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Myocardial infarction by percutaneous embolization coil deployment in a swine model

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TITLE:

Myocardial Infarction by Percutaneous Embolization Coil Deployment in a Swine Model

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KEYWORDS:

myocardial infarction; swine; coil deployment; pre-clinical model.

SUMMARY:

Myocardial infarction (MI) animal models that emulate the natural process of the disease in humans are crucial to understanding pathophysiological mechanisms and testing the safety and efficacy of new emergent therapies. Here, we describe an MI swine model created by deploying a percutaneous embolization coil.

ABSTRACT:

Myocardial infarction (MI) is the leading cause of mortality worldwide. Despite the use of evidence-based treatments, including coronary revascularization and cardiovascular drugs, a significant proportion of patients develop pathological left-ventricular remodeling and progressive heart failure following MI. Therefore, new therapeutic options, such as cellular and gene therapies, among others, have been developed to repair and regenerate injured myocardium. In this context, animal models of MI are crucial in exploring the safety and efficacy of these experimental therapies before clinical translation. Large animal models such as swine are preferred over smaller ones due to the high similarity of swine and human hearts in terms of coronary artery

anatomy, cardiac kinetics, and the post-MI healing process. Here, we aimed to describe an MI model in pig by permanent coil deployment. Briefly, it comprises a percutaneous selective coronary artery cannulation through retrograde femoral access. Following coronary angiography, the coil is deployed at the target branch under fluoroscopic guidance. Finally, complete occlusion is confirmed by repeated coronary angiography. This approach is feasible, highly reproducible, and emulates the pathogenesis of human non-revascularized MI, avoiding the traditional open-chest surgery and the subsequent postoperative inflammation. Depending on the time of follow-up, the technique is suitable for acute, sub-acute, or chronic MI models.

INTRODUCTION

Myocardial infarction (MI) is the most prevalent cause of mortality, morbidity, and disability worldwide[1]. Despite current therapeutic advances, a significant proportion of patients develop adverse ventricular remodeling and progressive heart failure following MI, resulting in poor prognosis due to ventricular dysfunction and sudden death[2–4]. New therapeutic options to repair and/or regenerate injured myocardium are thus under scrutiny, and translational MI animal models are crucial in testing their safety and efficacy. Although several models have been used for cardiovascular research, including rats[5,6], mice[7,8], dogs[9], and sheep[10], pigs are one of the best choices for modeling cardiac ischemia studies because of their high similarity to humans in terms of heart size, coronary artery anatomy, cardiac kinetics, physiology, metabolism, and the post-MI healing process[11–15].

In this context, many different open-surgical and percutaneous approaches are available to develop MI swine models. The open-chest approach involves a left lateral thoracotomy procedure and is useful in performing surgical coronary artery ligation[16,17], myocardial cryo-injury, cauterization[12], and coronary artery placement of a hydraulic occlude[18] or an ameroid constrictor[19], among others. Surgical coronary occlusion has been extensively used to test new therapeutic options such as cardiac tissue engineering and cell therapy, as it allows wide access and visual assessment of the heart; however, in contrast to human MI, it can result in surgical adhesions, adjacent scarring, and postoperative inflammation[17]. Myocardial cryo-injury and cauterization are easily reproducible techniques but do not reproduce the pathophysiological MI progression observed in humans[12]. On the other hand, several percutaneous techniques have been developed to produce temporary or permanent coronary blocking. These comprise transcoronary or intracoronary ethanol ablation[20,21], occlusion by balloon angioplasty[22], or delivery of thrombogenic materials such as agarose gel beads[23], fibrinogen mixtures[9,] or coil embolization[17,24]. While balloon angioplasty is better suited for ischemia/reperfusion studies, coronary coil deployment is one of the best choices for modeling non-revascularized MI. This percutaneous approach is feasible, consistently reproducible, and avoids open-chest surgery. It allows precise control of the infarct location and results in pathophysiology similar to that of a human non-reperfused MI. Moreover, coil embolization is suitable for modeling acute, sub-acute, or chronic MI; chronic congestive heart failure; or valvular disease[17].

The present protocol aims to describe how to develop an MI swine model by permanent coil deployment. Briefly, it comprises a percutaneous selective coronary artery

cannulation through retrograde femoral access. Following coronary angiography, a coil is deployed at the target branch artery under fluoroscopic guidance. Finally, complete occlusion is confirmed by repeated coronary angiography.

PROTOCOL:

This study was approved by the Animal Experimentation Unit Ethical Committee of the Germans Trias i Pujol Health Research Institute (IGTP) and Government Authorities (Generalitat de Catalunya; Code: 10558 and 11208), and complies with all guidelines concerning the use of animals in research and teaching as defined by the Guide for the Care and Use of Laboratory Animals[25].

1. Preprocedural preparation of animals

1.1. Use crossbred Landrace X Large White pigs (30–35 kg) of either sex.

1.2. Keep the animals in a fasting state for 12 h prior to the procedure.

2. Sedation, anesthesia, and analgesia

2.1. Sedate the animal with an intramuscular (IM) injection of ketamine (3 mg/kg), midazolam (0.3 mg/kg), and dexmedetomidine (0.03 mg/kg). Wait for approximately 10–15 min.

2.2. Once the pig is sedated, ventilate it with an oxygen (90–100%)-isoflurane (1–2%) mixture and a face mask to ensure optimal sedation.

2.3. Place vet ointment on the pig's eyes to prevent dryness.

NOTE: Repeat every 20 min.

2.4. Place intravenously (IV) a 20 G catheter in a lateral ear vein. Administer propofol (1–2 mg/kg) to induce anesthesia.

2.5. Once the pig has no swallowing reflex, intubate the animal using an endotracheal tube (size 6.5–7.0 for 30–35 kg).

NOTE: Adjust the size of the endotracheal tube according to the size of the pig. Intubation should be carried out rapidly to prevent a deeper anesthetic plane and prolonged apnea.

2.6. Administer IV buprenorphine (0.01 mg/kg) for intra-surgical analgesia. Use a fentanyl transdermal patch (100 μg/h) for post-operative analgesia.

NOTE: The fentanyl patch is applied to the inguinal skin, and it is active for 72 h to limit postoperative pain. Its pharmacological effect does not start immediately after delivery, thus apply it before starting the procedure.

2.7. Perform airway mask bag unit–ventilation (20 inflations/min) during the transport of the pig to the vascular interventional radiology (VIR) room.

2.8. Connect the endotracheal tube to the anesthesia machine equipped with an airway sensor and capnography recording.

2.9. Start mechanical positive pressure ventilation with FiO_2 0.50, using a tidal volume of 10 mL/kg and a frequency of 16–20 breaths/min. Maintain the anesthesia with isoflurane (1–3%).

NOTE: To confirm the correct surgical anesthetic plane, the animal should not be respiring spontaneously nor have corneal or pupillary light reflexes.

3. Hemodynamic monitoring and preparation of the surgical area

3.1. Place the animal on the operating table in the supine position and fix the limbs to the table with tape or bandage.

3.2. Place electrocardiogram (ECG) probes subcutaneously in the animal's extremities for recording changes in ST-segment, T-waves, and heart rate during the experimental procedure.

3.3. Place a pulse oximeter on the tongue or a corner of the lip of the animal and the non-invasive pressure cuff on the forelimb.

3.4. Measure the rectal/esophageal temperature with a probe.

3.5. Clean the right femoral area with surgical soap and antiseptic povidone-iodine solution under sterile conditions.

3.6. Ensure that the surgeon performs surgical handwashing and wears a sterile gown and sterile gloves.

3.7. Cover the animal with a sterile surgical drape.

3.8. Prepare and flush with heparinized saline solution the needle, a 6F vascular sheath, a 0.035-inch J-tipped wire, a 6F JR4 90-cm guiding catheter, a 0.014-inch 200-cm guidewire, a 150-cm length/0.017-inch inner diameter microcatheter, and the contrast medium injection manifold kit.

4. Vascular access

4.1. Puncture the right femoral artery via a percutaneous approach with ultrasound-guided puncture. Locate the bifurcation between the superficial femoral artery and the deep femoral artery.

4.2. Position the transducer 2–3 cm proximal to the bifurcation, in the common femoral artery, and align the center of the transducer with the common femoral artery.

4.3. Position the needle in the center of the transducer and puncture the artery at an angulation of approximately 45°. Subsequently, insert a 6F vascular sheath using the modified Seldinger technique[26].

NOTE: In case of significant spasm or hematoma, crossover to the contralateral femoral artery.

4.4. Flush the catheters with heparinized saline solution. (5000 IU unfractionated heparin/1000 mL of 0.9% NaCL).

4.5. Administer heparin through the sheath (300 IU/kg).

5. Coronary angiography

5.1. Insert the J-tip wire into the JR4 guide catheter and advance the wire through the sheath into the ascending aorta, and then place the catheter up over the valvular surface.

5.2. Remove the wire and connect the catheter to the injection manifold system. Purge the entire system.

5.3. Under fluoroscopy, engage the catheter into the left main coronary artery and inject 10 mL of iodinated contrast medium to visualize the left coronary system (**Figure 1A**,**C**).

NOTE: It is important to ensure that the arterial pressure waveform is not damped before injecting to avoid the risk of coronary dissection.

5.4. Perform angiograms in two orthogonal views: left anterior oblique 40° and right anterior oblique 30° projections.

5.5. Advance a 0.014-inch guidewire pre-assembled on the microcatheter to the middle left anterior descending (LAD) or distal left circumflex (LCX) coronary artery under fluoroscopic guidance.

6. Coil implantation

6.1. Under fluoroscopic guidance, advance the microcatheter through the wire to the desired location where the coil implant should be deployed. In the case of LAD occlusion, place the coil distal to the first diagonal branch, and for LCX, place the coil distal to the first marginal branch.

NOTE: Proximal approaches (before the first diagonal or first marginal branches) have very low survival rates.

6.2. Remove the wire and select the coil.

NOTE: It is important to select the optimal coil size and length. A small or short coil may not position well in the vessel lumen and has a very high risk of distal migration due to contrast injections or spontaneous, resulting in smaller infarct size. A large or long coil may prolapse proximal to the vessel and produce a larger infarct than desired. The choice of the correct coil is especially important if non-detectable coils are used, as they cannot be removed. The optimal size is 1–2 mm larger than the lumen of the vessel to be embolized, and the length between 20–30 mm is usually adequate for 30–40-kg pigs.

6.3. Deliver the coil via microcatheter and slowly inject 5 mL of iodinated contrast medium under fluoroscopy to visualize the correct position of the coil.

6.4. Remove the microcatheter inside the guide catheter and place the guide in a side branch to perform control injections and to ensure access to the artery in case a second coil needs to be implanted.

6.5. Wait for the coil to thrombose and occlude the artery.

NOTE: When the artery is occluded, changes in the electrocardiogram can be observed. Another way to check complete arterial occlusion is to perform slow injections of iodinated contrast every 10 min (**Figure 1B**,**D**). If the artery does not occlude within 20–30 min, another coil implant may be required.

7. End of procedure

7.1. Once the artery is occluded, administer a continuous IV infusion of lidocaine (50–100 µg/kg/min) for at least 1 h to prevent arrhythmic episodes.

7.2. Perform an angiogram to ensure that there is no flow distal to the occlusion.

7.3. Remove the wire, microcatheter, and guiding catheter.

7.4. Remove the sheath and perform manual compression for 20 min.

8. Postoperative procedure and animal recovery

8.1. Monitor the animal until it is fully recovered, using ECG, rectal temperature, pulse oximetry, and capnography.

NOTE: In case of arrhythmias, administer a bolus of lidocaine (1.5–3.5 mg/kg).

8.2. Administer an IM injection of tulathromycin (2.5 mg/kg) as prophylactic postoperative antibiotic therapy. For post-surgical analgesia, a transdermal fentanyl patch is administered before the surgical procedure (step 2.6).

8.3. Turn off the isoflurane and maintain mechanical ventilation until the animal begins to breathe spontaneously.

8.4. When the pig recovers the swallowing reflex, remove the endotracheal tube. NOTE: Check if the animal has a good SpO2 (more than 95%) before and after extubation.

8.5. Transport the animal to an individual cage. Position the animal over a hot water blanket and cover it with a thermal drape to avoid post-surgical hypothermia.

NOTE: Do not return the pig to the company of other animals until it has fully recovered.

8.6. Monitor the animal until it has regained sufficient consciousness to maintain sternal recumbency.

9. Euthanasia method

9.1. Under previous sedation and anesthesia, as previously described, administer an IV sodium thiopental overdose (200 mg/kg).

9.2. Confirm cardiorespiratory arrest and death by monitoring vital signs (electrocardiogram, blood pressure, capnography).

REPRESENTATIVE RESULTS:

MI survival rates and location

Fifty-seven pigs underwent coronary coil implantation in the LCX marginal branch (n = 25; 12 females and 13 males) or in the LAD between the first and the second diagonal branches (n = 32; 16 females and 16 males) of the coronary artery and were followed up for 30 days. The survival rate of animals submitted to an MI at the LCX marginal branch was 80% (n = 20). Three pigs died as a result of fatal complications related to atrioventricular (AV) block and asystole before coil deployment, and 2 pigs died after ventricular fibrillation (VF) related to transmural MI after coil placement. The survival rate of animals submitted to MI at LAD was 72% (n = 23): 1 pig died due to an AV block and asystole after coil deployment and 8 animals after VF (5 after coil deployment, 2 at 12–48 h post-MI, and one 26 days post-MI). The survival rates differed between the LCX marginal branch (2–2.5 mm in diameter) and middle LAD (2.5–3 mm in diameter) MI, probably due to the larger infarct extension in the LAD model.

Magnetic resonance imaging (MRI) analysis was performed in all animals 30 days post-MI. **Figure 2** illustrates late gadolinium-enhanced MRI images of the LCX marginal branch (**Figure 2A**,**C**) and distal LAD (**Figure 2B**,**D**) infarct models. As depicted, coil deployment in the LCX marginal coronary artery affects the LV lateral wall, while the interventricular septum is the most affected area in distal LAD placement. These results were also confirmed after heart sectioning (**Figure 2E**,**F**).

FIGURE AND TABLE LEGENDS:

Figure 1: Coronary angiography, anteroposterior projection. Representative images of pre- (**A**,**B**) and post-coil (white arrows) deployment (**C**,**D**) in the LCX marginal branch and distal LAD coronary artery. Scale bar = 1 cm.

Figure 2: Magnetic resonance imaging and cardiac tissue sections. Representative T1 3-chamber (**A**,**B**) and short-axis (**C**,**D**) delayed enhancement images for LCX marginal and distal LAD infarction. Images reveal healthy (black) and infarcted (white) myocardium. Photographs of heart sections after LCX marginal (**E**) and distal LAD MI (**F**). Arrows indicate the location and extension of the infarcted area. Scale bar = 1 cm

DISCUSSION:

A coil deployed in a coronary artery provides a reproducible and consistent pre-clinical non-reperfused MI model in swine that can be used to develop and test new cardiovascular therapeutic strategies.

In our hands, mortality at follow-up was 21% related to complications of MI, mostly within the first 24 h of the procedure. All these deaths are related to the natural history of the non-reperfused MI and were the primary outcomes of the study. One of the most critical steps in this protocol relies on the entry of the microcatheter into the coronary arteries. In some cases, microcatheter advancement caused a vagal reaction leading to severe hypotension, AV block, and finally asystole. Nevertheless, this can be avoided by administering a diluted bolus of adrenaline (0.001 mg/kg) before advancing the microcatheter. Another complication is the occurrence of malignant arrhythmias that can lead to VF. These episodes usually occur 30 min after MI instauration. We recommend delivering a bolus of lidocaine (1.5–3.5 mg/kg), atropine for bradycardia (0.01 mg/kg), noradrenaline perfusion (0.05–3 µg/kg/min) for mild or moderate hypotension, and adrenaline (0.03 mg/kg) for severe hypotension, electromechanical dissociation, AV block, or asystole. However, when a VF occurs, a 320J ventricular defibrillation has to be applied with a monophasic cardiac defibrillator and repeated until the animal recovers its cardiac rhythm. When several ventricular defibrillations are needed or asystole occurs, perform manual chest compressions (80–90 compressions/min), depressing the ribcage 4 inches, and connect the animal to the mechanical ventilator under 100% O_2.

Many other occlusion models have been described to simulate MI based on cessation of coronary flow by arterial ligation, an ameroid constrictor, or balloon inflation. However, a deployed coil sets off the coagulation cascade with thrombus formation that occludes the coronary artery. This mechanism simulates as closely as possible the pathophysiology of human MI, compared with other non-invasive techniques like balloon occlusion. Despite the fact that non-reperfused MI results in more extensive scarring, less viable myocardium, and a greater reduction in terms of cardiac function than ischemia-reperfusion models[27], it is more suitable for screening anti-inflammatory therapies, reverse cardiac remodeling, and gene or stem cell therapy for the treatment of cardiovascular disease[28].

ACKNOWLEDGMENTS:

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DISCLOSURES:

The authors have nothing to disclose

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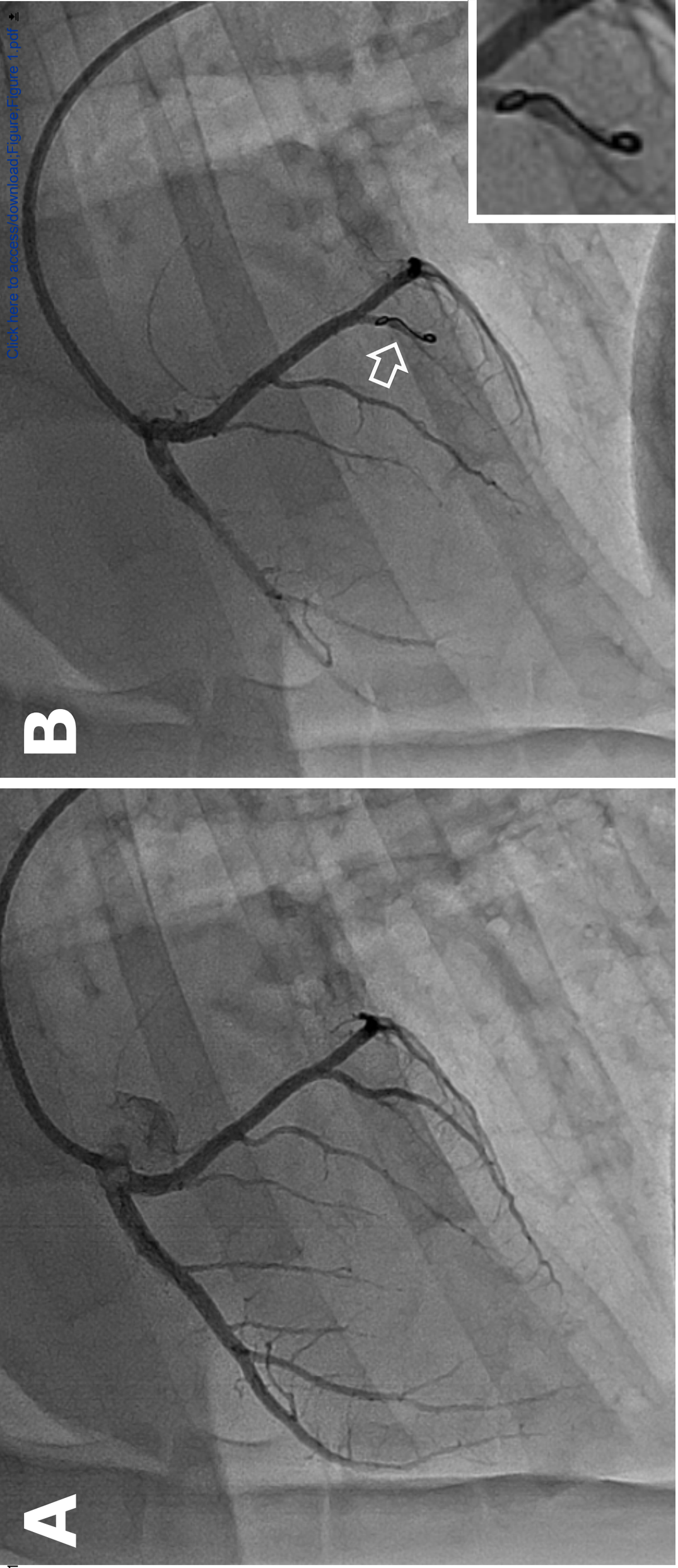
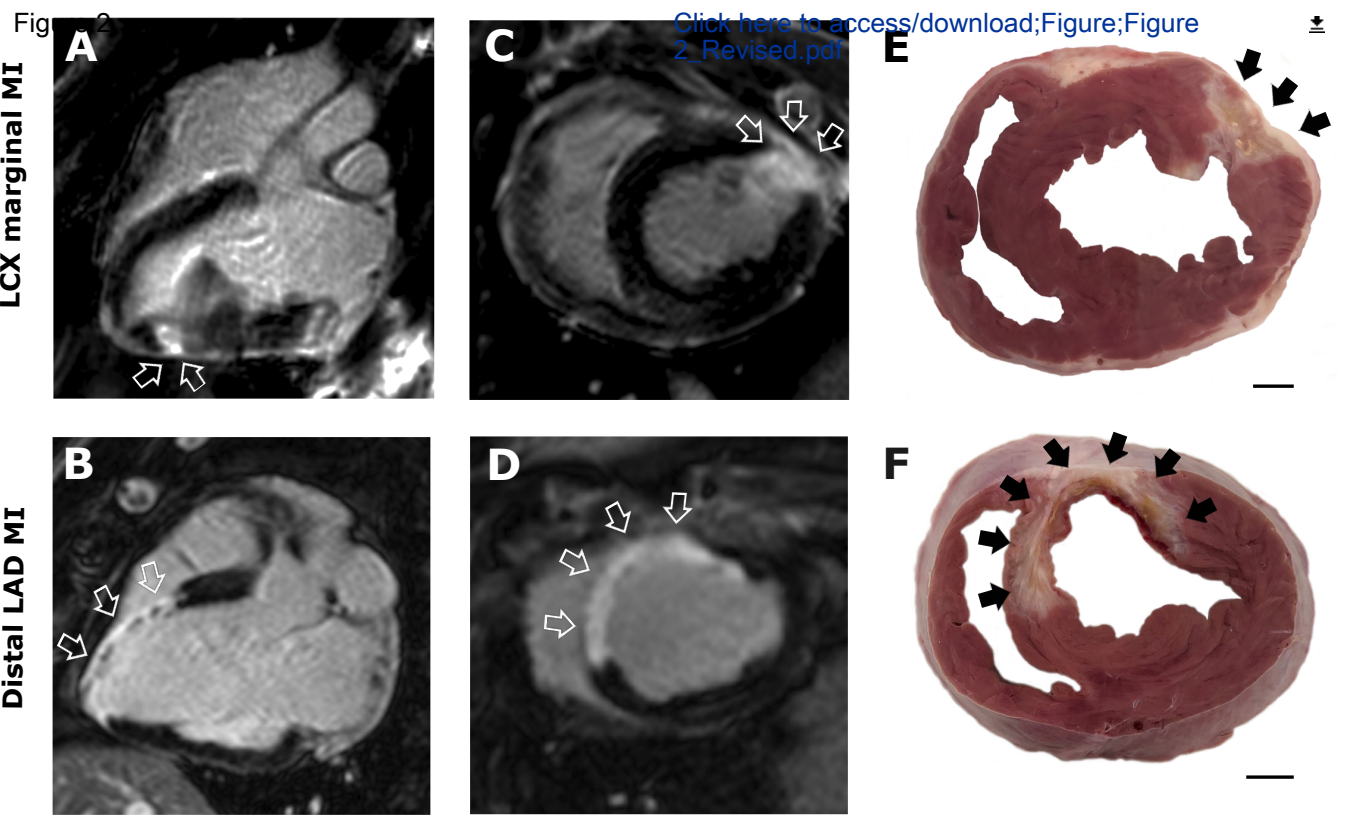


LCX marginal MI

Distal LAD MI

Figure 1

Fig. 2



Badalona, 19th September 2021

Dr. Amit Krishnan
Review Editor
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Dear Editor,

Please find enclosed a revised version of manuscript No. **JoVE63172** entitled "**Myocardial infarction by percutaneous embolization coil deployment in swine model**" by Martinez-Falguera *et al*., modified according to the editor and reviewers´ requests. We hope that you will consider this improved version suitable for publication in *JoVE*. All the authors have read and approved submission of this revised version.

Thank you very much for your consideration.

We look forward to hearing from you soon. Please let us know if you have any questions.

Sincerely,

Dr. Carolina Gálvez-Montón
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First of all, we would like to thank the editor and reviewers for their valuable comments and suggestions that helped us improve the clarity and relevance of this manuscript. We have modified the manuscript according to their comments. All changes made in the text are highlighted, as requested by the Editor. Here, we provide a detailed list of answers to the specific comments. The original editor's and reviewers' comments are reproduced in black before our responses in blue.

Editorial Comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

We have revised in detail the manuscript by a proofreading service.

2. Please provide an institutional email address for each author.

The list of the correct institutional emails is:

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3. Will you have an animal for filming the procedure? This is important. If yes, please confirm whether filming of live surgery is possible at your institute. Are there any restrictions for filming?

Yes. We will have two myocardial infarction surgeries programmed for this next October and they can be used for filming the live surgery for JoVE. There are no restrictions for filming at our center.

4. Please revise the following lines to avoid previously published work: 141-145, 150-152, 154-155. Please refer to the iThenticate report attached.

Thank for your comment. We have rewritten the indicated lines as follows:

Page 9, Line 187: *…"5.1. Insert the J-tip wire into the JR4 guide catheter and advance the wire through the sheath into the ascending aorta, and then place the catheter up over the valvular surface. Remove the wire and connect the catheter to the injection manifold system. Purge the entire system.*

5.2. Under fluoroscopy, engage the catheter into the left main coronary artery and inject 10 mL of iodinated contrast medium to visualize the left coronary system."…

5. 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 or dashes.

We have revised these items, and to make the protocol easier to understand, we have also numbered the different parts according JoVE Instructions.

6. Being a video based journal, JoVE authors must be very specific when it comes to the humane treatment of animals. Regarding animal treatment in the protocol, please add the following information to the text:

We really appreciate this comment and realize the importance of this issue. Then, according with the editor's suggestions, we have added the following information in the revised manuscript as follows:

a) Please include an ethics statement before all of the numbered protocol steps indicating that the protocol follows the animal care guidelines of your institution.

Page 6, Line 99: *…" This study was approved by the Animal Experimentation Unit Ethical Committee of the Germans Trias i Pujol Health Research Institute (IGTP) and Government Authorities (Generalitat de Catalunya; Code: 10558 and 11208), and complies with all guidelines concerning the use of animals in research and teaching as defined by the Guide for the Care and Use of Laboratory Animals.[25]"…*

Page 16, Line 415: … "*25. Animals, N.R.C. (US) C. for the U. of the G. for the C. and U. of L. Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. doi: 10.17226/12910 (2011).*"…

b) Please specify the euthanasia method.

Page 11, Line 265: *…"9. Euthanasia method*

9.1. Under previous sedation and anesthesia, as previously described, administrate an IV sodium thiopental overdose (200 mg/kg).

9.2 Confirm cardiorespiratory arrest and death by monitoring vital signs (electrocardiogram, blood pressure, capnography).”…

c) Please mention how animals are anesthetized and how proper anesthetization is confirmed.

Page 7, Line 136: *…”2.7 Connect the endotracheal tube to the anesthesia machine equipped with an airway sensor and capnography recording. Start mechanical positive pressure ventilation with FiO_2 0.50, using a tidal volume of 10 mL/kg and a frequency of 16–20 breaths/min. Maintain the anesthesia with isoflurane (1–3%). NOTE: To confirm the correct surgical anesthetic plane, the animal should not be respiring spontaneously, nor have corneal or pupillary light reflexes.”…*

d) Please specify the use of vet ointment on eyes to prevent dryness while under anesthesia.

Page 7, Line 118: *..."2.3 Place vet ointment on the pig’s eyes to prevent dryness. NOTE: Repeat every 20 minutes.”…*

e) For survival strategies, discuss post-surgical treatment of animal, including recovery conditions and treatment for post-surgical pain.

Page 7, Line 127: …” *Use a fentanyl transdermal patch (100 µg/h) for post-operative analgesia. NOTE: The fentanyl patch is applied to the inguinal skin and it is active for 72 h to limit post-operative pain. Its pharmacological effect does not start immediately after delivery, thus we strongly recommend applying it before starting the procedure.”…*

Page 11, Line 248: *…” 8.2 Administer an IM injection of tulathromycin (2.5 mg/kg) as prophylactic post-operative antibiotic therapy. For post-surgical analgesia, a transdermal fentanyl patch is administered before the surgical procedure (Protocol Section 2.5.).”…*

Page 11, Line 258: *…” 8.5. Transport the animal to an individual cage. Position the animal over a hot water blanket and cover it with a thermal drape to avoid post-surgical hypothermia. NOTE: Do not return the pig to the company of other animals until it has fully recovered.”…*

f) Discuss maintenance of sterile conditions during survival surgery.

Page 8, Line 159: …” *The operator performs surgical hand washing and wears a sterile gown and sterile gloves.”…*

g) Please specify that the animal is not left unattended until it has regained sufficient consciousness to maintain sternal recumbency.

Page 11, Line 262: *…"8.6 Monitor the animal until it has regained sufficient consciousness to maintain sternal recumbency."…*

h) Please specify that the animal that has undergone surgery is not returned to the company of other animals until fully recovered.

Page 11, Line 258: *…"8.5 Transport the animal to an individual cage. Position the animal over a hot water blanket and cover it with a thermal drape to avoid post-surgical hypothermia. NOTE: Do not return the pig to the company of other animals until it has fully recovered."…*

i) Please do not highlight any steps describing anesthesia/euthanasia.

We have improved the revised version of the manuscript according editor's suggestions.

7. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

We appreciate this comment. We have ensured that all necessary protocol steps to generate the script are included in the revised version of the manuscript.

8. 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 and a maximum of 4 sentences per step. Please use "NOTES" to include

Following editor's suggestions, we have simplified some steps of the protocol.

9. Please ensure that the Protocol contains only action items that direct the reader to do something. Please move the discussion about the protocol to the Discussion or consider adding "NOTEs".

We have revised in detail all the steps and have ensure that Protocol contains only action items. Moreover, some extra necessary information is included in NOTEs.

10. Please include a one-line space between each protocol step and then highlight up to 3 pages 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. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

Following editor's suggestions, we have included a one-line space between each protocol steps and have highlighted in grey the essential steps of the protocol for the video.

11. Please ensure that the Representative results section explains the results in the context of the technique you have described, e.g., how do these results show the technique, suggestions about how to analyze the outcome, etc. The paragraph text should refer to all of the figures. Data from both successful and sub-optimal experiments can be included.

We have confirmed that all the necessary results relative from our technique are included in the Representative Results section.

12. Line 220-254: Please move to the Discussion section.

We really appreciate this comment and have realized that this information refers better to discussion section. Then, following editor's suggestion, we have simplified and moved it to the Discussion section as follows:

Page 13, Line 299: *…" One of the most critical steps in this protocol relies on the entry of the microcatheter into the coronary arteries. In some cases, microcatheter advancement caused a vagal reaction leading to severe hypotension, AV block, and finally asystole. Nevertheless, this can be avoided by administering a diluted bolus of adrenaline (0.001 mg/kg) before advancing the microcatheter. Another complication is the occurrence of malignant arrhythmias that can lead to VF. These episodes usually occur 30 minutes after MI instauration. We recommend delivering a bolus of lidocaine (1.5–3.5 mg/kg), atropine for bradycardia (0.01 mg/kg), noradrenaline perfusion (0.05–3 mcg/kg/min) for mild or moderate hypotension, and adrenaline (0.03 mg/kg) for severe hypotension, electromechanical dissociation, AV block, or asystole. However, when a VF occurs, a 320J ventricular defibrillation has to be applied with a monophasic cardiac defibrillator and repeated until the animal recovers its cardiac rhythm. When several ventricular defibrillations are needed or asystole occurs, perform*

manual chest compressions (80–90 compressions/minute), depressing the ribcage 4 inches, and connect the animal to the mechanical ventilator under 100% O_2."…

13. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:

a) Critical steps within the protocol

b) Any modifications and troubleshooting of the technique

c) Any limitations of the technique

d) The significance with respect to existing methods

e) Any future applications of the technique

We have rewritten the Discussion section, according editor's suggestions including all the above-mentioned points:

Page 12, Line 294: …" *A coil deployed in a coronary artery provides a reproducible and consistent pre-clinical non-reperfused MI model in swine that can be used in the development and testing of new cardiovascular therapeutic strategies.*

In our hands, mortality at follow-up was 21% related to complications of MI, mostly within the first 24 hours of the procedure. All these deaths are related to the natural history of the non-reperfused MI and were primary outcomes of the study. One of the most critical steps in this protocol relies on the entry of the microcatheter into the coronary arteries. In some cases, microcatheter advancement caused a vagal reaction leading to severe hypotension, AV block, and finally asystole. Nevertheless, this can be avoided by administering a diluted bolus of adrenaline (0.001 mg/kg) before advancing the microcatheter. Another complication is the occurrence of malignant arrhythmias that can lead to VF. These episodes usually occur 30 minutes after MI instauration. We recommend delivering a bolus of lidocaine (1.5–3.5 mg/kg), atropine for bradycardia (0.01 mg/kg), noradrenaline perfusion (0.05–3 mcg/kg/min) for mild or moderate hypotension, and adrenaline (0.03 mg/kg) for severe hypotension, electromechanical dissociation, AV block, or asystole. However, when a VF occurs, a 320J ventricular defibrillation has to be applied with a monophasic cardiac defibrillator and repeated until the animal recovers its cardiac rhythm. When several ventricular defibrillations are needed or asystole occurs, perform manual chest compressions (80–90 compressions/minute), depressing the ribcage 4 inches, and connect the animal to the mechanical ventilator under 100% O_2.

Many other occlusion models have been described to simulate MI based on cessation of coronary flow by arterial ligation, an ameroid constrictor, or balloon inflation. However, a deployed coil sets off the coagulation cascade with thrombus formation that occludes the coronary artery. This mechanism simulates as closely as possible the pathophysiology of human MI, compared with other non-invasive techniques like balloon occlusion. Despite the fact that non-reperfused MI results in more extensive scarring, less viable myocardium, and a greater reduction in terms of cardiac function than ischemia-reperfusion models,[27] it is more suitable for screening anti-inflammatory therapies, reverse cardiac remodeling, and gene or stem cell therapy for the treatment of cardiovascular disease.[28] "…

14. Figure 1: Please mention what the white arrow in 1B,1D indicates in Figure Legends. Please include scale bars wherever applicable.

Many thanks for this comment. Following the editor's suggestion, we have added a sentence refer to white arrow indication:

Page 18, Line 431: …"*Representative images of pre- (A,B) and post-coil (white arrows) deployment (C,D) on the LCX marginal branch (C), and distal LAD coronary artery (D). Scale bar = 1 cm"…*

15. Figure 2: Please include scale bars in the images representing histology.

We have included scale bars in histology images in the revised version of the Figure 2.

16. Please ensure that the Table of Materials includes all the essential items (reagents, chemicals, consumables, equipment) used in this study.

We really appreciate this comment. We have revised in detail the Table of Materials to improve the manuscript.

Reviewers' comments:

Reviewer #1:

Manuscript summary:

The authors describe a protocol for inducing transmural MI in pigs using catheter-based coil embolization procedure. The protocol is easy to read and with sufficient information for most of the parts. Some minor edits and modifications are suggested as below.

1. Although the size of the coil is discussed, length and the shape (tapered, straight, with feather etc) are not discussed. These are also important factors that determine the success.

We really appreciate this comment and understand the relevance of these issues. Then we have included information relative to length and type of coil.

Page 10, Line 211: *…"6.2. Remove the wire and select the coil. NOTE: It is important to select the optimal coil size and length. A small or short coil may not position well in the vessel lumen and has a very high risk of distal migration, due to contrast injections or spontaneous, resulting in a smaller infarct size. A large or long coil may prolapse proximal to the vessel and produce a larger infarct than desired. The choice of the correct coil is especially important if non-detectable coils are used, as they cannot be removed. The optimal size is 1–2 mm larger than the lumen of the vessel to be embolized, and the length between 20–30 mm is usually adequate for 30–40-kg pigs."…*

2. No description of chest compression and defibrillation procedures were found. Efficient resuscitation is the key for successful MI induction in pigs and this should be described in detail.

We really appreciate this comment and understand the relevance of these issues. Then we have included information relative to the chest compression and defibrillation procedures, as follows:

Page 13, Line 309: *…"However, when a VF occurs, a 320J ventricular defibrillation has to be applied with a monophasic cardiac defibrillator and repeated until the animal recovers its cardiac rhythm. When several ventricular defibrillations are needed or asystole occurs, perform manual chest compressions (80–90 compressions/minute), depressing the ribcage 4 inches, and connect the animal to the mechanical ventilator under 100% O_2."…*

3. Line 211, "The survival rates differed between the LCX marginal and LAD MI, probably due to the larger infarct extension in the distal LAD model." I don't think they are different, at least statistically. So this statement is misleading.

We apologize for this misunderstanding. The infarct size of an LCX marginal branch is smaller than infarct after LAD occlusion. The diameter of a marginal branch is about 2-2.5mm while the diameter of middle LAD is about 2.5-3mm, and the myocardial areas that depend of these two arteries, in terms of oxygen and blood supply, also differ in extension. For this reason, we can conclude that survival rates differ due to the larger infarct extension in the middle LAD model. In order to avoid this confusion, we have rewritten the sentence as follows:

Page 12, Line 283: *…" The survival rates differed between the LCX marginal branch (2–2.5 mm in diameter) and middle LAD (2.5–3 mm in diameter) MI, probably due to the larger infarct extension in the LAD model."…*

4. Line 252 "adrenaline" in pig MI usually induces drug-induced arrhythmias. Did the authors try norepinephrine or phenylephrine instead of adrenaline?

We really appreciate this comment. In cases of mild or moderate hypotension we use noradrenaline perfusion (0.05-3 μcg/Kg/min) and in cases of severe hypotension or electromechanical dissociation we use adrenaline (0.03 mg/Kg).

We have included this new information as follows:

Page 13, Line 305: …"*We recommend delivering a bolus of lidocaine (1.5–3.5 mg/kg), atropine for bradycardia (0.01 mg/kg), noradrenaline perfusion (0.05–3 mcg/kg/min) for mild or moderate hypotension, and adrenaline (0.03 mg/kg) for severe hypotension, electromechanical dissociation, AV block, or asystole.*"…

5. Line 269, "it is more suitable for screening of thrombolytic drugs, anti-inflammatory therapy, reverse cardiac remodeling, and gene or stem cell therapy for the treatment of cardiovascular disease" - I disagree that this is a good model for screening thrombolytic drugs as there is a foreign body (coil) inside the coronary and it is clearly different from human MI.

According to reviewer's suggestion we have remove this information.

6. Line 146 (heparin 25000U/L) - this seems excessive. Do the authors use that much for just flushing the catheters?

We have revised this item and rewritten.

Page 9, Line 181: …" *4.2. Flush the catheters with heparinized saline solution. (5000 IU unfractionated heparin/1000 mL NaCL 0.9%).*"…

Reviewer #2:

Manuscript Summary:

It will be of great help to practitioners of porcine myocardial infarction research.

Minor Concerns:

1. Please add oxygen supply per hour in "Sedation, anesthesia and analgesia-2. Once sedated, ventilate the pig with an oxygen-isoflurane (1-2%) mixiture".

(ex. Turn on the oxygen to flow at 1-2 liters per hour.)

We have added this information as follows:

Page 7, Line 115: *..."2.2. Once the pig is sedated, ventilate it with an oxygen (90–100%)-isoflurane (1–2%) mixture and a face mask to ensure optimal sedation."…*

Page 7, Line 137: *…" Start mechanical positive pressure ventilation with FiO2 0.50, using a tidal volume of 10 mL/kg and a frequency of 16–20 breaths/min."…*

2. It would be better to move the contents of pig ventilation (Page 4-line 102) to the "Sedation, anesthesia and analgesia-5. Perform airway mask bag unit-ventilation (frequency 20 inflations/min) and~" (Page 5-line 115). I thought that the order has changed. In pig, respiratory anesthesia using anesthesia machine and ventilator (tracheal tube insertion, isoflurane, and oxygen) is possible after intramuscular injection drug induced anesthesia.

The comments are well received. We agree with the reviewer that the contents of pig ventilation were unclear. According to the reviewer's suggestions, we have changed the order and we have specified that ventilation with airway mask bag unit-ventilation has to be applied during the transport of the animal to the vascular interventional radiology (VIR) room, while the animal cannot be connected to a mechanical ventilator.

3. In page 10-line 253. FV is VF (ventricular fibrillation), right?

Thank you for your correction. We have amended it.