perivascular fibrosis in the sterile pericarditis animals compared to shams. (**A**) Left: Masson's trichrome staining of left atrial tissue. Blue color = fibrotic tissue. Sterile pericarditis induces more perivascular and interstitial fibrosis in atrial tissue than sham surgery. Upper: 4x magnification; scale bars = 500 μm. Lower: 20x magnification; scale bars = 50 μm. (**B**) Blinded quantification of the % of blue area relative to the total myocardial area using ImageJ software shows a mean of 8.84 ± 0.95% in the sham group (n=4) and 13.16 ± 1.03% in the sterile pericarditis group (n = 3; p = 0.0022, unpaired *t*-test; mean ± SD).

Table 1: Emergency medications, including indications and dosages, to be available during surgery[15-17]. Abbreviations: CPR = cardiopulmonary resuscitation; AF = atrial fibrillation.

DISCUSSION:

A reliable large animal model is a major asset for the study of atrial myopathy and AF and the development of novel therapies for AF. Implantation of pacemaker leads on the atrial epicardium allowed a longitudinal follow-up and repetitive electrophysiologic testing, which is difficult in small animals. Minipigs are easy to handle, and their hearts are structurally and physiologically similar to the human heart[10].

The sterile pericarditis model is relatively straightforward compared to continuous atrial tachypacing because no customized programmed pacemakers are needed. The pathophysiology induced in this model also more closely resembles the pathophysiology often observed in humans, as inflammation and fibrosis precedes the induction of AF[2]. Other models, wherein AF is secondary to ventricular dysfunction or mitral valve regurgitation, tend to be more complicated to develop, and the presence of a non-atrial primary disease confounds the interpretation of effects induced by therapeutic interventions.

To the best of our knowledge, Schwartzman et al.[14] were the only other investigators who induced sterile pericarditis in pigs. In that study, AF inducibility was higher (10%) immediately after surgery and rose to 80% after 1 week postoperatively. In contrast, AF inducibility only rose after 2 weeks and did not exceed 40% in our model. A possible explanation is the older age and greater body weight of their pigs, as well as the higher talcum dose that they used, which makes their model a more acute and aggressive model. Lower talcum dose and younger animals are probably also why the AF inducibility rises later and is lower in this study.

For smooth execution of this protocol, an experienced (cardiac) surgeon and animal anesthesiologist should be involved. Surgically, the anatomy of the minipig is close to that of humans. As described in the protocol, an ultrasound-guided placement of the arterial catheter makes the procedure less invasive, painful, and time-consuming[18].

In the earlier stages of the project, a pacing lead was tunneled to the back of the animal and externalized to connect it to a programmable external cardiac stimulator (see the **Table of Materials**). However, despite the rigorous fixation of these leads, they were often extracted by the animals themselves, and some leads got infected, leading to purulent pericarditis. Therefore, the strategy was adapted to the described two-pacemaker strategy. Critical steps are intubation, central venous catheter placement, pacing lead implantation, and recovery after anesthesia.

Principal anesthetic concerns are hypotension, hypothermia, and cardiac dysrhythmia caused by manipulation. These must be monitored closely and managed by administering fluid boluses and norepinephrine, heating pads, and the presence of emergency drugs and a defibrillator. Some tips and tricks have been included throughout the protocol, with an emphasis on the importance of a supervised postoperative recovery (requiring patience) and temperature management to ensure a rapid and full recovery. The length of the procedure from sedation until extubation

ranges from 3 to 6 h.

There are some limitations to the present protocol. As with any large-animal model, a major limitation is the overall cost. Substantial investments must be made in specialized infrastructure for the housing of the animals and equipment of the operating theater. The animals and consumables are expensive as well. Nevertheless, the sterile pericarditis model is substantially cheaper than atrial tachypacing models because of the short duration and because no modification has to be made to the pacemakers. Compared to small-animal models, the present protocol is also labor-intensive, limiting the overall *N*-value that can be achieved. However, this model has a higher translational value, based on the larger size of the atria and anatomy and physiology closer to that of humans.

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DISCLOSURES:

None of the authors have any conflict of interest to disclose.

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Figure 1

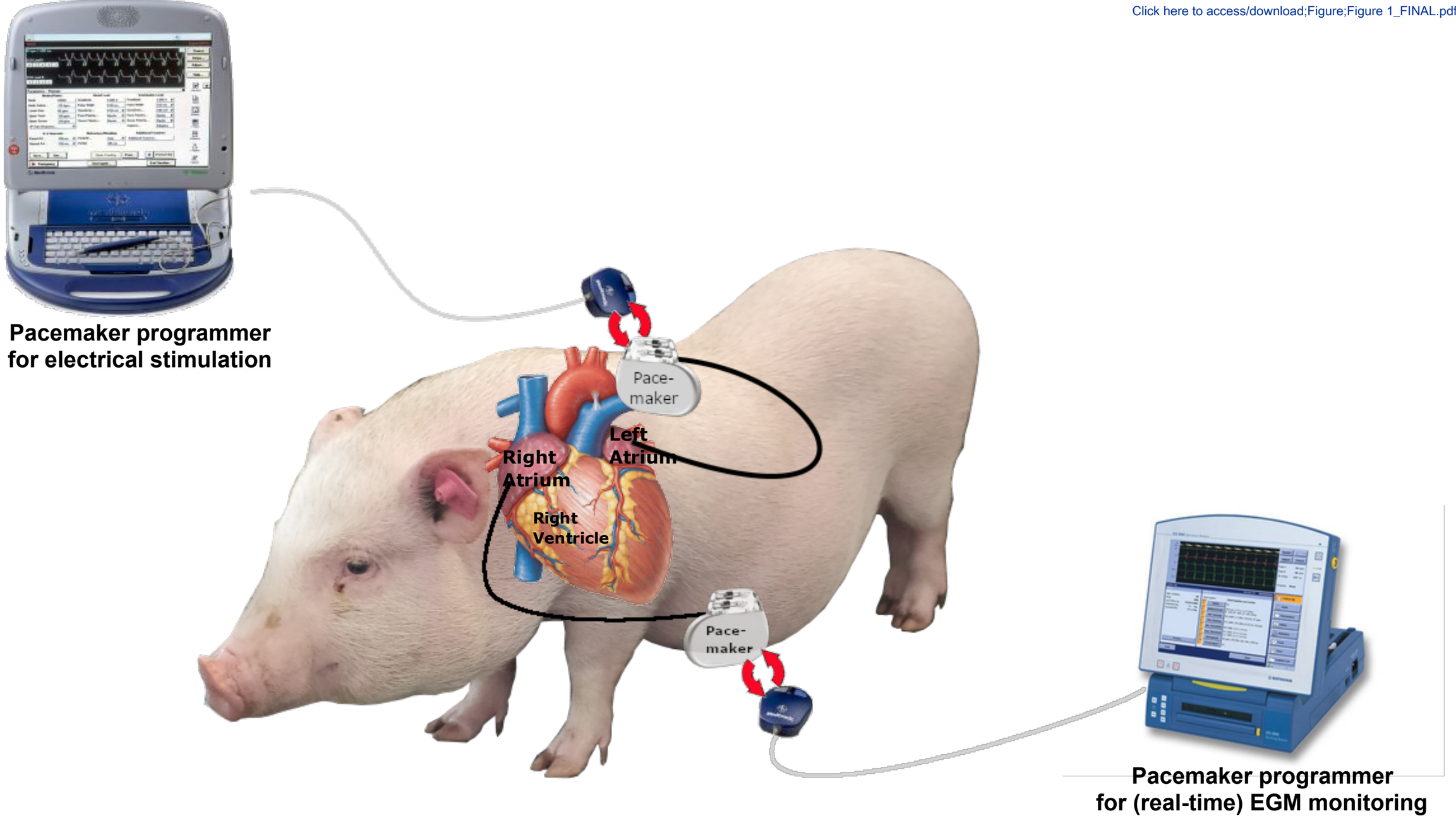
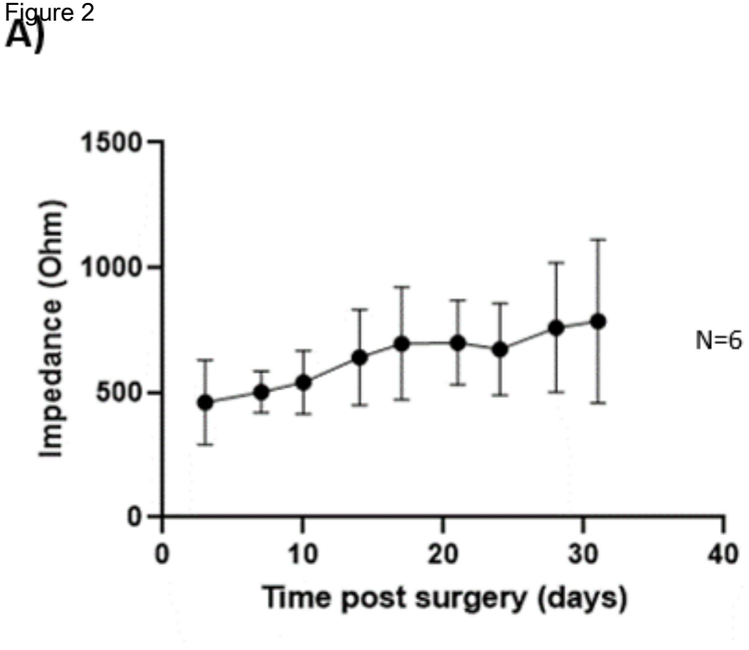
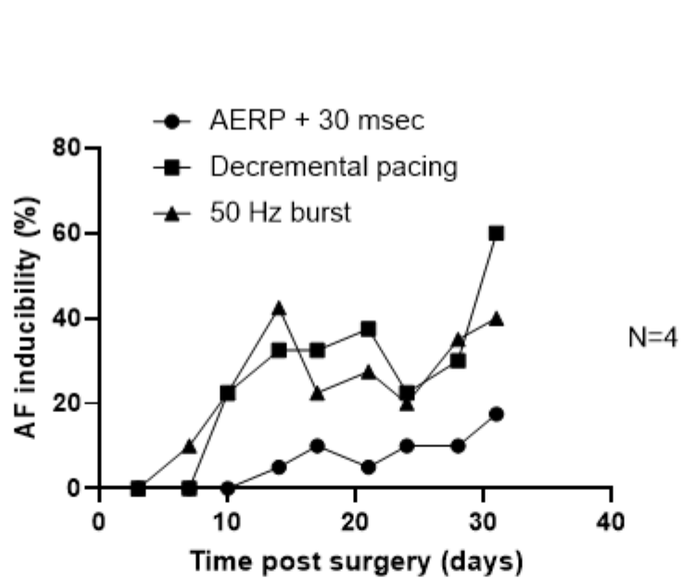
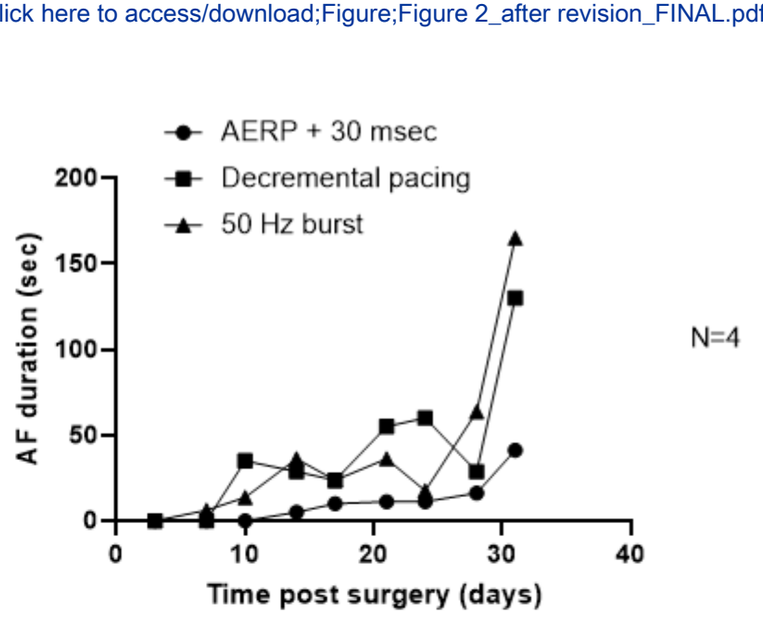
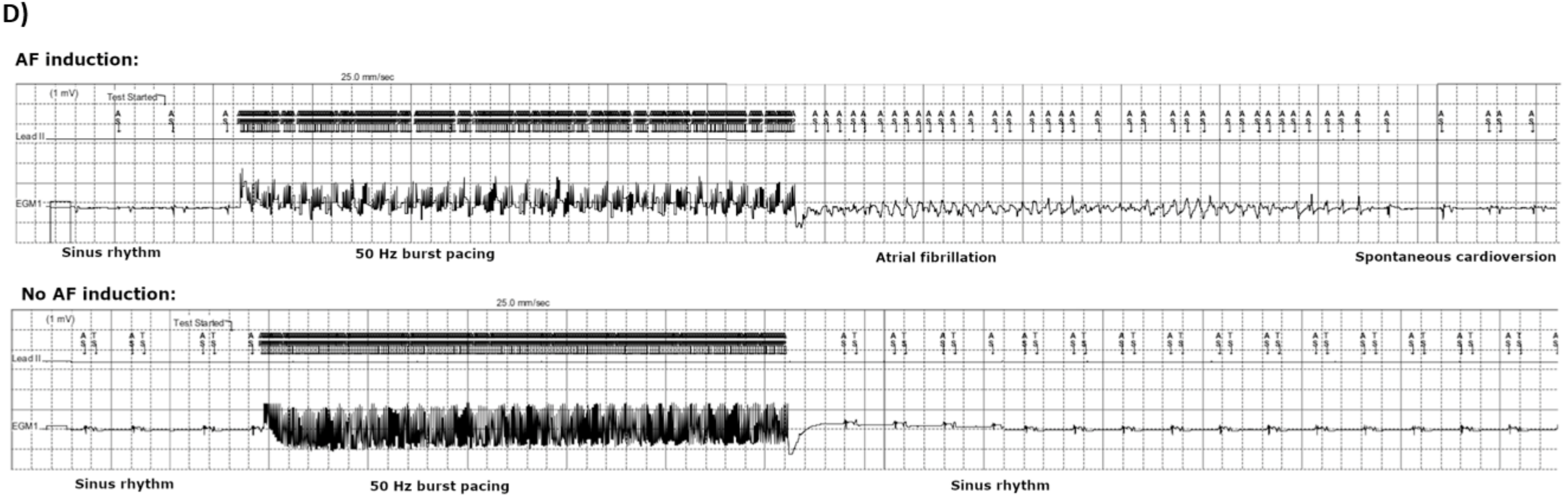


Figure 2









Figure

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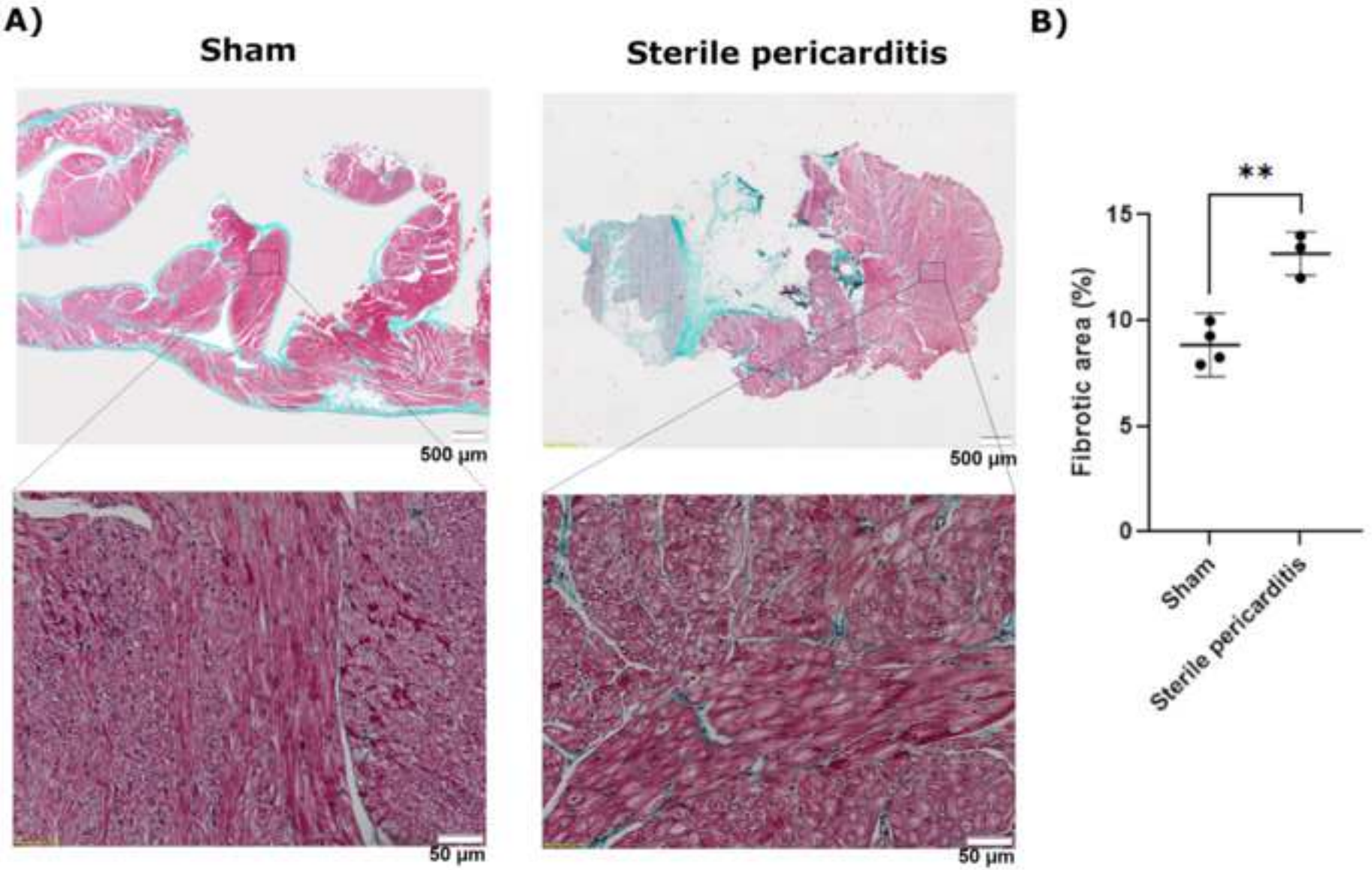

EMERGENCY MEDICATION	Indication	Dose (Bolus)
Adrenaline	Life-threatening situations such as severe hypotension, anaphylactic shock, and resuscitation	15 µg/kg
Amiodarone	Resuscitation, ventricular arrhythmia	7.5 mg/kg
Atracurium	Neuromuscular blocking agent	0.75 mg/kg
Atropine	Bradycardia & CPR	0.02–0.05 mg/kg IM, SC, IV
Clonidine	Malignant hyperthermia / hypertension	0.06 µg/kg
Digoxine	AF with fast ventricular response	12.5 µg/kg
Dobutamine	Cardiogenic shock, hypotension	
Metoprolol	AF with fast ventricular response	50–250 µg/kg
Nitroglycerine	Malignant hyperthermia / hypertension	50 µg/kg
Noradrenaline	Hypotension	
Electrical defibrillation	Sustained ventricular arrhythmia	50–150 J DC biphasic

Dose (Continuous infusion)
0.05–1 μg·(kg·min)$^{-1}$
15 mg·(kg·24 h)$^{-1}$
1 mg·(kg·h)$^{-1}$
2.5 –10 μg·(kg·min)$^{-1}$
0.45 mg·(kg·h)$^{-1}$
0.05–1 μg·(kg·min)$^{-1}$

Antwerp, Sept 1, 2021

Dear editor,

Thank you for your carefully reviewing our manuscript. We revised the text based on your suggestions.

Sincerely,

Michiel Tubeeckx, M.D.
First author

Vincent Segers, M.D., Ph.D.
Last and corresponding author

Also on behalf of all co-authors