sciendo

J Vet Res 66, 435-447, 2022
DOI: 10.2478/jvetres-2022-0046

The impact of a modified anaesthetic protocol on animal survival and the characteristics of ventricular arrhythmias in the course of acute myocardial infarction in a domestic pig model

Piotr Frydrychowski[1]✉, Marcin Michałek[1], Wiktor Kuliczkowski[2], Krzysztof Nowak[3], Piotr Skrzypczak[4], Iwona Bil-Lula[5], Agnieszka Noszczyk-Nowak[1]

[1]Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats, [4]Department and Clinic of Surgery, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, 50-366 Wrocław, Poland
[2]Department of Cardiology, [3]Department of Heart Diseases, Faculty of Health Sciences,
[5]Department of Medical Laboratory Diagnostics, Division of Clinical Chemistry and Laboratory Haematology, Wrocław Medical University, 50-556 Wrocław, Poland
piotr.frydrychowski@upwr.edu.pl

Received: March 07, 2022 *Accepted: August 18, 2022*

Abstract

Introduction: Acute myocardial infarction (MI) is one of the most common causes of death in humans in highly developed countries. Among its most frequent complications affecting the patient's prognosis are cardiac arrhythmias: ventricular tachycardia (VT) and ventricular fibrillation (VF). **Material and Methods:** The study aimed to characterise arrhythmias in 19 pigs subjected to experimentally induced MI obtained by occlusion of the proximal left anterior descending (LAD) coronary artery using an angioplasty balloon. The anaesthetic protocol was modified to reduce mortality by including procedures stabilising haemodynamic disorders which develop during episodes of ischaemia and arrhythmia. During 30 min of experimentally induced ischaemia, the heart rhythm was recorded using a 12-lead ECG. The time, frequency, and type of arrhythmias were analysed. **Results:** Ventricular arrhythmias were found in 94.74% of the treated pigs. The most common were ventricular premature complexes, reported in 88.89% of pigs with arrhythmia. Ventricular tachycardia was recorded in 66.67% and ventricular fibrillation in 50% of pigs with arrhythmias. **Conclusion:** Myocardial infarction due to proximal LAD occlusion is characterised by a high incidence of ventricular arrhythmias, especially VT and VF. Because of the high survival rate, this MI porcine model may serve as a model for research on acute ischaemic ventricular arrhythmias in humans. Additionally, it reduces the total number of animals required for testing while yielding meaningful results, which is in line with the 3R principle.

Keywords: acute myocardial infarction, domestic pig model, proximal left anterior descending coronary artery occlusion, ventricular arrhythmias.

Introduction

Abnormal biomarker levels with signs of acute myocardial ischaemia satisfy the clinical definition of myocardial infarction (MI) (54). Obstruction of one of the coronary arteries results in ischaemia of the area supplied by this artery (54). The most common site of occlusion is the left anterior descending coronary artery (LAD). Studies on MI in humans show that occlusion in the proximal section of the LAD results in a greater area of necrosis and more significant mortality than medial or distal LAD occlusion (8, 18). The common complications of MI are life-threatening arrhythmias – ventricular tachycardia (VT) and ventricular fibrillation (VF) – which are considered the leading causes of death in the acute phase of myocardial infarction (5, 17). These arrhythmias usually occur in the early stages of ischaemia, but the reperfusion process also significantly increases the possibility of their development. Possible causes include hypoxia of cells resulting in metabolic acidosis which disturb the ion balance of the cells (15, 22), arrhythmias in the re-entry

© 2022 P. Frydrychowski et al. This is an open access article distributed under the Creative Commons Attribution-NonCommercial-NoDerivs license (http://creativecommons.org/licenses/by-nc-nd/3.0/)

mechanism (33), or foci of cardiac arrhythmias (15, 22).

The use of animal models plays a significant role in MI research, enabling experiments in the pathophysiology, prevention, diagnosis, and therapy of MI. Generally, large animals are good material for arrhythmia studies because of similarities in the action potential and ion channel profiles (11). Since the pig heart's anatomy and size, and the structure of its coronary circulation are similar to those of the human heart, it is a suitable research model for studying the pathophysiology and treatment of human myocardial ischaemia (2, 19, 27, 31, 32, 60). Additionally, pigs have similar body weight and vital parameters to humans, such as the heart rate (24, 40, 43). Myocardial ischaemia caused by occlusion of the coronary arteries in pigs produces similar changes to the pathological changes reported in humans with MI, which further justifies using the porcine model. This feature significantly impedes the development of collateral circulation and the heart stimulatory system that is sensitive to ischaemia and hypoxia (10, 13, 17, 41, 53). In summary, LAD occlusion in the pig model leads to the formation of relatively large infarct zones and various types of arrhythmias (28, 29).

The porcine model of myocardial ischaemia is most often obtained by transvascular or thoracic occlusion of the coronary vessels. Models of MI in the pig with a closed chest include occlusion of the coronary arteries using an angioplasty balloon (9, 39, 44, 47, 51, 52, 57, 61, 62), coils (14, 21), agarose gel balls (17), a purpose-made foam sponge (46), or balls with an attached filament (36). Closed-chest models are less invasive than open-chest models and more similar to human MI pathophysiology. They are more survivable by animals and – in most cases – need shorter procedure times and cost less (37). Studies using LAD occlusion to induce MI in pigs have used different anaesthetic protocols (28, 35, 47, 51, 52). Usually, anaesthetic care consisted of appropriate premedication of the animal, then induction of general anaesthesia and its maintenance. For premedication, most often tiletamine (52) or a combination of tiletamine with zolazepam (51), ketamine (28), midazolam (35), atropine (28) or their mixtures (28, 47) were used. To induce anaesthesia, propofol (47), isoflurane (35, 51, 52), sodium pentobarbital (28) or thiopental (35) were used. The sinus rhythm was restored with appropriate drugs, such as amiodarone (28, 47, 51, 52) and lignocaine (28, 35, 47, 51) or with defibrillation (28, 35, 47, 51, 52). However, the available publications do not describe anaesthetic procedures stabilising the animal's condition and correcting the haemodynamic disorders caused by MI and the development of cardiac arrhythmias. Haemodynamic disturbances due to MI induced by LAD occlusion may be hypotension, hypovolaemia, or shock, which if left untreated may result in the death of the animal during the procedure or a shorter survival time after its completion. Appropriate action to compensate for these disorders is essential, especially in the procedure of LAD closure in its proximal part, which is characterised by high mortality (28, 35, 52).

So far, the porcine infarction model has been mainly used to study ventricular arrhythmias (39, 41, 55), antiplatelet drugs for myocardium cardioprotection (57, 61, 62), VF management (30, 39, 45, 58), post-reperfusion injuries (9) and therapies for heart failure following MI (51). However, pigs have a predisposition to ventricular arrhythmias in MI that are resistant to treatment, which results in the high mortality rate of this model in standard anaesthetic management (37, 41, 49, 59). In pig MI models generated with an angioplasty balloon, LAD occlusion leading to ischaemia is performed in the medial or distal LAD (9, 25, 28, 30, 39, 44, 45, 48, 52, 55, 57, 58), which increases the survival rate of the animals used in the experiment. To the best of our knowledge, there are no reports in the literature on an effective way to achieve MI in pigs by occluding the proximal part of the LAD using an angioplasty balloon. The high mortality associated with MI due to proximal LAD occlusion in humans recommends the development of a stable porcine MI model induced by the same LAD occlusion as good material for research on the therapeutic and protective management of the myocardium during ischaemia. Hence, the presented study aimed firstly to evaluate a porcine model of MI obtained by proximal LAD occlusion under a modified anaesthetic protocol intended to stabilise the condition of the pigs during MI and secondly to characterise the ventricular arrhythmias developing during the procedure.

Material and Methods

Animals. The study was carried out on 19 female pigs of the Polska Biała Zwisłoucha breed (National Research Institute of Animal Production Experimental Station, Żerniki Wielkie, Poland), aged 16–20 weeks and weighing 33–44 kg. The pigs were all fed an identical complete feed meeting nutritional standards and kept under the same breeding conditions. Before starting the tests, all animals underwent acclimation to become used to handling and grooming activities.

Protocol for establishing a model of acute myocardial infarction – preparation of animals. The animals' access to food was restricted for 12 h before the procedures started. All procedures and measurements were performed according to the same anaesthesia scheme and the same operating procedure.

Premedication and induction and maintenance of general anaesthesia. Pigs were premedicated with an intramuscular injection of a mixture containing ketamine (Vetaketam; Vet-Agro, Lublin, Poland) (10 mg/kg b.w.), midazolam (Midanium; Polfa Warszawa, Warsaw, Poland) (0.3 mg/kg b.w.) and medetomidine (Sedator; Eurovet Animal Health BV,

Bladel, The Netherlands) (0.03 mg/kg b.w.). The drugs used in the premedication differ from those in the schemes described in the literature (23, 30, 32, 33, 39), and to our knowledge, this combination was used for the first time. After immobilisation, the animals were placed in the sternum position, and vascular access was obtained to the marginal vein of the ear. After general anaesthesia induction, propofol (Provive; Claris Lifesciences UK, Crewe, UK) was administered intravenously (2 mg/kg b.w.), and the animal was intubated with a size 8 endotracheal tube (Murphy Tracheal Tube; SUMI, Sulejówek, Poland). After intubation, the animals were mechanically ventilated using 100% oxygen and a closed gas system with a carbon dioxide absorber. Ventilation was initiated in the pressure-swing mode, and after the animal was stabilised and the ventilation and capnometry parameters were assessed, it was switched to the volumetric-swing mode. The oxygen flow was maintained at 2 L/min (Primus; Dräger Medical AG & Co., Lübeck, Germany) with a tidal volume of 10 mL/kg b.w. and the number of breaths was 12/min. The tidal volume was appropriately adjusted to maintain the end-tidal CO_2 concentration between 35 and 45 mmHg. Anaesthesia was maintained with isoflurane (Forane; Abbott Laboratories, Warsaw, Poland) administered using an appropriately calibrated vaporiser (Vapor 2000; Dräger Medical AG & Co). The isoflurane concentration (1.5% to 2.5% in 100% oxygen) was monitored (Lifepack 12, Medtronic, Redmond, WA, USA) and regulated using a vaporiser based on capnometry values to keep the final concentration at 1 minimum alveolar concentration. Intraoperative analgesia was provided by intravenous administration of fentanyl (Fentanyl WZF; Polfa Warszawa), initially as a bolus (10 µg/kg b.w.) and then as a continuous infusion at a dose of 10 µg/kg b.w./h. During the procedure, fluid therapy was administered in the form of constant rate infusion (CRI) of a multi-electrolyte solution (Optilyte; Fresenius Kabi Polska, Kutno, Poland) at a dose of 6–12 mL/kg b.w./h, depending on the animal's hydration status and response to drugs. Throughout the procedure, complete anaesthetic monitoring was carried out by non-invasively measuring internal body temperature, saturation, pulse and blood pressure (Lifepack 12) and electrocardiography. The heart rhythm was continuously monitored with a 12-lead ECG test (BTL-08 MT Plus ECG; BTL Industries, Stevenage, UK).

Treatment of haemodynamic disorders. In the event of a drop in blood pressure during the procedure, the patients were haemodynamically stabilised. For this purpose, the animal was treated as needed with boluses of lactated Ringer's solution (Fresenius Kabi Polska) (10 mL/kg b.w.) and boluses of hydroxyethyl starch 130/0.4 (Voluven; Fresenius Kabi Deutschland, Bad Homburg vor der Höhe, Germany) (3–5 mL/kg b.w.). In the absence of a haemodynamic response to the applied fluid boluses, continuous infusion of dopamine (Dopaminum Hydrochloricum WZF; Polfa Warszawa) was started at an initial dose of 4 µg/kg b.w./min and subsequently adjusted depending on the operational needs and the animal's response to the treatment. There was no need to use dopamine at a dose above 10 µg/kg b.w./min during the procedures. The patient was put into the Trendelenburg position to increase blood flow to the heart and cardiac output until hypovolaemia was corrected.

Induction of myocardial infarction. Percutaneous access to the femoral artery was obtained under ultrasound control (F37; Hitachi Aloka Medical Ltd, Mure, Mitaka-shi, Tokyo, Japan) using a 21G femoral puncture needle (21G; Balton, Warsaw, Poland) and a 6F diameter vascular introducer sheath (Balton). Following insertion of a 6F diameter Judkins Left 3.5 curvature guide catheter (Launcher; Medtronic), heparin (Heparinum WZF; Polfa Warszawa) was administered (6,000 UI), and coronary angiography was performed (Fig. 1).

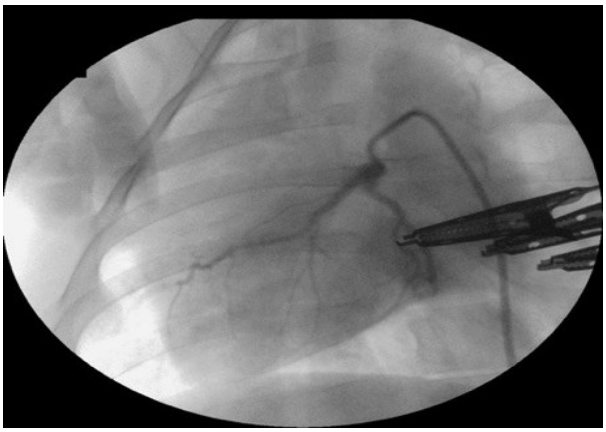


Fig. 1. Coronary angiography performed on a female pig subjected to myocardial infarction induced by 30 min occlusion of the proximal part of the left anterior descending coronary artery with an angioplasty balloon in a modified anaesthetic protocol

Then a 0.014″ balance middleweight 300 cm angioplasty guidewire (Abbott, Santa Clara, CA, USA) was inserted through the catheter and placed under fluoroscopic control (Symbol; General Medical Merate SpA, Seriate, Italy) in the proximal segment of the LAD. A 3.0 × 10 mm over-the-wire angioplasty balloon (Sprinter; Medtronic) was placed on the guidewire. The balloon was inflated to 6 atm and held at that pressure for 30 min for complete LAD occlusion. Arterial closure was confirmed by angiography (Fig. 2), while MI was diagnosed by ST-segment elevation on the 12-lead ECG (Fig. 3). Additionally, MI was confirmed by histopathological analysis of myocardial tissues collected from the animals when euthanised 4 weeks after the procedure.

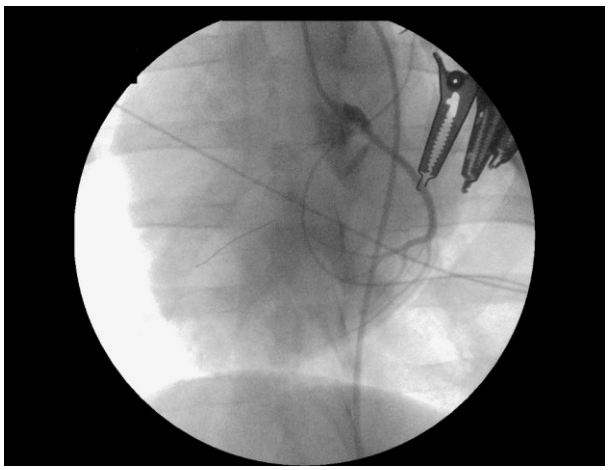


Fig. 2. Coronary angiography presented occlusion of the proximal segment of the left anterior descending artery achieved with an angioplasty balloon in a female pig subjected to myocardial infarction in a modified anaesthetic protocol

Anti-arrhythmic treatment. The VT onset was treated with an intravenous infusion of 2% lignocaine (Lignocainum Hydrochloricum WZF 2%; Polfa Warszawa) at 25–50 µg/kg b.w. (the dose was adjusted according to the animal's response to treatment) and an infusion of amiodarone (Cordarone; Sanofi-Aventis France, Paris, France) at 5 mg/kg b.w./h diluted in 250 mL of 5% glucose solution (B. Braun, Melsungen, Germany). Ventricular fibrillation was terminated with external defibrillation at 300 J or 360 J for recurrent VF episodes (Lifepack 12).

Statistical analysis. Data on the number and type of arrhythmias and their duration were statistically analysed using GraphPad Prism 5.0 (GraphPad, San Diego, CA, USA). The Shapiro–Wilk normality test was used to check the distribution and normality of the data distribution. The mean ± standard deviation was calculated for normally distributed data, while the median (range) was calculated for skewed data. Differences in the incidence of arrhythmias were calculated using nonparametric Kruskal–Wallis analysis followed by Dunn's multiple comparisons in a post-hoc test. A P-value of <0.05 was considered statistically significant. Charts presenting selected data were prepared in the free-to-use web wizard LiveGAP (https://charts.livegap.com).



Fig. 3. Features of acute myocardial ischemia – ST-segment elevation in a female pig subjected to myocardial infarction induced by 30 min occlusion of the proximal part of the left anterior descending coronary artery with an angioplasty balloon in a modified anaesthetic protocol. The sinus rhythm is shown at a rate of 106 bpm. V1 – costochondral junction of the right first intercostal space; V2 – sixth intercostal space, to the left of the sternum; V3 – midway point between leads V2 and V4; V4 – costochondral junction of the sixth intercostal space; V5 – sixth intercostal space, dorsal to V4 at a distance equal to the distance between V2 and V3 or V3 and V4; V6 – sixth intercostal space, dorsal to V5 at a distance equal to the distance between V2 and V3, V3 and V4 or V4 and V5. Paper speed: 50 mm/s; amplitude: 10 mm/mV; 25 Hz notch filter; Fuzzy+ software filter

Results

All 19 animals survived the 30 minutes LAD occlusion procedure, and 19 records were analysed. All pigs subjected to the procedure showed an elevated electrocardiographic ST-segment in the first 10 min of induction of LAD occlusion, with a median of 1 (1–5) min. Only one animal (5.26% of all pigs) showed no cardiac arrhythmias during an induced MI. In comparison, at least one type of arrhythmia was diagnosed in the rest of the 18 pigs (94.74% of all pigs) ($P < 0.0001$). Treatment aimed at correcting haemodynamic disturbances was administered to nine pigs which developed VF. The use of dopamine, in addition to fluid therapy, was required in seven pigs. Table 1 shows the mean values of the ECG parameters determined before, during, and after induction of MI.

Types of arrhythmias. Ventricular arrhythmias were found in all 18 animals with the described cardiac arrhythmias. The most common arrhythmia was ventricular premature complexes (VPCs) (Figs 4a and 4b), defined as a single ventricular beat occurring earlier than the expected sinus beat. It was found in the electrocardiographic records of 16 pigs (84.21% of all pigs and 88.89% of pigs with diagnosed arrhythmia). All 16 pigs with VPCs developed polymorphic VPCs of left ventricular origin (Fig. 4a), and additionally, two of them developed single monomorphic VPCs of right ventricular origin (Fig. 4b).

Table 1. Mean values of ECG parameters at different stages of the experiment

	Before MI induction	During MI induction procedure	After MI induction
HR (bpm)	91.84	89.32	88.53
PQ (ms)	101.79	109.16	129.06
QRS (ms)	82.00	80.74	69.53
QTc (ms)	490.95	480.11	458.00

MI – myocardial infarction; HR – heart rate; PQ – interval from the beginning of the P wave to the beginning of the Q wave; QRS – interval from the end of the PQ interval to the end of the S wave; QTc – interval from the start of the Q wave to the end of the T wave corrected for heart rate



Fig. 4a. Single ventricular premature complex (the 4th beat) of left ventricular origin in a female pig subjected to myocardial infarction induced by 30 min occlusion of the proximal part of the left anterior descending coronary artery with an angioplasty balloon in a modified anaesthetic protocol. I – bipolar limb lead, potential difference between the electrodes on the left superior limb and the right superior limb; II – bipolar limb lead, potential difference between the electrodes on the left inferior limb and the right superior limb; III – bipolar limb lead, potential difference between the electrodes on the left inferior limb and the left superior limb; aVR – augmented unipolar right limb lead; aVL – augmented unipolar left limb lead; aVF – augmented unipolar left hindlimb lead. The sinus rhythm is shown at a rate of 63 bpm. Paper speed: 50 mm/s; amplitude: 10 mm/mV; 25 Hz notch filter; Fuzzy+ software filter

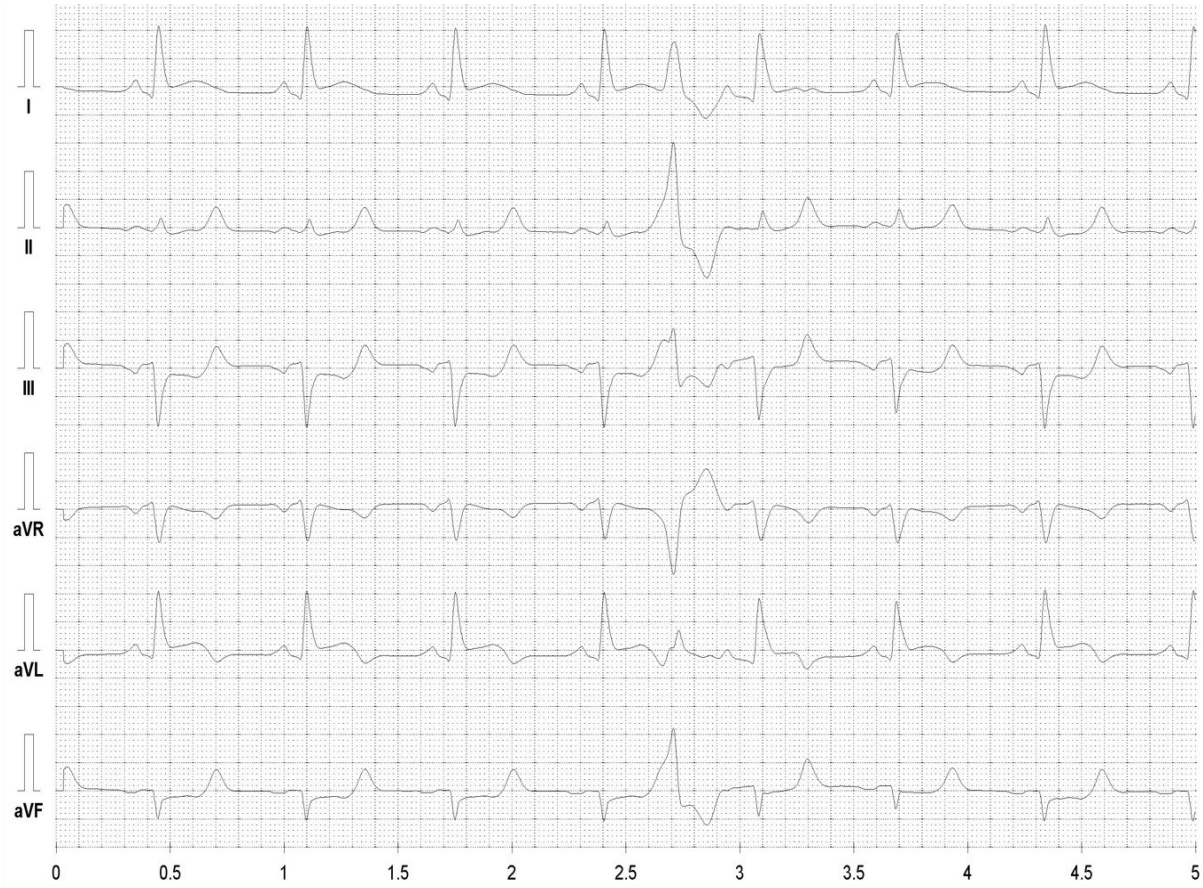


Fig. 4b. Single ventricular premature complex (the fifth beat) of right ventricular origin in a female pig subjected to myocardial infarction induced by 30 min occlusion of the proximal part of the left anterior descending coronary artery with an angioplasty balloon in a modified anaesthetic protocol. I – bipolar limb lead, potential difference between the electrodes on the left superior limb and the right superior limb; II – bipolar limb lead, potential difference between the electrodes on the left inferior limb and the right superior limb; III – bipolar limb lead, potential difference between the electrodes on the left inferior limb and the left superior limb; aVR – augmented unipolar right limb lead; aVL – augmented unipolar left limb lead; aVF – augmented unipolar left hindlimb lead. The sinus rhythm is shown at a rate of 92 bpm. Paper speed: 50 mm/s; amplitude: 10 mm/mV; 25 Hz notch filter; Fuzzy+ software filter

The most common type of arrhythmia seen concurrently with VPCs was ventricular tachycardia, recorded in 11 pigs with VPCs (68.75%). Ventricular premature complexes occurred alone only in two pigs (11.11% of pigs with arrhythmias and 10.53% of all pigs). Ventricular couplets were found in 9 out of 18 pigs with arrhythmias (50% of pigs with arrhythmias and 47.37% of all pigs). Three consecutive ventricular beats (triplets) occurred in three pigs (16.67% of pigs with arrhythmias and 15.79% of all pigs). Ventricular bigeminies, defined as regular sinus beats continuously alternating with premature ventricular beats, were reported in three pigs (16.67% of pigs with arrhythmias and 15.79% of all pigs). Equally frequently occurring ventricular trigeminies (two sinus beats alternating with one premature ventricular beat) were found in three pigs (16.67% of pigs with arrhythmias and 15.79% of all pigs).

Ventricular tachycardia (Fig. 5) was diagnosed in 12 pigs (66.67% of pigs with arrhythmias and 63.16% of all pigs) and all tachycardia episodes were classified as non-sustained VT (*i.e.* lasting less than 30 s). The median duration of ventricular complexes during VT was 106 (60–246) ms and the mean duration of QRS was 80.92 ± 9.34 ms as recorded during sinus rhythm in animals before MI induction.

Three of the VT episodes were diagnosed as Torsade de Pointes (25% of pigs with VT and 16.67% of pigs with arrhythmias). The number of VT episodes recorded in animals during MI varied from 1 to 9 (Fig. 6).

Ventricular fibrillation was reported in nine animals (50% of pigs with arrhythmias and 47.37% of all pigs). Those nine pigs had 18 episodes of VF, in six of which it developed without preceding VT (33.33% of all episodes) (Fig. 7), while in 12 episodes there was a direct transition from VT to VF (66.67% of all episodes).

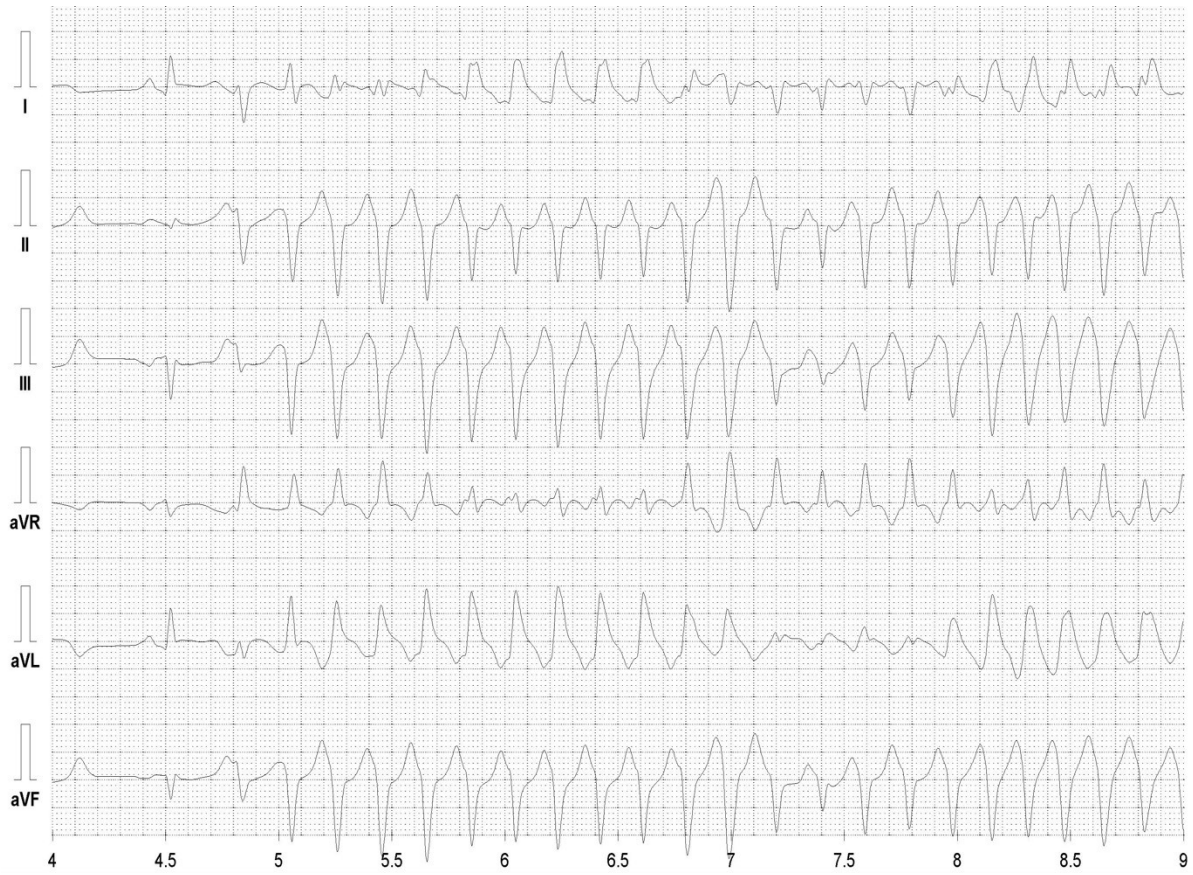


Fig. 5. Ventricular accessory R/T beat triggering ventricular tachycardia in a female pig subjected to myocardial infarction induced by 30 min occlusion of the proximal part of the left anterior descending coronary artery with an angioplasty balloon in a modified anaesthetic protocol. I – bipolar limb lead, potential difference between the electrodes on the left superior limb and the right superior limb; II – bipolar limb lead, potential difference between the electrodes on the left inferior limb and the right superior limb; III – bipolar limb lead, potential difference between the electrodes on the left inferior limb and the left superior limb; aVR – augmented unipolar right limb lead; aVL – augmented unipolar left limb lead; aVF – augmented unipolar left hindlimb lead. Heart rate: 303 bpm; Paper speed: 50 mm/s; amplitude: 10 mm/mV; 25 Hz notch filter; Fuzzy+ software filter

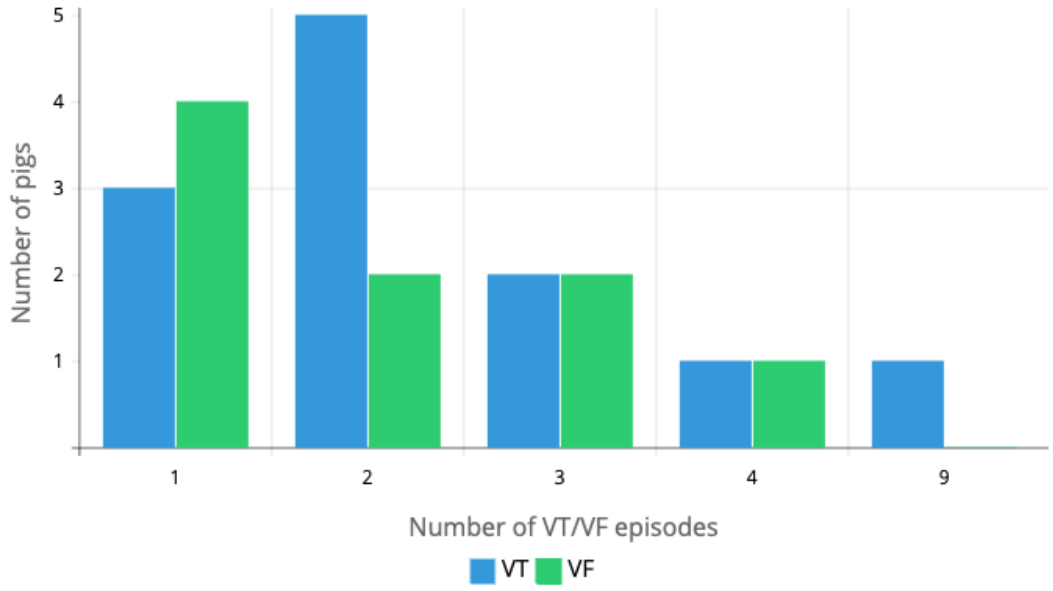


Fig. 6. The number of pigs with recorded ventricular tachycardia (VT) or ventricular fibrillation (VF) episodes during the induction of acute myocardial infarction (MI) by 30 min left anterior descending coronary artery occlusion with an angioplasty balloon in a modified anaesthetic protocol



Fig. 7. Ventricular fibrillation in a female pig subjected to myocardial infarction induced by a 30 min occlusion of the proximal part of the left anterior descending coronary artery with an angioplasty balloon in a modified anaesthetic protocol. I – bipolar limb lead, potential difference between the electrodes on the left superior limb and the right superior limb; II – bipolar limb lead, potential difference between the electrodes on the left inferior limb and the right superior limb; III – bipolar limb lead, potential difference between the electrodes on the left inferior limb and the left superior limb; aVR – augmented unipolar right limb lead; aVL – augmented unipolar left limb lead; aVF – augmented unipolar left hindlimb lead. Heart rate: 329 bpm; paper speed: 50 mm/s; amplitude: 10 mm/mV; 25 Hz notch filter; Fuzzy+ software filter

The number of VF episodes in individual pigs ranged from 1 to 4 (Fig. 6). The only arrhythmia not classified as ventricular was the first-degree atrioventricular (AV) block, diagnosed in 2 pigs (11.11% of pigs with arrhythmias and 10.53% of all pigs). The incidence of VPC was statistically significantly higher than the incidence of triplets, bigeminies, trigeminies, and the first-degree AV block ($P < 0.05$).

Coexistence of different types of arrhythmias. In pigs with cardiac arrhythmias, co-occurrence of three different types was most commonly diagnosed (6 pigs, 33.33% of pigs with arrhythmias, and 31.58% of all pigs). Simultaneous affliction with two types of arrhythmia was described in four pigs (22.22% of pigs with arrhythmias and 21.05% of all pigs). A single type of cardiac arrhythmia was found in three pigs (16.67% of pigs with arrhythmias and 15.79% of all pigs). Six different arrhythmias were reported in two pigs (11.11% of pigs with arrhythmias and 10.53% of all pigs). In contrast, multiplicities of 4, 5, or 7 different arrhythmias were diagnosed in one animal each (5.56% pigs with arrhythmias and 5.26% of all pigs).

Time of the first arrhythmia's occurrence. The first arrhythmia developed within the first 10 min of LAD occlusion in 15 (83.33%) pigs with arrhythmias, while in three pigs (16.67%) it did so between the 20th and 30th minute of LAD occlusion (median 4.5 (1–30) min). The appearance of the first VPC was also associated with two time periods. In 14 pigs with VPC (87.5%), it occurred during the first 10 minutes of ischaemia, and in two pigs with VPC (12.5%), it manifested between the 20th and 30th min of LAD occlusion (median 4.5 (1–30) min). Additionally, VPC was the first reported arrhythmia in 83.33% of pigs (n = 15) with arrhythmias. The first episodes of VT were recognised: in seven affected pigs within the first 10 min of LAD occlusion (58.33%), in two pigs between the 10th and 20th min (16.67%), and in three pigs between the 20th and 30th min (25%) (mean 13.25 ± 9.64 min). The first episodes of VF were recorded in one pig (11.11%) in the first 10 minutes, in three pigs (33.33%) between the 10th and 20th min, and in five pigs (55.56%) between the 20th and 30th min of LAD occlusion (mean 20.11 ± 6.68 min) (Fig. 8).

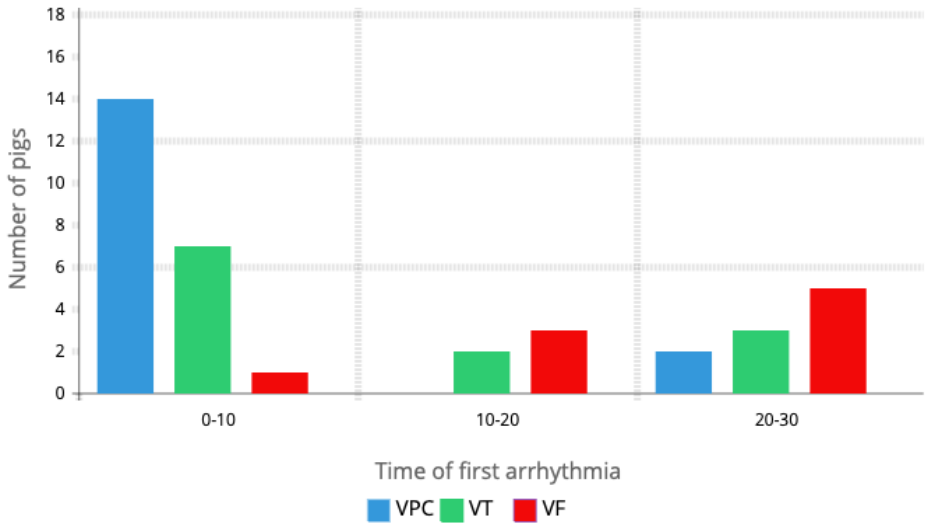


Fig. 8. The number of pigs with ventricular premature complexes (VPCs), ventricular tachycardia (VT), or ventricular fibrillation (VF) occurring for the first time within a specific time interval during induction of acute myocardial infarction by 30 min left anterior descending coronary artery occlusion with an angioplasty balloon in a modified anaesthetic protocol

Discussion

Our study aimed to characterise ventricular arrhythmias in a porcine MI model obtained by occluding the proximal part of the LAD. We used a modified protocol of animal anaesthesia consisting of a changed premedication scheme and appropriate management during the haemodynamic disorder's development, and we aimed to obtain a stable MI model with a high survival rate. In the changed premedication regimen, we used a combination of drugs from three different groups (ketamine, medetomidine and midazolam). This allowed us to use lower doses of drugs, reduce the risk of side effects characteristic for higher doses of drugs (synergism) and introduce appropriate anaesthetic management during haemodynamic stabilisation of subjects at the time of pressure drop, hypovolaemia, or shock. So far, the procedure of haemodynamic disorder correction has not been described in the literature for similar animal models. A combination of drugs from three different groups was described for premedication in pigs (47). However, the application of the substances used in our experiment was not previously reported.

In the conducted experiment, ventricular arrhythmias occurred in 94.74% of pigs subjected to myocardial ischaemia induction. The most common arrhythmia observed in our study was the VPC (88.89% of pigs with diagnosed arrhythmia), the occurrence of which was related to the time of ischaemia induction, as also reported by other studies (20, 50).

In our study, VT and VF, which may be fatal complications of acute MI, occurred in 66.67% and 50% of pigs with cardiac arrhythmias, respectively. The registered VT episodes were characterised as non-sustained and polymorphic. In some studies, the incidence of VF in pigs during LAD occlusion ranged from 50 to 75%. Other studies reported even up to 100% VF incidence (3, 4, 6, 12, 23, 26, 28, 37, 42, 52, 56). Therefore, the VF incidence rate of 50% obtained in our experiment is consistent with the previously published literature data. In this study, 83.33% of pigs with arrhythmias (15 out of 18) developed complexes in the form of two or more types of arrhythmia, which may be related to the extent of the ischaemic area created by the severely injurious LAD occlusion. In most of the studied animals, the first arrhythmias following the myocardial ischaemia induction developed within the first 10 min (83.33%). The first episodes of VPC or VT occurred in the initial 10 min of MI (87.5% and 58.33%, respectively). The second period in which a significant proportion of cardiac arrhythmias was recorded was within the last 10 min of the procedure (in 12.5% of animals VPC presented and in 25% VT). This distribution of arrhythmias over time is consistent with that noted in other studies (4, 7, 47). However, the VF reported during our study had a different distribution, as most episodes occurred at the end of ischaemia between the 20th and 30th min (55.56%).

Our experiment showed a 100% survival rate, which is very high compared to other studies on MI induced by the proximal LAD occlusion (35, 52) reporting that mortality of pigs in the MI group could reach 100%. In pig models using angioplasty balloons, acute myocardial ischaemia was usually achieved by LAD (9, 25, 26, 39, 45, 47, 52, 55, 61) or left coronary artery circumflex (LCX) balloon occlusion (44, 61, 62). Most often, LAD closure was performed in its central

part (9, 25, 28, 55, 57), mainly distally to the first septal branch (39) or diagonal branch (30, 44, 45, 48, 52, 58). The place where the LCX was closed was usually at its beginning (45, 58) or more distally (51). The mortality associated with the development of myocardial ischaemia reported in these studies ranged from 20.51% to 33%. In these studies, the LAD lumen occlusion was not performed in its proximal part, most probably because it results in a larger area of induced ischaemia. Larger ischaemia may potentially affect the course of the procedure and undoubtedly decreases the animal survival rate because of episodes of VF and haemodynamic disorders (28, 35, 52). In their study, Suzuki *et al.* (52) found that LAD occlusion in the proximal part resulted in higher animal mortality than mid-LAD occlusion. Moreover, they proved that LAD proximal lumen occlusion led to the greatest myocardial necrosis (52). Also, the mortality rate for the proximal LAD occlusion group was as high as 50%, while in the mid-LAD occlusion group, no deaths occurred (52). In another study, Munz *et al.* (35) permanently ligated the proximal LCX or LAD in three different sections, namely proximal, medial, and distal, to achieve MI (35). In the group of animals with the proximal LAD occlusion, the mortality was 100%. The authors established that the optimal place of LAD occlusion to induce MI in a pig model is its middle part (35). The effects of LAD closure location on MI development, the size of the myocardial ischaemia area, and the development of arrhythmias were confirmed by Li *et al.* (28). In this experiment, the LAD was closed in its middle and bottom-third parts. The results indicated that LAD occlusion in its middle part promotes more frequent VF and more serious haemodynamic disturbances (28). The high survival rate of the animals in our MI model could have been influenced by timely management of the disturbances caused by arrhythmias and myocardial dysfunction caused by MI. However, it may also result from using only young and healthy pigs in the presented study. A relatively homogeneous group of young and healthy pigs may be considered the main limitation of the presented study, since they do not constitute the best material to be analogous to people suffering from acute MI in terms of health and age.

Another limitation is the sex of the animals used. Mortality from coronary events in women at younger age is lower than in men of the same age group, although the differences become less pronounced in older age groups (34). The INTERHEART study showed that the first MI in women occurred on average 9 years later than in men (1). Although women tend to be less frequently affected by MI, clinical evidence suggests that they have higher mortality and a worse prognosis after an acute cardiovascular event (16).

The choice to use juvenile females in our study is related to our previous experience using a porcine model. According to our observations, females are more resistant to the stress of new housing conditions and perioperative time. Moreover, they show less aggression towards other animals and staff and become accustomed to human investigators faster than males. In addition, the use of young animals is also due to the need for venepuncture to perform the test and for effective defibrillation if VF develops. These activities in adult males are significantly hampered by their size and large body mass. Thus, experimentation on young females by choice was intended to reduce the impact of stress associated with daily maintenance and handling and the perioperative period and to facilitate the efficient performance of the planned procedures.

Based on the cited literature data, we may conclude that LAD occlusion in the proximal segment is not optimal for establishing a porcine MI model. The procedure results in high mortality of the animals, which makes it impossible to monitor a sufficient number of them or assess the effects of LAD closures over the long term. However, it is essential to develop a stable model for MI induction *via* proximal LAD occlusion conducive to research on arrhythmias and their outcomes and prognosis, which is vital for people who develop MI because of proximal LAD occlusion. Such a model would enable researchers to better understand the pathophysiological changes in the myocardium due to occlusion in the LAD proximal segment. The anaesthetic protocol used in our study (consisting of haemodynamic stabilisation of patients at the time of pressure drop, hypovolaemia or shock) allowed for the effective treatment of life-threatening haemodynamic disorders and arrhythmias caused by myocardial ischaemia and for the correction of the condition of animals with already developing hypotension, hypovolaemia, or shock. Thanks to haemodynamic stabilisation, we achieved 100% survival of the animals subjected to the ischaemia induction and reperfusion procedure.

In conclusion, myocardial ischaemia obtained by LAD proximal segment occlusion is characterised by a high rate of cardiac arrhythmias. The most frequently recorded cardiac arrhythmias were ventricular tachycardia and ventricular fibrillation, which usually pose a direct threat to life. The applied modified anaesthesia management, aiming to stabilise haemodynamic disorders caused by myocardial infarction and arrhythmias, ensured 100% ischaemia and reperfusion survival. Such results qualify the described MI model as worthwhile to adopt for research on arrhythmias in MI due to proximal LAD occlusion in humans. The proposed protocol significantly affects the survival of animals in the presented MI model, which enhances its potential for use in the study of ventricular arrhythmias in acute myocardial ischaemia.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: This study was supported by the Wrocław University of Environmental and Life Sciences (Poland) as part of the 'Innovative doctorate' ("Innowacyjny Doktorat") Ph.D. research programme no. N070/0005/2020. The APC was met by the Wrocław University of Environmental and Life Sciences.

Animal Rights Statement: The study was carried out according to the guidelines of the National Institute of Health for the care and use of laboratory animals. All the experimental protocols involving animals were approved by the Local Ethical Committee for Animal Experiments at the Wrocław University of Environmental and Life Sciences (Resolution no. 081/2019, 11.12.2019).

References

1. Anand S.S., Islam S., Rosengren A., Franzosi M.G., Steyn K., Yusufali A.H., Keltai M., Diaz R., Rangarajan S., Yusuf S.: INTERHEART Investigators. Risk factors for myocardial infarction in women and men: insights from the INTERHEART study. Eur Heart J 2008, 29, 932–940, doi: 10.1093/eurheartj/ehn018.
2. Barkagan M., Leshem E., Rottmann M., Sroubek J., Shapira-Daniels A., Anter E.: Expandable lattice electrode ablation catheter. Circ Arrhythm Electrophysiol 2019, 12, e007090, doi: 10.1161/CIRCEP.118.007090.
3. Barrabés J.A., García-Dorado D., González M.A., Ruiz-Meana M., Solares J., Puigfel Y., Soler-Soler J.: Regional expansion during myocardial ischemia predicts ventricular fibrillation and coronary reocclusion. Am J Physiol 1998, 274, H1767–H1775, doi: 10.1152/ajpheart.1998.274.5.H1767.
4. Barrabés J.A., García-Dorado D., Padilla F., Agulló L., Trobo L., Carballo J., Soler-Soler J.: Ventricular fibrillation during acute coronary occlusion is related to the dilation of the ischemic region. Basic Res Cardiol 2002, 97, 445–451, doi: 10.1007/s003950200051.
5. Berg R.A., Sanders A.B., Kern K.B., Hilwig R.W., Heidenreich J.W., Porter M.E., Ewy G.A.: Adverse hemodynamic effects of interrupting chest compressions for rescue breathing during cardiopulmonary resuscitation for ventricular fibrillation cardiac arrest. Circulation 2001, 104, 2465–2470, doi: 10.1161/hc4501.098926.
6. Bergey J.L., Nocella K., McCallum J.D.: Acute coronary artery occlusion–reperfusion-induced arrhythmias in rats, dogs, and pigs: antiarrhythmic evaluation of quinidine, procainamide, and lidocaine. Eur J Pharmacol 1982, 81, 205–216, doi: 10.1016/0014-2999(82)90438-1.
7. Bergey J.L., Wendt R.L., Nocella K., McCallum J.D.: Acute coronary artery occlusion-reperfusion arrhythmias in pigs: antiarrhythmic and antifibrillatory evaluation of verapamil, nifedipine, prenylamine and propranolol. Eur J Pharmacol 1984, 97, 95–103, doi: 10.1016/0014-2999(84)90516-8.
8. Brener S.J., Witzenbichler B., Maehara A., Dizon J., Fahy M., El-Omar M., Dambrink J.-H., Genereux P., Mehran R., Oldroyd K., Parise H., Gibson C.M., Stone G.W.: Infarct size and mortality in patients with proximal versus mid left anterior descending artery occlusion: the Intracoronary Abciximab and Aspiration Thrombectomy in Patients With Large Anterior Myocardial Infarction (INFUSE-AMI) trial. Am Heart J 2013, 166, 64–70, doi: 10.1016/j.ahj.2013.03.029.
9. Chen Y., Shao D.-B., Zhang F.-X., Zhang J., Yuan W., Man Y.-L., Du W., Liu B.-X., Wang D.-W., Li X.-R., Cao K.-J.: Establishment and evaluation of a swine model of acute myocardial infarction and reperfusion-ventricular fibrillation-cardiac arrest using the interventional technique. J Chin Med Assoc 2013, 76, 491–496, doi: 10.1016/j.jcma.2013.05.013.
10. Cherry B.H., Nguyen A.Q., Hollrah R.A., Olivencia-Yurvati A.H., Mallet R.T.: Modeling cardiac arrest and resuscitation in the domestic pig. World J Crit Care Med 2015, 4, 1–12, doi: 10.5492/wjccm.v4.i1.1.
11. Clauss S., Bleyer C., Schüttler D., Tomsits P., Renner S., Klymiuk N., Wakili R., Massberg S., Wolf E., Kääb S.: Animal models of arrhythmia: classic electrophysiology to genetically modified large animals. Nat Rev Cardiol 2019, 16, 457–475, doi: 10.1038/s41569-019-0179-0.
12. Conradie S., Coetzee A., Coetzee J.: Anesthetic modulation of myocardial ischemia and reperfusion injury in pigs: comparison between halothane and sevoflurane. Can J Anaesth 1999, 46, 71–81, doi:10.1007/bf03012519.
13. Crick S.J., Sheppard M.N., Ho S.Y., Gebstein L., Anderson R.H.: Anatomy of the pig heart: comparisons with normal human cardiac structure. J Anat 1998, 193, 105–119, 10.1046/j.1469-7580.1998.19310105.x
14. Dib N., Diethrich E.B., Campbell A., Gahremanpour A., McGarry M., Opie S.R.: A percutaneous swine model of myocardial infarction. J Pharmacol Toxicol Methods 2006, 53, 256–263, doi: 10.1016/j.vascn.2005.10.005.
15. Di Diego J.M., Antzelevitch C.: Ischaemic ventricular arrhythmias: Experimental models and their clinical relevance. Heart Rhythm 2011, 8, 1963–1968, doi: 10.1016/j.hrthm.2011.06.036.
16. Di Giosia P., Passacquale G., Petrarca M., Giorgini P., Marra A.M., Ferro A.: Gender differences in cardiovascular prophylaxis: focus on antiplatelet treatment. Pharmacol Res 2017, 119, 36–47, doi: 10.1016/j.phrs.2017.01.025.
17. Eldar M., Ohad D., Bor A., Varda-Bloom N., Swanson D.K., Battler A.: A closed-chest pig model of sustained ventricular tachycardia. Pacing Clin Electrophysiol 1994, 17, 1603–1609, doi: 10.1111/j.1540-8159.1994.tb02353.x.
18. Elsman P., van 't Hof A.W.J., Hoorntje J.C.A., de Boer M.-J., Borm G.F., Suryapranata H., Ottervanger J.P., Gosselink A.T.M., Dambrink J.-H.E., Zijlstra F.: Effect of coronary occlusion site on angiographic and clinical outcome in acute myocardial infarction patients treated with early coronary intervention. Am J Cardiol 2006, 97, 1137–1141, doi: 10.1016/j.amjcard.2005.11.027.
19. Ewy G.A., Zuercher M., Hilwig R.W., Sanders A.B., Berg R.A., Otto Ch.W., Hayes M.M., Kern K.B.: Improved neurological outcome with continuous chest compressions compared with 30:2 compressions-to-ventilations cardiopulmonary resuscitation in a realistic swine model of out-of-hospital cardiac arrest. Circulation 2007, 116, 2525–2530, doi: 10.1161/CIRCULATIONAHA.107.711820.
20. Garcia-Dorado D., Theroux P., Elizaga J., Galinanes M., Solares J., Riesgo M., Gomez M.J., Garcia-Dorado A., Fernandez Aviles F.: Myocardial reperfusion in the pig heart model: infarct size and duration of coronary occlusion. Cardiovasc Res 1987, 21, 537–544, doi: 10.1093/cvr/21.7.537.
21. Gavira J.J., Perez-Ilzarbe M., Abizanda G., Garcia-Rodriguez A., Orbe J., Paramo J.A., Belzunce M., Rabago G., Barba J., Herreros J., Panizo A., de Jalon J.A., Martinez-Caro D., Prosper F.: A comparison between percutaneous and surgical transplantation of autologous skeletal myoblasts in a swine model of chronic myocardial infarction. Cardiovasc Res 2006, 71, 744–753, doi: 10.1016/j.cardiores.2006.06.018.
22. Gorenek B., Blomström Lundqvist C., Brugada Terradellas J., Camm A.J., Hindricks G., Huber K., Kirchhof P., Kuck K.-H., Kudaiberdieva G., Lin T., Raviele A., Santini M., Tilz R.R., Valgimigli M., Vos M.A., Vrints Ch., Zeymer U., Kristiansen S.B.: Cardiac arrhythmias in acute coronary syndromes: position paper from the joint EHRA, ACCA and EAPCI task force. EuroIntervention 2015, 10, 1095–1108, doi: 10.4244/EIJY14M08_19.

23. Gourine A.V., Bulhak A., Gonon A.T., Pernow J., Sjoquist P.: Cardioprotective effect induced by brief exposure to nitric oxide before myocardial ischemia–reperfusion in vivo. Nitric Oxide 2002, 7, 210–216, doi: 10.1016/s1089-8603(02)00114-3.
24. Hohmann S., Deisher A.J., Suzuki A., Konishi H., Rettmann M.E., Merrell K.W., Kruse J.J., Newman L.K., Parker K.D., Monahan K.H., Foote R.L., Herman M.G., Packer D.L.: Left ventricular function after noninvasive cardiac ablation using proton beam therapy in a porcine model. Heart Rhythm 2019, 16, 1710–1719, doi: 10.1016/j.hrthm.2019.04.030.
25. Kren L., Meluzin J., Pavlovsky Z., Mayer J., Kala P., Groch L., Hornacek I., Rauser P., Vlasin M.: Experimental model of myocardial infarction: histopathology and reperfusion damage revisited. Pathol Res Pract 2010, 206, 647–650, doi: 10.1016/j.prp.2010.03.008.
26. Krombach G.A., Kinzel S., Mahnken A.H., Günther R.W., Buecker A.: Minimally invasive closed-chest method for creating reperfused or occlusive myocardial infarction in swine. Invest Radiol 2005, 40, 14–18.
27. Leshem E., Zilberman I., Tschabrunn C.M., Barkagan M., Contreras-Valdes F.M., Govari A., Anter E.: High-Power and short-duration ablation for pulmonary vein isolation: biophysical characterization. JACC Clin Electrophysiol 2018, 4, 467–479, doi: 10.1016/j.jacep.2017.11.018.
28. Li X., Shao D., Wang G., Jiang T., Wu H., Gu B., Cao K., Zhang J., Qi L., Chen Y.: Effects of different LAD-blocked sites on the development of acute myocardial infarction and malignant arrhythmia in a swine model. J Thorac Dis 2014, 6, 1271–1277, doi: 10.3978/j.issn.2072-1439.2014.07.22.
29. Li X.D., Yang Y.J., Geng Y.J., Zhao J.L., Zhang H.T., Cheng Y.T., Wu Y.L.: Phosphorylation of endothelial NOS contributes to simvastatin protection against myocardial no-reflow and infarction in reperfused swine hearts: partially via the PKA signaling pathway. Acta Pharmacol Sin 2012, 33, 879–887, doi: 10.1038/aps.2012.27.
30. Li Y., Ristagno G., Bisera J., Tang W., Deng O., Weil M.H.: Electrocardiogram waveforms for monitoring effectiveness of chest compression during cardiopulmonary resuscitation. Crit Care Med 2008, 36, 211–215, doi: 10.1097/01.CCM.0000295594.93345.A2.
31. Mader T.J., Kellogg A.R., Walterscheid J.K., Lodding C.C., Sherman L.D.: A randomized comparison of cardiocerebral and cardiopulmonary resuscitation using a swine model of prolonged ventricular fibrillation. Resuscitation 2010, 81, 596–602, doi: 10.1016/j.resuscitation.2010.01.013.
32. Manninger M., Zweiker D., van Hunnik A., Alogna A., Prassl A.J., Schipke J., Zeemering S., Zirngast B., Schönleitner P., Schwarzl M., Herbst V., Thon-Gutschi E., Huber S., Rohrer U., Ebner J., Brussee H., Pieske B.M., Heinzel F.R., Verheule S., Antoons G., Lueger A., Mühlfeld C., Plank G., Schotten U., Post H., Scherr D.: Arterial hypertension drives arrhythmia progression via specific structural remodeling in a porcine model of atrial fibrillation. Heart Rhythm 2018, 15, 1328–1336, doi: 10.1016/j.hrthm.2018.05.016.
33. Marchlinski F.E., Waxman H.L., Buxton A.E., Josephson M.E.: Sustained ventricular tachyarrhythmias during the early postinfarction period: electrophysiologic findings and prognosis for survival. J Am Coll Cardiol 1983, 2, 240–250, doi: 10.1016/s0735-1097(83)80159-4.
34. Millett E.R.C., Peters S.A.E., Woodward M.: Sex differences in risk factors for myocardial infarction: cohort study of UK Biobank participants. BMJ 2018, 363, k4247, doi: 10.1136/bmj.k4247.
35. Munz M.R., Faria M.A., Monteiro J.R., Águas A.P., Amorim M.J.: Surgical Porcine Myocardial Infarction Model through Permanent Coronary Occlusion. Comp Med 2011, 61, 445–452.
36. Naslund U., Haggmark S., Johansson G., Marklund L., Reiz S.: A closed-chest myocardial occlusion–reperfusion model in the pig: techniques, morbidity and mortality. Eur Heart J 1992, 13, 1282–1289, doi: 10.1093/oxfordjournals.eurheartj.a060350.
37. Naslund U., Haggmark S., Johansson G., Pennert K., Reiz S., Marklund S.L.: Effects of reperfusion and superoxide dismutase on myocardial infarct size in a closed chest pig model. Cardiovasc Res 1992, 26, 170–178, doi: 10.1093/cvr/26.2.170.
38. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals: *Guide for the Care and Use of Laboratory Animals*, 8th edition, National Academies Press (US), Washington (DC), 2011.
39. Niemann J.T., Rosborough J.P., Youngquist S.T., Shah A.P.: Transthoracic defibrillation potential gradients in a closed chest porcine model of prolonged spontaneous and electrically induced ventricular fibrillation. Resuscitation 2010, 81, 477–480, doi: 10.1016/j.resuscitation.2009.12.027.
40. Noszczyk-Nowak A., Cepiel A., Janiszewski A., Pasławski R., Gajek J., Pasławska U., Nicpoń J.: Normal values for heart electrophysiology parameters of healthy swine determined on electrophysiology study. Adv Clin Exp Med 2016, 25, 1249–1254, doi: 10.17219/acem/65808.
41. Odenstedt J., Mansson C., Jansson S.O., Grip L.: Endocardial electromechanical mapping in a porcine acute infarct and reperfusion model evaluating the extent of myocardial ischemia. J Invasive Cardiol 2003, 15, 497–501.
42. Parker G.W., Michael L.H., Hartley C.J., Skinner J.E., Entman M.L.: Central β-adrenergic mechanisms may modulate ischemic ventricular fibrillation in pigs. Circ Res 1990, 66, 259–270, doi: 10.1161/01.RES.66.2.259.
43. Pasławska U., Noszczyk-Nowak A., Pasławski R., Janiszewski A., Kiczak L., Zyśko D., Nicpoń J., Jankowska E.A., Szuba A., Ponikowski P.: Normal electrocardiographic and echocardiographic (M-mode and two-dimensional) values in Polish Landrace pigs. Acta Vet Scand 2014, 56, 54–66, doi: 10.1186/s13028-014-0054-2.
44. Perez de Prado A., Cuellas-Ramon C., Regueiro-Purrinos M., Gonzalo-Orden J.M., Perez-Martinez C., Altonaga J.R., Garcia-Iglesias M.J., Orden-Recio M.A., Garcia-Marin J.F., Fernandez-Vazquez F.: Closed-chest experimental porcine model of acute myocardial infarction-reperfusion. J Pharmacol Toxicol Methods 2009, 60, 301–306, doi: 10.1016/j.vascn.2009.05.007.
45. Qin H., Walcott G.P., Killingsworth C.R., Rollins D.L., Smith W.M., Ideker R.E.: Impact of myocardial ischemia and reperfusion on ventricular defibrillation patterns, energy requirements, and detection of recovery. Circulation 2002, 105, 2537–2542, doi: 10.1161/01.cir.0000016702.86180.f6.
46. Reffelmann T., Sensebat O., Birnbaum Y., Stroemer E., Hanrath P., Uretsky B.F., Schwarz E.R.: A novel minimal-invasive model of chronic myocardial infarction in swine. Coron Artery Dis 2004, 15, 7–12, doi: 10.1097/00019501-200402000-00002.
47. Regueiro-Purriños M., Fernández-Vázquez F., Perez de Prado A., Altónaga J.R., Cuellas-Ramón C., Ajenjo-Silverio J.M., Orden A., Gonzalo-Orden J.M.: Ventricular arrhythmias and mortality associated with isoflurane and sevoflurane in a porcine model of myocardial infarction. J Am Assoc Lab Anim Sci 2011, 50, 73–78.
48. Saeed M., Martin A.J., Saloner D., Do L., Wilson M.: Noninvasive MR characterization of structural and functional components of reperfused infarct. Acta Radiol 2010, 51, 1093–1102, doi: 10.3109/02841851.2010.520025.
49. Savage R.M., Guth B., White F.C., Hagan A.D., Bloor C.M.: Correlation of regional myocardial blood flow and function with myocardial infarct size during acute myocardial ischemia in the conscious pig. Circulation 1981, 64, 699–707, doi: 10.1161/01.CIR.64.4.699.
50. Smart S.C., Sagar K.B., Warltier D.C.: Differential roles of Ca++ channels and Na+–Ca++ exchange in myocardial reperfusion injury in open chest dogs: relative roles during ischemia and reperfusion. Cardiovasc Res 1997, 36, 337–346, doi: 10.1016/s0008-6363(97)00187-9.
51. Sun S., Jiang Y., Zhen Z., Lai W.-H., Liao S., Tse H.-F.: Establishing a swine model of post-myocardial infarction heart

failure for stem cell treatment. J Vis Exp 2020, 159, e60392, doi: 10.3791/60392.
52. Suzuki Y., Lyons J.K., Yeung A.C., Ikeno F.: In vivo porcine model of reperfused myocardial infarction: in situ double staining to measure precise infarct area/area at risk. Catheter Cardiovasc Interv 2008, 71, 100–107, doi: 10.1002/ccd.21329.
53. Teunissen P.F.A., Horrevoets A.J.G., van Royen N.: The coronary collateral circulation: genetic and environmental determinants in experimental models and humans. J Mol Cell Cardiol 2012, 52, 897–904, doi: 10.1016/j.yjmcc.2011.09.010.
54. Thygesen K, Alpert J.S., Jaffe A.S., Chaitman B.R., Bax J.J, Morrow D.A., White H.D., The Executive Group on behalf of the Joint European Society of Cardiology (ESC)/American College of Cardiology (ACC)/American Heart Association (AHA)/World Heart Federation (WHF) Task Force for the Universal Definition of Myocardial Infarction: Fourth universal definition of myocardial infarction (2018). Circulation 2018, 138, e618–e651, doi: 10.1161/CIR.0000000000000617.
55. Tschabrunn C.M., Roujol S., Nezafat R., Faulkner-Jones B., Buxton A.E., Josephson M.E., Anter E.: A swine model of infarct-related reentrant ventricular tachycardia: Electroanatomic, magnetic resonance, and histopathological characterization. Heart Rhythm 2016, 13, 262–273, doi: 10.1016/j.hrthm.2015.07.030.
56. Verdouw P.D., Remme W.J., Hugenholtz P.G.: Cardiovascular and antiarrhythmic effects of aprindine (AC 1802) during partial occlusion of a coronary artery in the pig. Cardiovasc Res 1977, 11, 317–325, doi: 10.1093/cvr/11.4.317.
57. Vilahur G., Gutiérrez M., Casani L., Lambert C., Mendieta G., Ben-Aicha S., Capdevila A., Pons-Lladó G., Carreras F., Carlsson L., Hidalgo A., Badimon L.: P2Y12 antagonists and cardiac repair post-myocardial infarction: global and regional heart function analysis and molecular assessments in pigs. Cardiovasc Res 2018, 114, 1860–1870, doi: 10.1093/cvr/cvy201.
58. Walcott G.P., Killingsworth C.R., Smith W.M., Ideker R.E.: Biphasic waveform external defibrillation thresholds for spontaneous ventricular fibrillation secondary to acute ischemia. J Am Coll Cardiol 2002, 39, 359–365, doi: 10.1016/s0735-1097(01)01723-5.
59. Walcott G.P., Kroll M.W., Ideker R.E.: Ventricular fibrillation: are swine a sensitive species? J Interv Card Electrophysiol 2015, 42, 83–89, doi: 10.1007/s10840-014-9964-1.
60. Wojakowski W., Tendera M., Cybulski W., Zuba-Surma E.K., Szade K., Florczyk U., Kozakowska M., Szymula A., Krzych L., Pasławska U., Pasławski R., Milewski K., Buszman P.P., Nabiałek E., Kuczmik W., Janiszewski A., Dzięgiel P., Buszman P.E., Józkowicz A., Dulak J.: Effects of intracoronary delivery of allogenic bone marrow-derived stem cells expressing heme oxygenase-1 on myocardial reperfusion injury. Thromb Haemost 2012, 108, 464–475, doi: 10.1160/TH12-05-0303.
61. Yang Y.-J., Zhao J.-L., You S.-J., Wu Y-J., Jing Z.-C., Yang W.-X., Meng L., Wang Y.-W., Gao R.-L.: Different effects of tirofiban and aspirin plus clopidogrel on myocardial no-reflow in a mini-swine model of acute myocardial infarction and reperfusion. Heart 2006, 92, 1131–1137, doi: 10.1136/hrt.2005.077164.
62. Zhao J.-L., Yang Y.-J., Wu Y.-J., Jing Z.-C., You S.-J., Yang W.-X., Meng L., Tian Y., Chen J.-L., Gao R.-L., Chen Z.-J.: Effects of anti-platelet drugs on myocardial no-reflow after acute myocardial infarction and reperfusion: experiment with mini-swine model. Zhonghua Yi Xue Za Zhi 2005, 85, 2187–2191, doi: 10.3760/j:issn:0376-2491.2005.31.008.