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Cover Letter SJTREM

Dear Editor

I am pleased to submit an original research article entitled “A standardized model of ventricular fibrillation and advanced cardiac life support in swine” by R. Ruemmler, A. Ziebart, A. Garcia-Bardon, J.Kamuf, and E.K. Hartmann for consideration for publication in *The Journal of Visualized Experiments.* We present in this animal study a standardized experimental protocol of ventricular fibrillation and resuscitation that allows for a more scientific approach to those medically challenging situations.

In this manuscript, we show that ventricular fibrillation can be reliably induced in large animals and can afterwards be treated adequately while adhering to internationally accepted resuscitation guidelines. Additionally, the presented model may help to evaluate novel treatment options in critically ill patients, with a dramatically reduced ethically ambiguous threshold, which usually tends to confound resuscitation studies in humans.

We believe that this manuscript is appropriate for publication in *JoVE* because it describes a standardized model that has not been filmed for other research groups to benefit from the more practical approaches of visualized experimental protocols. Additionally, the possibility to actually see the resuscitation process in particular step by step is, in our opinion, extremely helpful for understanding, which factors are crucial in real life.

This manuscript has not been published and is not under consideration for publication elsewhere. The study and all animal experiments were conducted in adherence to the ARRIVE guidelines. We have no conflicts of interest to disclose. We declare that all authors listed above contributed substantially to conducting the study, analyzing the data and/or drafting this manuscript. If you feel that the manuscript is appropriate for your journal, we suggest the following reviewers:

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Thank you for your consideration!

TITLE:

Standardized Model of Ventricular Fibrillation and Advanced Cardiac Life Support in Swine

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KEYWORDS:

resuscitation, chest compressions, advanced cardiac life support, ventricular fibrillation, pig, animal model

SUMMARY:

Cardiopulmonary resuscitation and defibrillation are the only effective therapeutic options during cardiac arrest caused by ventricular fibrillation. This model presents a standardized regimen to induce, assess, and treat this physiological state in a porcine model, thus providing a clinical approach with various opportunities for data collection and analysis.

ABSTRACT:

Cardiopulmonary resuscitation after cardiac arrest, independent of its origin, is a regularly encountered medical emergency in hospitals as well as preclinical settings. Prospective randomized trials in human subjects are difficult to design and ethically ambiguous, which results in a lack of evidence-based therapies. The model presented in this report represents one of the most common causes of cardiac arrests, ventricular fibrillation, in a standardized setting in a large animal model. This allows for reproducible observations and various therapeutic interventions under clinically accurate conditions, hence facilitating the generation of better evidence and eventually the potential for improved medical treatment.

INTRODUCTION:

Cardiac arrest and cardiopulmonary resuscitation (CPR) are regularly encountered medical emergencies in hospital wards as well as preclinical emergency provider scenarios[1,2]. While there have been extensive efforts to characterize the optimal treatment for this situation[3-6],

international guidelines and expert recommendations (e.g., ERC and ILCOR) usually rely on low-grade evidence due to the lack of prospective randomized trials[3-5,7-9]. This is in part due to obvious ethical reservations regarding randomized resuscitation protocols in human trials[10]. However, this may also point towards a lack of strict protocol adherence when confronted with a life-threatening and stressful situation[11,12]. The protocol presented in this report aims to provide a standardized resuscitation model in a realistic clinical setting, which generates valuable, prospective data while being as valid and accurate as possible without the need for human subjects. It adheres to common resuscitation guidelines, can be easily applied, and enables researches to examine and characterize various aspects and interventions in a critical but controlled setting. This will lead to 1) a better understanding of the pathological mechanisms underlying cardiac arrest and ventricular fibrillation and 2) higher quality evidence in order to optimize treatment options and increase survival rates.

PROTOCOL:

The experiments in this protocol were approved by the State and Institutional Animal Care Committee (Landesuntersuchungsamt Rheinland-Pfalz, Koblenz, Germany; Chairperson: Dr. Silvia Eisch-Wolf; approval no. G16-1-042). The experiments were conducted in accordance with the ARRIVE guidelines. Seven anesthetized male pigs (*sus scrofa domestica*) with a mean weight of 30 ± 2 kg and 12–16 weeks in age were included in the protocol.

1. Anesthesia, intubation, and mechanical ventilation[13,14]

1.1. Maintain animals in their normal environment as long as possible to minimize stress. Withhold food 6 h before the scheduled experiment to reduce the risk of aspiration, but do not refuse water access.

1.2. Sedate pigs with a combined injection of ketamine (4 mg/kg) and azaperone (8 mg/kg) in the neck or gluteal muscle with a needle (20 G) for intramuscular injection. Leave the animals undisturbed in their stables until sedation sets in (15–20 min).

CAUTION: Gloves are absolutely necessary when handling animals.

1.3. Transport the sedated animals to the laboratory. Transport time should not exceed effective sedation time (here, 30–60 min).

1.4. Monitor the peripheral oxygen saturation (SpO_2) with a sensor clipped to the tail or ear.

1.5. Disinfect the skin with an alcoholic disinfectant before insertion of a peripheral vein catheter (20 G) into an ear vein. Spray the area, wipe 1x, spray again, and let the disinfectant dry.

1.6. Administer analgesia via intravenous injection of fentanyl (4 µg/kg). Induce anesthesia with intravenous injection of propofol (3 mg/kg)

1.7. Place the pig in supine position on a stretcher with a vacuum mattress and fix it with bandages. Apply muscle relaxant via intravenous injection of atracurium (0.5 mg/kg)

1.8. Directly start noninvasive ventilation with a dog ventilation mask (size 2). Ventilation parameters are as follows: FiO_2 (inspiratory oxygen fraction) = 100%, respiratory rate = 18–20 breaths/min, peak inspiratory pressure = <20 cmH_20, PEEP (positive end-expiratory pressure) = 5 cmH_20.

1.9. Maintain anesthesia via continuous infusion of fentanyl (0.1–0.2 mg kg^{-1} h^{-1}) and propofol (8–12 mg kg^{-1} h^{-1}). Start a continuous infusion of balanced electrolyte solution (5 mL kg^{-1} h^{-1}).

1.10. Secure the airway via intubation with a common endotracheal tube (ID 6-7) and an introducer. Use a common laryngoscope with a Macintosh blade (size 4). Two people are necessary for this step.

1.10.1. Ensure that one person fixes the tongue outside with a piece of tissue and opens the snout with the other hand.

1.10.1.1. Ensure that the second person performs a laryngoscopy of the porcine larynx. When the epiglottis comes into view, move the laryngoscope ventrally. The epiglottis should be lifted up and the vocal cords will be visible.

NOTE: If the epiglottis does not move ventrally, it will stick to the soft palatine and can be mobilized by the tip of the tube.

1.11. Move the tube carefully through the vocal cords.

NOTE: The narrowest point of the trachea is not on the level of the vocal cords but is subglottic. If tube insertion is not possible, try to rotate the tube clockwise or use a smaller tube.

1.12. Pull the introducer out of the tube. Use a 10 mL syringe to block cuff with 10 mL of air. Control the cuff pressure with a cuff manager (30 cmH_2O).

1.13. Start mechanical ventilation after tube connection with a ventilator (PEEP = 5 cmH_2O, tidal volume = 8 mL/kg, FiO_2 = 0.4, I:E [inspiration to expiration ratio] = 1:2, respiratory rate = variable to achieve an end-tidal CO_2 of <6 kPa, usually 20–30/min). Make sure that tube position is correct by regular and periodic exhalation of carbon dioxide via capnography.

1.14. Check double-sided ventilation via auscultation.

NOTE: In case of incorrect placement of the tube, an air-filled stomach rapidly forms a clearly visible bulge through the abdominal wall. In this case, immediate replacement of the tube and insertion of a gastric tube is necessary. If intubation is not successful, switch back to mask

ventilation and try a smaller tube or better positioning of the snout.

1.15. Place gastric tube into the stomach to avoid reflux and vomiting with two people.

1.15.1. Fix the tongue outside with a piece of tissue and open the snout with the other hand.

1.15.1.1. Ensure that a second person performs a laryngoscopy of the porcine larynx then visualizes the esophagus. Push the gastric tube inside the esophagus with a Magill's forceps until gastric fluid is drained.

NOTE: Visualization may be difficult. In this case, lift the tube with the laryngoscope ventrally to open the esophagus.

2. Instrumentation

2.1. Use bandages to pull back the hindlegs to smooth the folds in the femoral area for vessel catherization.

2.2. Prepare the following materials: syringes (5 mL, 10 mL, and 50 mL), Seldinger needle, introducer sheaths (6 Fr, 8 Fr, 8 Fr), guidewires for the sheaths, central venous catheter with three ports (7 Fr, 30 cm) with guidewire, cardiac output monitor (**Table of Materials**), and a catheter (5 Fr, 20 cm).

2.3. Disinfect the inguinal area (see step 1.6). Repeat this process 2x.

2.4. Fill all catheters with saline solution. Apply ultrasound gel on the ultrasound probe. Cover the inguinal area with a sterile fenestrated drape.

2.5. Scan the right femoral vessels with ultrasound and use doppler technique to identify the artery and vein[15]. Visualize the right femoral artery axially. Switch to a longitudinal view of the arteria by rotating the probe 90°.

2.6. Puncture the right femoral artery under ultrasound visualization with the Seldinger needle under permanent aspiration with the 5 mL syringe.

NOTE: In our opinion, the ultrasound guided Seldinger's technique is associated with significantly less blood loss and tissue trauma than other methods of vascular access.

2.7. Confirm the desired needle position by observing bright red pulsating blood. Disconnect the syringe and quickly insert the guidewire into the right femoral artery.

2.8. Visualize the longitudinal axis of the right femoral vein. Insert the Seldinger needle under permanent aspiration with the 5 mL syringe. Aspirate any dark red non-pulsating venous blood.

NOTE: If the correct position of the needle in the different vessels cannot be visually confirmed, take blood samples and analyze the blood gas content. A high oxygen level is a good sign for arterial blood, while low oxygen saturation indicates intravenous position.

2.9. Insert the guidewire for the central venous catheter into the right femoral vein after disconnecting the syringe. Retract the Seldinger needle.

2.10. Visualize both right vessels using ultrasound to control the correct wire position. Push the arterial introducer sheath (6 Fr) over the guidewire into the right artery and secure the position with blood aspiration.

NOTE: Placing the sheath through the skin can be difficult. It can be helpful to perform a small incision along the wire to facilitate better placement.

2.11. Use Seldinger's technique to position the central venous line into the right femoral vein. Aspirate all ports and flush them with saline solution.

2.12. Perform the same procedure on the left inguinal side to insert the other introducer sheaths in Seldinger's technique into the left femoral artery (8 Fr) and femoral vein (8 Fr).

2.13. Connect the right arterial introducer sheath and the central venous catheter with two transducer systems for measurement of invasive hemodynamics. Position both transducers at heart level.

2.14. Switch the tree-way stopcocks of both transducers open to the atmosphere to calibrate the system to zero.

NOTE: It is necessary to avoid any air bubbles and bloodstains in the system to generate plausible values.

2.15. Switch all infusions for maintaining anesthesia from the peripheral vein to a central venous line. Take baseline values (hemodynamics, spirometrics, and other output from the cardiac monitor; see section 3) after a 15 min recovery.

2.16. Initiate ventricular fibrillation (see section 4).

3. Pulse contour cardiac output

3.1. Insert transpulmonal thermodilution catheter into the right arterial introducer sheath.

NOTE: In clinical medicine, thermodilution catheters are directly placed by Seldinger's technique. However, placement via an introducer sheath is also feasible. In the proposed protocol, sheaths are placed as a standardized vascular access for maximum flexibility in instrumentation throughout different experiments.

3.2. Connect the catheter with the arterial wire of the cardiac monitor system. Switch the arterial transducer directly with the cardiac monitor port and recalibrate as described in step 2.14. Connect the venous measuring unit of the cardiac monitor system with the left venous introducer sheath.

NOTE: It is necessary to connect the venous and arterial probes as far apart as possible; otherwise, the measurement will be disturbed, because the application of cold water into the venous system will affect the arterial measurement. More details regarding $PiCCO_2$ have been provided previously[16].

3.3. Turn on the cardiac monitor system. Confirm that a new patient is being measured. Enter the size and weight.

3.4. Switch the category to adults. Enter the protocol name and ID. Click on **Exit**.

3.5. Set the injection volume to 10 mL.

NOTE: The volume of chosen injection solution can be changed in the software. A higher volume makes the measured values more valid. A small volume was chosen for this experiment to avoid any hemodilution effects.

3.6. Enter the central venous pressure.

3.7. Open the three-way stopcock to the atmosphere.

3.8. Click on **Zero** for system calibration. Click on **Exit**.

3.9. Calibrate the continuous cardiac output measurement.

3.9.1. Click on **TD** (thermodilution). Prepare a physiological saline solution with a temperature of 4 °C in a 10 mL syringe. Click on **Start**.

3.9.2. Inject 10 mL of cold saline solution quickly and steadily into the venous measuring unit. Wait until the measurement is completed and the system requests a repetition.

3.9.3. Repeat the previous step until three measurements are completed. The system will calculate the mean of all parameters. Click on **Exit**.

NOTE: Measurements will start immediately after calibration has been completed. Although cardiac output measurements during CPR are not performed regularly, plausible results have been able to be affirmed after adequate calibration[17,18].

4. Ventricular fibrillation and mechanical resuscitation

4.1. Place defibrillator patch electrodes in anterior-posterior position on the torso. The posterior electrode should be positioned on the central left hemithorax.

NOTE: Use a razor to remove excess hair and dirt to facilitate optimal conduction.

4.2. Connect the electrodes to a defibrillator and establish an ECG.

4.3. Immobilize the pig inside the vacuum mattress. Deflate the mattress to prevent unwanted movement during CPR. Control fixation of the limbs.

4.4. Place chest compression device (here, LUCAS-2) around the chest and under the vacuum mattress according to the manufacturer's recommendations. Adjust the pressure pad to the lower third of the sternum in median position.

4.5. Turn on chest compression device ("power" button) and lower the pressure pad to skin level. Set the compression frequency to 100/min, if not otherwise defined in the protocol. Press the **Pause** button to prepare the compression device for chest compressions.

4.6. Insert a fibrillation/pacing catheter into the left femoral vein through the i.v. sheath.

4.7. Inflate the catheter cuff with 1–2 mL of air. Slowly push the inflated cuff further until it is placed next to the right atrium (usually about a 50 cm distance).

4.8. Connect catheter electrodes to an adequate oscilloscope/function generator. Adjust fibrillation parameters to the desired values (here, a 13.8 V current with frequencies between 50–200 Hz).

4.9. Turn on generator and monitor ECG changes. Move the catheter slowly forward until arrhythmias can be detected in the ECG.

CAUTION: Prevent the separate electrodes at the end of the catheter from touching human skin or each other to prevent short circuits and possibly life-threatening situations.

4.10. Carefully vary the catheter position until ventricular fibrillation can be detected.

NOTE: It can be difficult to induce fibrillation right away. If a position is reached at which ECG effects can be seen, changing the frequency or repeatedly turning the generator on and off can sometimes be helpful.

4.11. Once ventricular fibrillation is confirmed, turn off the generator, deflate the balloon, and remove the fibrillation catheter. Maintain fibrillation with or without ventilation for as long as required.

4.12. Start mechanical chest compressions by pressing the **Play** button on the compression device. To interrupt chest compressions, press the **Pause** button on the compression device.

4.13. Analyze ECG patterns. If ventricular fibrillation persists, prepare defibrillation.

4.13.1. Enter **Manual** mode in the defibrillator menu. Adjust the energy to 200 J biphasic.

4.13.2. Press the **Load** button. Wait until acoustic signal turns on to indicate a prepared shock value. Initiate electric shock.

CAUTION: Only experienced users should handle defibrillators and fibrillation catheters. No shocks should be initiated if there is any indication for faulty or worn materials. The initiation of an electric shock must always be announced clearly audible to every person in the room, and the person launching the defibrillation is responsible for ensuring that nobody is touching the animal or stretcher prior to releasing the shock.

NOTE: Here, guideline-based resuscitation protocol was used (i.e., 2 min of chest compressions, ECG assessment, shock, 2 min of chest compressions, adrenaline administration, etc.). For more information, consult with the guidelines[4].

4.14. In the case of return of spontaneous circulation (ROSC), stop chest compressions, continue ventilation, and apply monitoring as extensively and for as long as needed.

NOTE: Anesthetic drug administration may or may not be interrupted during CPR, depending on the protocol. If sedation is discontinued, infusion should be restarted upon confirmed ROSC.

4.15. A goal-directed approach for the guidance of fluid and catecholamine administration as well as standardized respiratory and ventilation settings are recommended to prevent cardiorespiratory deterioration in the ROSC phase leading to experimental failure.

5. End of experiment and euthanasia (in the case of ROSC)

5.1. Inject 0.5 mg of fentanyl into the central venous line. Wait 5 min. Inject 200 mg of propofol into the central venous line.

5.2. Euthanize the animal with a 40 mmol potassium chloride injection.

5.3. Perform organ removal/fixation or analyses as needed.

REPRESENTATIVE RESULTS:

Cardiac arrest was induced in seven pigs. Return of spontaneous circulation following CPR was achieved in four Pigs (57%) with a mean of 3 ± 1 biphasic defibrillations. Healthy and adequately anesthetized pigs should stay in supine position without shivering and signs of agitation

throughout the entire experiment. Mean arterial blood pressures should not drop below 50 mmHg before initiation of fibrillation[18]. For optimal results, blood gas analyses can be performed and all values including temperature should be normalized.

If placed in the right position, the pacing catheter should start to influence heart rhythm. This can result in extrasystoles, tachycardia and all forms of ventricular and supraventricular arrhythmias. Cardiac arrest can be assumed if 1) the ECG reading shows ventricular fibrillation and 2) no cardiac output or pressure variations are measured by the arterial line (**Figure 1**). If this state persists with the generator turned off, fibrillation is likely not to spontaneously subside anymore[17].

Once chest compressions are started, sufficient cardiac output generation is indicated by a mean arterial pressure of 30–50 mmHg. (**Figure 1**) If adhering to resuscitation guidelines, the administration of adrenaline (1 mg) should result in a substantial rise in blood pressure within 1 min.

ROSC is confirmed by a dramatic increase in expiratory carbon dioxide measurements (usually increasing from 10–20 mmHg during arrest to 45 mmHg and above), organized heart rhythm in the ECG, and respective cardiac output as shown by arterial measurement. Hypercapnia and a decreased Horovitz index (PaO_2/FiO2) are commonly observed after ROSC. Reestablishment of controlled mechanical ventilation leads to recompensation and stable respiratory conditions (**Figure 2**). A ROSC rate of 50%–70% can be expected depending on the time between cardiac arrest and the start of chest compressions.

FIGURE LEGENDS:

Figure 1: Typical hemodynamic values. (**A**) Heart rate monitoring during trial (depicted as mean values with standard deviation [SD] error bars). Heart rate drops to zero at cardiac arrest (CA) and is standardized during CPR according to the specifications of the chest compression device (here, 100 bpm). Tachycardia is regularly seen after achieving ROSC, initially as a result of adrenaline administration and metabolic acidosis compensation. Values usually normalize over a period of 1–2 h. (**B**) Mean intra-arterial blood pressure values. At cardiac arrest (CA), pressure does not drop below 10–20 mmHg but loses all signs of effective output. During CPR, especially before vasopressor effects are registered, adequate chest compressions are indicated by pressure values between 30–50 mmHg. Post-ROSC, norepinephrine might be necessary to cover low blood pressure intervals during metabolic recompensation.

Figure 2: Oxygenation and decarboxylation parameters during and after resuscitation. (**A**) Arterial partial pressure values of carbon dioxide ($PaCO_2$) during and after CPR (depicted as mean values with standard deviation error bars). Under guideline-based ventilation, no significant differences should be detected. An increase in CO_2 levels directly after ROSC is to be expected but should normalize within 1 h. (**B**) Typical values of Horovitz index (arterial partial pressure of oxygen [PaO_2]/inspiratory oxygen fraction [F_iO_2]; depicted as mean values with SD error bars). During CPR, oxygenation is often highly impaired but usually fully recovers post-

ROSC during the first 2 h.

DISCUSSION:

Some major technical issues regarding anesthesia in a porcine model have previously been described by our group[13,14]. These include the strict avoidance of stress and unnecessary pain for the animals, possible anatomical problems during airway management, and specific personnel requirements[19].

Additionally, the benefits of ultrasound-guided catheterization was highlighted previously and remains the preferable approach to prevent vascular damages during instrumentation. However, only professionally trained users should work with this technique to yield its advantages[20]. For this experimental model, it must be stressed that handling electrical frequency generators as well as defibrillators should only be handled by specifically trained personnel or under their direct supervision. Failure to provide adequate expertise while conducting such trials may result in serious injury and can be life-threatening.

Correct positioning of the pacing catheter and initiation of ventricular fibrillation may prove difficult and can require reinsertion of the catheter or frequency variation. When repositioning or removing the catheter, the balloon should be deflated first to prevent internal injuries as well as damage to the catheter itself. If frequency variations are used, the catheter should be placed near the myocardium in order to detect ECG changes, then frequency should slowly be changed according to the manufacturer's instructions.

Porcine models have been successfully used in critical care studies for decades[17,21-23]. Similar anatomic and physiologic properties comparable to humans allow for reasonably accurate deductions regarding patient reactions to certain stimuli or clinical situations. The presented resuscitation model has been used and modified in various trials[18, 24-26]. It provides an experimental setting that enables the evaluation of guideline effectiveness, since (in contrast to resuscitation models in rodents) equal chest compression intervals, blood pressure thresholds, blood gas values, and defibrillation energies can be used for human comparisons as recommended by ILCOR and ERC, respectively. This facilitates internationally comparable and comprehensible study designs, thus generating a higher quality of evidence overall. The model additionally allows for adequate assessment of drug effects not only qualitatively, but also in a dose-dependent fashion.

Assuming guideline-based resuscitation with intervals of 2 min between defibrillations, pigs usually achieve ROSC within the first four shocks or within 8–10 min[27]. A ROSC rate of 50%–70% can be expected depending on the time between cardiac arrest and the start of chest compressions. If acceptable ROSC rates or adequate blood pressure values cannot be achieved, it is possible to add vasopressine (0.5 IU/kgBW) to the therapy regimen during CPR. During and directly after CPR, pulmonary gas exchange is heavily impaired. This is largely dependent on the ventilation mode used during chest compressions and can have long-term effects on end organ damage and inflammation[18,25,28]. Additionally, metabolic acidosis and stunned myocardium can

lead to persistent hypotension, especially in the first 1 h following ROSC. This can be treated by fluid administration (20–30 mL/kgBW) and continuous norepinephrine infusion. Excessive acidosis can also be treated with 8.4% sodium bicarbonate solution with a maximum of 4 mL/kgBW.

This experimental protocol provides a standardized setting for resuscitation research in which the aspects of hemodynamic effects of specific drug treatments, influence of ventilation modes on ROSC rates, end-organ damage, and post-resuscitation reactions can be analyzed and evaluated under various circumstances. This will help further scientific insight into the pathophysiologic mechanisms underlying ventricular fibrillation and may lead to more effective treatment options.

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DISCLOSURES:
The LUCAS-2 device was provided unconditionally by Stryker/Physio-Control, Redmond, WA, USA for experimental research purposes. No authors report any conflicts of interest.

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Figure 1

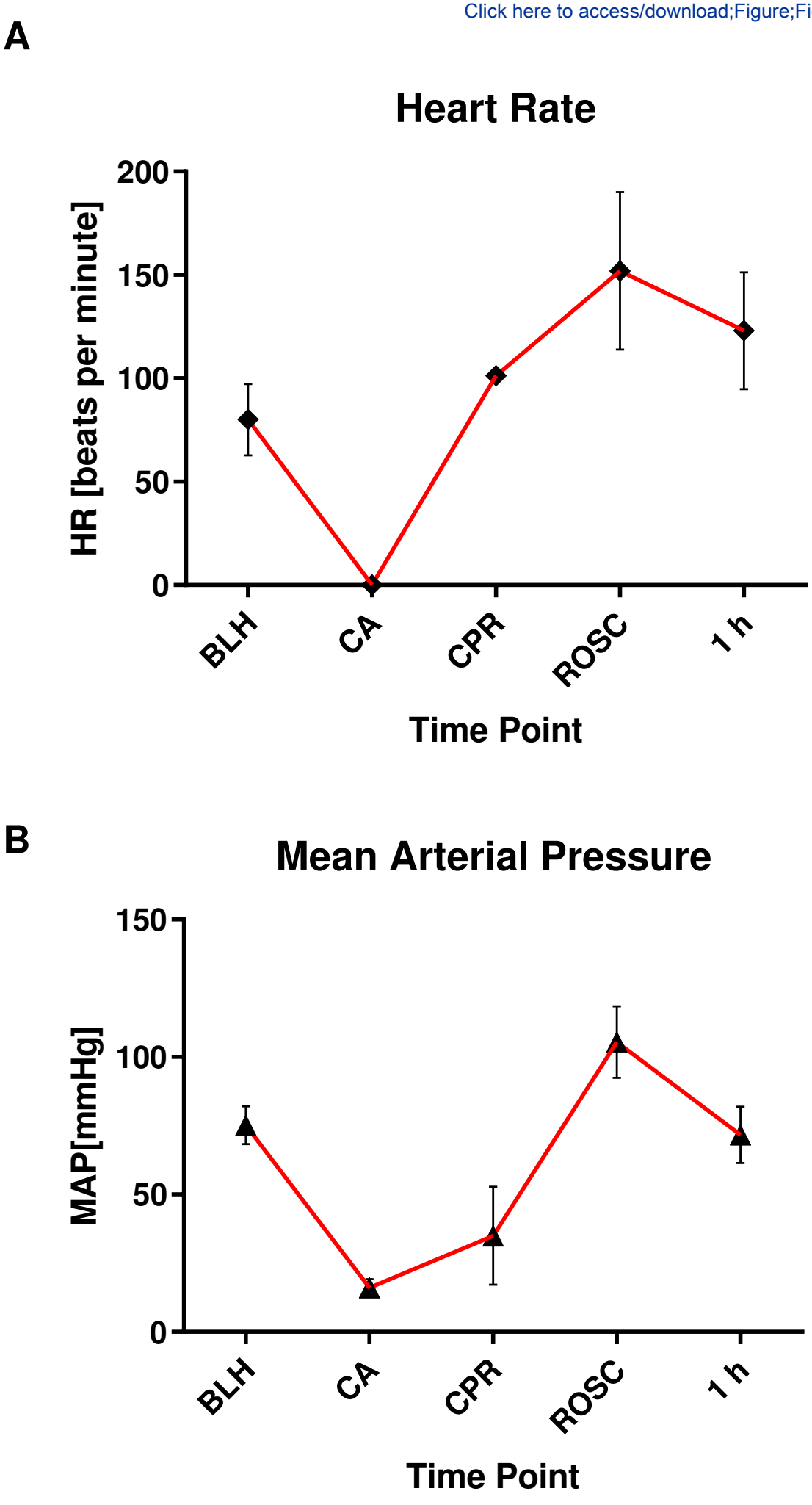
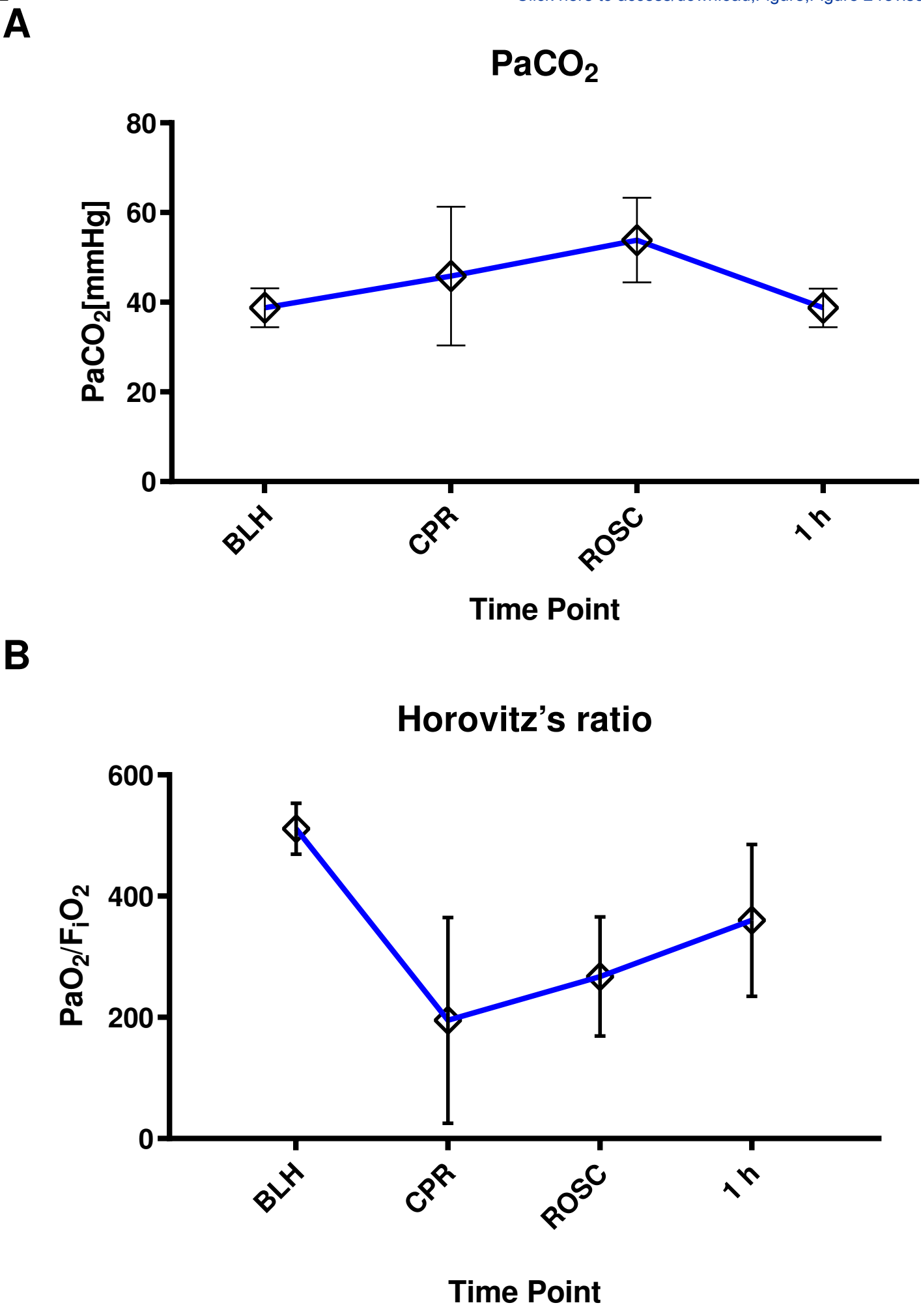


Figure 2



Name of Material/ Equipment	Company	Comments/Description
1 M- Kaliumchlorid-Lösung 7,46% 20ml	Fresenius, Kabi Deutschland GmbH	potassium chloride
Arterenol 1mg/ml 25 ml	Sanofi- Aventis, Seutschland GmbH	norepinephrine
Atracurium Hikma 50mg/5ml	Hikma Pharma GmbH , Martinsried	atracurium
BD Discardit II Spritze 2,5,10,20 ml	Becton Dickinson S.A. Carretera Mequinenza Fraga, Spain	syringe
BD Luer Connecta	Becton Dickinson Infusion Therapy AB Helsingborg, Schweden	3-way-stopcock
BD Microlance 3 20 G	Becton Dickinson S.A. Carretera Mequinenza Fraga, Spain	canula
CorPatch Easy Electrodes	CorPuls, Kaufering, Germany	defibrillator electrodes
Corpuls 3	Corpuls, Kaufering, Germany	defibrillator
Datex Ohmeda S5	GE Healthcare Finland Oy, Helsinki, Finland	hemodynamic monitor
Engström Carestation	GE Heathcare, Madison USA	ventilator
Fentanyl-Janssen 0,05mg/ml	Janssen-Cilag GmbH, Neuss	fentanyl
Führungsstab, Durchmesser 4.3	Rüsch	endotracheal tube introducer
Incetomat-line 150 cm	Fresenius, Kabi Deutschland GmbH	perfusorline
Ketamin-Hameln 50mg/ml	Hameln Pharmaceuticals GmbH	ketamine
laryngoscope	Rüsch	laryngoscope
logicath 7 Fr 3-lumen 30cm lang	Smith- Medical Deutschland GmbH	central venous catheter
LUCAS-2	Physio-Control/Stryker, Redmond, WA, USA	chest compression device
Masimo Radical 7	Masimo Corporation Irvine, Ca 92618 USA	periphereal oxygen saturation
Neofox Oxygen sensor 300 micron fiber	Ocean optics Largo, FL USA	ultrafast pO2-measurements
Ölsäure reinst Ph. Eur NF C18H34O2 M0282,47g/mol Dichte 0,9	Applichem GmbH Darmstadt, Deutschland	oleic acid
Original Perfusor syringe 50ml Luer Lock	B.Braun Melsungen AG, Germany	perfusorsyringe
Osypka pace, 110 cm	Osypka Medical GmbH, Rheinfelden-Herten, Germany	Pacing/fibrillation catheter

PA-Katheter Swan Ganz 7,5 Fr 110cm	Edwards Lifesciences LLC, Irvine CA, USA	PAC
Percutaneous sheath introducer set 8,5 und 9 Fr, 10 cm with integral haemostasis valve/sideport	Arrow international inc. Reading, PA, USA	introducer sheath
Perfusor FM Braun	B.Braun Melsungen AG, Germany	syringe pump
Propofol 2% 20mg/ml (50ml flasks)	Fresenius, Kabi Deutschland GmbH	propofol
Radifocus Introducer II, 5-8 Fr	Terumo Corporation Tokio, Japan	introducer sheath
Rüschelit Super Safety Clear >ID 6/ 6,5 /7,0 mm	Teleflex Medical Sdn. Bhd, Malaysia	endotracheal tube
Seldinger Nadel mit Fixierflügel	Smith- Medical Deutschland GmbH	seldinger canula
Sonosite Micromaxx Ultrasoundsystem	Sonosite Bothell, WA, USA	ultrasound
Stainless Macintosh Größe 4	Welsch Allyn69604	blade for laryngoscope
Stresnil 40mg/ml	Lilly Deutschland GmbH, Abteilung Elanco Animal Health	azaperone
Vasofix Safety 22G-16G	B.Braun Melsungen AG, Germany	venous catheter
Voltcraft Model 8202	Voltcraft, Hirschau, Germany	oscilloscope/function generator

JoVE rebuttal letter to "A standardized model of ventricular fibrillation and advanced cardiac lifesupport in swine"

Editorial comments:

We thank the editors for consideration of the manuscript. General concerns have been addressed in the manuscript file. Protocol lines with previously used methods that still belong to the presented protocol should not be changed, because they are standardized in our research group and referenced respectively. Citation rules have been updated, material list should be comprehensible.

Reviewer 1:

The authors want to thank reviewer 1 for the favourable review. The minor semantic errors that were addressed have been corrected in the reviewed manuscript. Results section has not been changed, since submission guidelines require a description of expectable results and reactions as well as exemplary data.

Reviewer 2:

The authors want to thank reviewer 2 for the time invested and the detailed review provided. However, we feel the intention of the submitted protocol as well as the scope of the journal that is being reviewed for has been misconceived substantially:

1. Neither do the authors claim that this is a new experimental model nor that it is the only one. In the contrary, other experimental groups are cited in the article (e.g. Tan et al., Kill et al.). Additionally, there is no mention of this approach being a comprehensible summary of all possible experimental protocols in porcine resuscitation experiments.
2. However, JoVE aims to provide video protocols of standardized experiments to enable more research groups to establish their own scientific environments. There is no video protocol of resuscitation trials in the JoVE data base and no standardized model could be found in an easily accessible video format elsewhere, why we deem is suitable and sensible to publish in this journal.
3. Obviously, the reviewer is not familiar with submission protocols of JoVE, which explains why he/she does not know, that highlighted text passages are required in every manuscript to indicate the protocol aspects, that are to be filmed eventually.

Following points 1-3, no additional changes have been made to the manuscript.

Reviewer 3:

The authors want to thank reviewer 3 for the detailed review and the in depth analysis and suggestions for style improvements.

1. Since we cannot control, at which level of expertise potential readers are situated when reading our protocol or trying to implement it, we have to make sure necessary caution is taken. Adequate knowledge of the technique of defibrillation and its use and dangers is required and not every scientist is inherently familiar with that fact, which is why we repeatedly mention the proper preparation necessary to prevent harm from investigators as well as animals.

2. Since notes are required to always follow the respected passage, we did not change it here. Generally, we feel it is a matter of individual taste, but might be considered in the future.
3. Semantic errors have been addressed in the manuscript.
4. Ad 1.1 Restrictions in infrastructure and animal care conventions for our laboratory do not allow us to keep animals for more than 24 hours before being euthanized. So stress reduction here only means sedation during transport (~30 minutes from farm to lab).
5. Ad 1.2 major respiratory complications are generally possible, although we have not encountered described problems by the reviewer
6. Ad 1.4-1.21 Fentanyl and propofol will induce hypoventilation. However, mask ventilation with standard dog masks – at least in our experience – is very rarely difficult. Atracurium is used because it is cheaper and the time between repositioning the pig and preparing intubation is long enough for atracurium to work. We feel relaxants should be used to enable easier tracheal access. We do not use frovas regularly, but in difficult cases it might help pass the subglottic plane. Adequate placement of gastric tubes using Magill prongs and laryngoscopes is usually feasible and does not necessarily cause major injuries.
7. Ad 2 We strongly feel that ultra-sound guided catheter placement should be the gold standard in terms of safe application and less unnecessary vascular and tissue damage, which is why we recommend it. If other groups are not able to provide ultra-sound probes, there are other recommendations available but will not be added here. Inguinal veins were used due to experimental standardization in our lab. Additionally, resuscitation and mechanical chest compressions can compromise cranial catheters or thoracic measure approaches. In or lab, we never use Miller catheters and feel, that standard invasive measurements yield adequate results.
8. Ad 3 Level of detail and thorough description for untrained users is recommended for JoVE submissions in order to maximize accessibility.
9. Ad 4 The vacuum mattress does not pose positioning problems of the compression device, at least not in pigs of sizes described in the manuscript. Patch electrode positioning description has been updated. Thumper positioning is key to effective chest compressions. This is highly dependent on pig anatomy, which might differ race by race. Universally, thumper positioning should be as adherent to resuscitation guidelines as possible, i.e. located in the median sternal axis over the lower third of the sternum. This is described in the manufacturer's recommendation and might differ, once you use a different compression device. Repositioning during CPR might prove difficult and the need for repositioning of animal and/or compression device should be reduced to a minimum. In our experience, vacuum mattresses help reduce unwanted movements. Drug dosing is an important issue. Pediatric dosages would be 0.01mg/kgBW or in our case 300µg adrenaline. In our experience, this often did not prove effective. Generally, "accurate" dosing might not always prove useful depending on what the study focus is supposed to be.
10. Ad 5 The line has been changed. In our experience, 40 mmol is a high enough dose to quickly induce cardiac arrest, independent from injection site or flushing.

Reviewer 4:

The authors want to thank reviewer 4 for the positive verdict and interesting insights and impulses.

1. Alternative sedative strategies are generally feasible and dependent on researcher experience or veterinary support. Volatile anesthetics require respective infrastructure

including gas suction and sufficient disposal mechanisms. This is not provided in our lab, which is why we rely on intravenous anesthesia.

2. We do place PACs in our animal models regularly for the here mentioned benefits. CPR and ROSC outcomes could theoretically be affected by a positioned catheter though, which is why we only use them in special cases (e.g. mixed-venous blood sampling for MIGET) and did not include them in our protocol.
3. Correct positioning is, of course, indicated by ventricular arrhythmias. In our experience, the distance between supraventricular and ventricular responses is often very short and transitions can be fluent, which is why we did not specify further.
4. Adrenaline is regularly used during all resuscitation experiments according to guidelines as described in the manuscript.
5. This is a very interesting point. While we do not take post mortem blood samples, direct effects of potassium chloride should be considered. Although we do not use this method, a second induction of ventricular fibrillation might be helpful to eliminate confounding factors depending on special scientific questions.

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Author(s):	Robert Ruemmler1, Alexander Ziebart1, Andreas Garcia-Bardon1, Jens Kamuf1, Erik K. Hartmann1

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