Impact of the inspiratory oxygen fraction on the cardiac output during jugulo-femoral venoarterial extracorporeal membrane oxygenation in the rat

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Abstract

Background: Venoarterial extracorporeal membrane oxygenation (V-A ECMO) with femoral access has gained wide acceptance in the treatment of critically ill patients. Since the patient’s cardiac output (CO) can compete with the retrograde aortic ECMO-flow, the aim of this study was to examine the impact of the inspiratory oxygen fraction on the cardiac function during V-A ECMO therapy.

Methods: Eighteen male Lewis rats (350–400 g) received V-A ECMO therapy. The inspiratory oxygen fraction on the ventilator was randomly set to 0.5 (group A), 0.21 (group B), or 0 in order to simulate apnea (group C), respectively. Each group consisted of six animals. Arterial blood pressure, central venous saturation (ScvO2), CO, stroke volume, left ventricular ejection fraction (LVEF), end diastolic volume, and pressure were measured. Cardiac injury was determined by analyzing the amount of lactate dehydrogenase (LDH).

Results: During anoxic ventilation the systolic, mean and diastolic arterial pressure, CO, stroke volume, LVEF and ScvO2 were significantly impaired compared to group A and B. The course of LDH values revealed no significant differences between the groups.

Conclusion: Anoxic ventilation during V-A ECMO with femoral cannulation leads to cardiogenic shock in rats. Therefore, awake V-A ECMO patients might be at risk for hypoxia-induced complications.

Keywords: ECMO, Cardiogenic shock, Apnea, Ventilation during ECMO, Harlequin phenomenon

Background

Extracorporeal membrane oxygenation (ECMO) has been widely accepted for the treatment of pulmonary or cardiac failure in critically ill patients, which has led to increased implementation in intensive care medicine over the last decade [1]. While venoarterial ECMO (V-A ECMO) is used in patients suffering from cardiac failure with and without severe respiratory dysfunction, venovenous ECMO (V-V ECMO) is indicated in patients suffering from severe respiratory failure with preserved cardiac function [2].

In contrast to complete cardiopulmonary bypass (CPB) perfusion with cardioplegic cardiac arrest, during ECMO therapy the heart continues beating [3]. Knowledge of the altered hemodynamic effects of ECMO compared to CPB is crucial for the management of extracorporeal cardiac life support. Particularly, the type and location of arterial
therapy, a decrease of pCO2 might lead to apnea resulting with jugulo-femoral access. cal ventilation affect pCO2 values. During awake ECMO therapy has been developed and offers the opportunity to reduce the incidence of VILI in patients suffering from severe respiratory failure [10–12].

Antegrade perfusion is not chosen on a regular basis because it involves open-chest cannulation of aorta, subclavian, or carotid arteries leaving the patient at higher risk of infection, ischemic events, and bleeding [2]. Even though femoral cannulation offers a preferable risk profile, the competition between the retrograde ECMO-perfusion of the aorta and the body’s own cardiac output (CO) leads to the distinct risk of the development of a watershed within the aorta branch [5]. This mechanism is known as the harlequin phenomenon and potentially results in a critical perfusion/oxygenation mismatch of the upper body. Radiologic studies using computed tomography were able to visualize the watershed within the aortic arch [5–7]. Typically, this phenomenon can be observed during pulmonary dysfunction with recovered left ventricular function [6]. This is of particular importance because patients in need of V-A ECMO therapy often also suffer from respiratory complications which is addressed with ultraprotective ventilation strategies [8, 9]. Otherwise, positive pressure ventilation implies the risk of ventilator-induced lung injury (VILI). Particularly, high driving pressures and cyclic re- and decruitment result in severe lung injury. Therefore, the concept of awake ECMO therapy has been developed and offers the opportunity to reduce the incidence of VILI in patients suffering from severe respiratory failure [10–12].

Recent studies showed the beneficial effects of awake V-A ECMO therapy but especially these patients are at an increased risk for the development of ventilator-associated pneumonia [13, 14]. As a result, their pulmonary function might be impaired which is of high clinical importance because arterial femoral cannulation can lead to a harlequin phenomenon in spontaneously breathing ECMO patients. While the respiratory rate is centrally regulated by the arterial partial pressure of carbon dioxide (pCO2), both ECMO and mechanical ventilation affect pCO2 values. During awake ECMO therapy, a decrease of pCO2 might lead to apnea resulting in a severe deoxygenation with consecutive ischemia of the coronary and cerebral tissue caused by the harlequin phenomenon. For this reason, the aim of our study was to examine the impact of the inspiratory oxygen fraction (FiO2) on cardiac function during V-A ECMO therapy with jugolo-femoral access.

Methods

Animals

All procedures involving animals were conducted in compliance with standards for animal care and the ARRIVE guidelines, and were approved by the responsible single local committee for animal care (animal welfare commission of the department of veterinary medicine at the regional council Giessen—Gl 20/26 G45/2018; Regierungspräsidium Giessen, Germany).

Twenty-two male Lewis rats (350–400 g) obtained from Janvier Labs (Le Genest St. Isle, France) were housed at 22 °C, 55% relative humidity, and a day/night cycle of 14/10 h, with access to standard chow and water ad libitum. The rats were randomly divided into three groups. The FiO2 was adjusted at 0.5 (group A, n = 6), 0.21 (group B, n = 6), or 0 in order to simulate apnea (group C; n = 6).

Since the measurements of the pressure volume (PV) catheter are influenced by the ventilation, apnea would have affected the results of the PV catheter. Therefore, anoxic ventilation with 100% of nitrogen was performed as a surrogate for simulated apnea.

Four animals had to be euthanized before the end of the study due to perforation of the draining cannula into the chest cavity during the process of cannulation. These animals were excluded from the study.

Induction and maintenance of anesthesia

After inhalative induction of anesthesia (5% isoflurane (Baxter, Unterschleißheim, Germany) balanced with 100% oxygen) the rats were intubated endotracheally (16 G cannula, B. Braun, Melsungen, Germany) and ventilated volume-controlled (Harvard Inspira, Harvard Apparatus, Cambridge, UK) in a weight-adjusted manner (tidal volume (ml) = mass (kg)0.71 × 0.0062; respiratory rate (/minute) = mass (kg)−0.26 × 53.5). The animals were placed on an automated heating pad and a rectal temperature probe was inserted. Monitoring included end-tidal carbon dioxide (MicroCapStar, CWE, Ardmore, PA, USA), continuous electrocardiogram, heart rate (HR), CO, stroke volume (SV), left ventricular end diastolic volume (LVEDV) and pressure (LVEDP), left ventricular ejection fraction (LVEF), and arterial blood pressure (systolic, diastolic and mean). After the percutaneous cannulation of the lateral tail vein (24 G cannula, B. Braun, Melsungen, Germany) a continuous balanced crystallloid infusion at a rate of 5 ml/kg/h (Sterofundin B. Braun, Melsungen, Germany) with fentanyl (10 µg/kg/h, Albrecht GmbH, Aulendorf, Germany) was started.

Additionally, midazolam (2 mg/kg/h, Roche, Basel, Switzerland) and pancuronium (0.1 mg/kg/h, Inresa, Freiburg, Germany) were administered for the maintenance of anesthesia.
Extracorporeal membrane oxygenation

The vascular accesses were placed surgically and consisted of the tail artery cannula for measurement of the arterial blood pressure and intermittent blood gas analysis (24 G, B. Braun, Melsungen, Germany), the right femoral artery cannula for ECMO return (22 G catheter, Terumo, Eschborn, Germany), and the right jugular vein cannula for ECMO drainage (modified multi-orifice 17 G cannula, B. Braun, Melsungen, Germany) [15]. Further, for the continuous measurement of left ventricular pressure and volume, a 2 F PV catheter (SPR-838, Millar, Houston, TX, USA) was placed into the left ventricle through the right carotid artery. Prior to cannulation of the right jugular vein, the animals received a heparin bolus (400 IU/kg, Merckle GmbH, Blaubeuren, Germany) through the lateral tail vein.

As previously described, the ECMO circuit consisted of a venous reservoir (M. Humbs, Valley, Germany), a roller-pump (Verderflex Vantage 3000, Castleford, UK), and a membrane oxygenator (Micro-1, Keweii Rising Medical, Shenzhen, China) [15]. The whole circuit was primed with 250 international units of Heparin (Ratiopharm, Ulm, Germany) and 9 ml hydroxyethyl starch 6% (Volufen, Fresenius Kabi, Bad Homburg, Germany). At first, the blood flow was initiated at a rate of 45 ml/kg/minute and then continuously increased to 90 ml/kg/minute. Subsequently, the FIO₂ was adjusted to 0.5, 0.21 or 0, accordingly. Sweep gas flow on the membrane was regulated between 20 and 30 ml/minute to adjust the pCO₂ levels between 35 and 45 mmHg. The oxygen fraction on the ECMO membrane was set to 1.0. Further, the animals received no vasopressor support. Moreover, the volume of blood sampling was replaced with hydroxyethyl starch 6%.

Timepoints of hemodynamic measurements

Baseline values were captured before commencing the ECMO after implementation of all cannulas and catheters at baseline (t₀). While a new timepoint was defined every 7.5 min, arterial blood pressure and invasive hemodynamic parameters were recorded up to 120 min (t₀–t₁₆). Depending on the procedural injury, the animals in groups A and B were observed for two hours, whereas the observation period of group C was one hour due to animal welfare.

Euthanasia

After the observation period all animals received a bolus of 5 µg fentanyl, 0.8 mg midazolam and 0.04 mg pancuronium. Further, isoflurane was adjusted to 5%. After 5 min, the animals were euthanized by collection of the whole blood through the draining ECMO cannula and removal of the heart and lungs.

Blood analyses

Blood samples were collected at baseline (t₀), and every 30 min after commencing the ECMO till t₁₀. At each observation point the oxygen partial pressure (pO₂), pCO₂, hemoglobin, hematocrit, pH, bicarbonate, base excess (BE), lactate, glucose, sodium, potassium, calcium, and chloride were measured (ABL800, Radiometer, Copenhagen, Denmark). In addition, blood samples were centrifuged at 3000 g for 5 min and the plasma samples were stored at −80 °C for further analysis.

Lactate dehydrogenase (LDH) measurements were performed using a commercial enzyme-linked immune sorbent assay (ELISA) according to the manufacturer’s instructions (ab102526, Abcam, Cambridge, UK). The probes were unfrozen only once.

Statistics

The sample size calculation was performed using R statistical software version 3.6.2 (http://www.r-project.org). A group size of ten animals revealed a measurable difference of a twofold standard deviation regarding the LVEF (alpha and beta error of 0.05 and 0.2, respectively). According to animal welfare regulations, intermittent statistical analyses were performed. Following the regulations of the animal welfare board, the experiments were stopped after six animals of each study group because statistical significance of the results had already been reached. All data are expressed as median with 25th and 75th percentile. Homogeneity of variance was verified with the Levene test. If the test of homogeneity was positive, the ANOVA and post hoc Tukey test were used for comparison of intergroup differences at identical time points, while the Welch and post hoc Games-Howell test were applied during inhomogeneity of variance. Due to the fact that group C ended after t₁₀, groups A and B were compared with Wilcoxon–Mann–Whitney test between t₈ and t₁₀. The analysis of variations in LDH levels across different time points within the same group was calculated by the Friedmann test. All statistical analyses were performed using SPSS Version 20 (IBM, Stuttgart, Germany). GraphPad Prism Version 7 was used for data presentation (GraphPad Software, San Diego, CA, USA).

Results

Hemodynamic measurements

Compared to groups A (FIO₂ 0.5) and B (FIO₂ 0.21) the systolic, diastolic, and mean arterial pressure were significantly decreased during anoxic ventilation (group C). Contrary, no differences were found between group A and B after commencing the ECMO (Fig. 1). Furthermore, measurements of the HR did not reveal significant differences between groups. While LVEF, SV and CO were significantly impaired during anoxic ventilation.
Fig. 1 Invasive blood pressure during V-A ECMO. Animals were ventilated with inspiratory oxygen fraction of 0.5 (group A), 0.21 (group B) or 0 (group C). Values were recorded every 7.5 min until the end of the experiment. The systolic (A), mean (B) and diastolic (C) arterial pressure were significantly reduced during anoxic ventilation. Results are presented as median with interquartile range. Asterisks, rhombus and crosses display the degree of statistical significance: A vs. C: **p < 0.01; ***p < 0.001; B vs. C: #p < 0.05; ###p < 0.001; A vs B: ++p < 0.01. Abbreviations: SAP: systolic arterial pressure; MAP: mean arterial pressure; DAP: diastolic arterial pressure; FiO2: inspiratory oxygen fraction.
(group C), groups A and B did not show alterations of LVEF, SV and CO (Fig. 2). However, LVEDP was significantly reduced in group B at t5 and t15. Additionally, no differences in LVEDV were found between all groups.

**LDH**

The measured concentration of LDH was rising continuously within groups B and C during the observation period (A: \(p = 0.199\); B: \(p < 0.001\); C: \(p = 0.007\); Fig. 3A). Further, no significant differences were found between the three groups.

**Blood gas analyses**

Significantly lower \(pO_2\) and elevated \(pCO_2\) values were measured during pulmonary hypoxia (group C) at t4 and t8 compared to groups A and B (Table 1). Furthermore, potassium, and calcium were significantly increased in group C at t4 and t8 (Additional file 1: Table S1), while pH, bicarbonate, and BE were significantly reduced in group C at t4 and t8 (Table 1). Glucose and lactate were significantly elevated, whereas the central venous saturation \((SvO_2)\) was significantly reduced in group C during t4 and t8 (Table 1, Fig. 3B).

**Discussion**

This study aimed to investigate the impact of pulmonary hypoxia during anoxic ventilation on the LVEF during V-A ECMO with jugulo-femoral cannulation. Anoxic ventilation (group C) resulted in severe hemodynamic alterations which was defined by a significant reduction of systolic, diastolic, and mean arterial pressure. Furthermore, a significant impairment of cardiac function determined by a reduction of LVEF and CO was observed during pulmonary hypoxia, which was mainly induced by a decrease of the SV but not the HR indicating cardiac failure.

Even though these results seem unsurprisingly, they are the first to describe systemically the relevance of apnea on the cardiac function during ECMO-therapy with femoral cannulation because commonly rat models of CPB are performed under asphyctic cardiac or deep hypothermic circulatory arrest \([16–19]\). Furthermore, the majority of studies did not measure LVEF or CO. Even though Ali et al. used a PV catheter in a rat model of V-A ECMO with femoral cannulation, their methodology was significantly different. Due to different study aims, the animals primarily underwent 15 min of hypoxic cardiac arrest before eventually commencing the ECMO and ventilation \([20]\). Fujii et al. recently published another rodent model of V-A ECMO; however, since arterial cannulation was performed through the carotid artery, the circulatory impact of the V-A ECMO differed from our model with femoral cannulation \([21]\). Even though Kato et al. demonstrated that the coronary blood flow is inversely proportional to the ECMO flow in a V-A ECMO model with carotid access, this is not necessarily applicable to femoral access with retrograde aortic perfusion \([22]\).

Cardiogenic shock during anoxic ventilation was reflected by the severely impaired \(SvO_2\) and increased lactate level. Next, loss of BE, decreased bicarbonate, and impaired pH demonstrated that the acid–base metabolism was not able to compensate for the lactate induced acidosis. These results are in accordance with the findings of Engels et al. who investigated rats under circulatory arrest. Despite the deep hypothermic circulatory arrest, bicarbonate levels of 13.8 mmol/l and a BE of -12.5 were measured during rewarming \([19]\). In addition, the elevated ionized calcium during pulmonary hypoxia is most likely caused by the acidosis. Since metabolic acidosis leads to insulin resistance, this might also explain the elevated glucose levels during pulmonary hypoxia \([23]\).

Lower \(pO_2\) and elevated \(pCO_2\) are also explainable with the anoxic ventilation within study group C. However, Koning et al. were able to adjust the \(pO_2\) between 150 and 250 mmHg within a rat model of CPB with femoral cannulation and apnea before the beginning of CPB \([17]\). These studies included blood flow rates of 150–200 ml/kg/minute which were higher than rates of 90 ml/kg/minute during our experiments. It must be highlighted that our approach focused on compensation of an anoxic ventilation during V-A ECMO with femoral cannulation and continuous CO by a \(FiO_2\) of 1.0 during ECMO therapy.

Due to the fact that the ventilator driving pressure during ECMO therapy is associated with increased inhospital mortality, ultraprotective lung ventilation under ECMO-supported oxygenation is widely adopted across ECMO centers \([9, 24, 25]\). Since V-V ECMO therapy displays the commonly used treatment option during severe respiratory failure, most studies concentrated on V-V ECMO rather than on V-A ECMO therapy. However, contrary to V-A ECMO with femoral cannulation, V-V ECMO leads to sufficient lung perfusion with oxygenated blood and, therefore, neither cardiac nor cerebral ischemia is to be expected. Since mechanical ventilation is known to induce lung injury by barotrauma and volutrauma, current approaches focus on awake ECMO without mechanical ventilation \([11, 12]\). For this reason, awake V-A ECMO therapy with cardiocirculatory indication offers a promising therapeutic option but also leaves the patients at risk for apnea \([26–29]\). Following this, our results indicate that these patients might be vulnerable to cardiac ischemia during apnea. Caused by the harlequin phenomenon these adverse effects were independent of the 100% oxygen insufflation of the ECMO. Following this, the oxygen content of the coronary arteries seems...
Fig. 2  Invasive hemodynamic parameters during V-A ECMO. Animals were ventilated with inspiratory oxygen fraction of 0.5 (group A), 0.21 (group B) or 0 (group C). Values were recorded every 7.5 min until the end of the experiment. The cardiac output (A), stroke volume (B) and ejection fraction (C) were significantly reduced during anoxic ventilation. Results are presented as median with interquartile range. Asterisks, rhombus and crosses display the degree of statistical significance: A vs. C: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; B vs. C: $^#p < 0.05$; $^{##}p < 0.01$; $^{###}p < 0.001$; A vs B: $^+p < 0.05$. Abbreviations: $\text{FiO}_2$: inspiratory oxygen fraction
to depend highly on the ventilatory FiO2. Therefore, the impaired cardiac function during anoxic ventilation appears to be caused by the low oxygen saturation of the coronary blood. However, to the knowledge of the authors, it is technically not possible to measure the coronary blood flow in rats. Further, it offers the risk of exsanguination due to additional aortic cannulation and probe placement. Last, the measurement of both—coronary blood flow and blood saturation—would have required an open chest cavity, which affects the measurements of the pressure volume catheter. We decided against increasing the ECMO flow because an enhanced ECMO flow might raise the already reduced mean arterial pressure and, thus, aggravate the shock signs by raising the left ventricular afterload. This is shown by König et al., even though they used no PV catheter in their model. In the description of their experiments, the blood pressure of the tail artery showed an almost flat line during CPB indicating low CO [17]. Contrarily, we were always able to detect a pulsatile arterial wave in the tail artery during V-A ECMO therapy. Furthermore, the LVEDV did not differ between the experimental groups, implying that the ECMO-induced watershed has not increased the afterload or moved towards the heart. Moreover, experiments with an in-vitro mock circulation loop demonstrated that an increased ECMO flow was unable to shift the watershed towards the aortic arch [30]. In summary, this study underlines the importance to avoid apnea during awake V-A ECMO with femoral cannulation in order to lower the risk for pulmonary and coronary hypoxia. For the same reason, it is crucial to monitor the respiratory rate and to adjust the sweep gas flow on the V-A ECMO restrictive to avoid hypocapnia.

Our experiments still have some limitations. First, positive intrathoracic pressures induced by the mechanical ventilation are lacking during awake V-A ECMO with spontaneous breathing. However, it is very difficult to perform good quality left ventricular PV measurements during spontaneous breathing. Furthermore, passive oxygenation with ambient air can occur during apnea. Since no passive oxygenation is possible during ventilation with a FiO2 of 0, our approach is different from apnea. Compared to apnea without ventilation, we opted for continuous ventilation without oxygen to maintain the ventilation-induced positive intrathoracic pressure. While the reduced intrathoracic pressure during spontaneous breathing could enhance the perfusion of the lungs and thus affect the left ventricular PV measurements, our experimental groups would not have been comparable. Therefore, we also opted for ventilation during V-A ECMO with neuromuscular blocking to avoid spontaneous breathing. Moreover, due to the limited diameter of the venous cannula, only blood flows of 90 ml/kg/minute were achieved. Third, this study was not able to proof cardiac ischemia in terms of increasing LDH blood levels only during anoxic ventilation. Lacking of data on the harlequin effect during V-A ECMO therapy in the rat, we designed this study as a pilot study and performed the sample size calculation on the primary endpoint “LVEF”. To reduce unnecessary harm to the animals, we stopped the inclusion of further rodents after the interim analysis according to the regulations of the local department of animal welfare (see statistical analysis). As a result, only a limited number of animals were available for the analyses of secondary endpoints such as the measurement of LDH. For this reason, the study is not able to clarify if a higher number of animals would have revealed significant alterations of the plasma LDH. Furthermore, LDH was rising continuously in all study groups indicating the increasing

![Fig. 3 Time course of LDH and Central venous saturation during V-A ECMO. Animals were ventilated with inspiratory oxygen fraction of 0.5 (group A), 0.21 (group B) or 0 (group C). Blood was withdrawn for analysis at baseline and every 30 min after commencing the V-A ECMO. While the LDH (A) was continuously rising in all group, no differences were analyzed between the groups. Anoxic ventilation was associated with a significant decrease of the central venous saturation. Results are presented as median with interquartile range. Asterisks and rhombus display the degree of statistical significance: A vs. C: ***p < 0.001; B vs. C: ###p < 0.001. Abbreviations: FiO2: inspiratory oxygen fraction; LDH: lactate dehydrogenase](image-url)
ECMO-induced cell damage. Clements et al. analyzed the time course of LDH and Troponin I levels after isoproterenol-induced myocardial injury in the rat and revealed serum peak levels of LDH after four and troponin I after three hours, respectively. Following this, the LDH was not significantly different in our experiments due to the short distance between myocardial damage and measurement of LDH. Therefore, we opted against measurement of troponin I. However, histological examination of cardiomyocytes may be useful to clarify this issue in further studies. Moreover, potassium was elevated during pulmonary hypoxia, which might be a surrogate of myocardial damage. Nevertheless, the lactate-induced acidosis could also be causative. Since the function of the lungs could be impaired during V-A ECMO therapy, histological examinations of the lungs could bring more insights into this topic in following experiments. Further, the impact of FiO2 on the brain during V-A ECMO therapy could be investigated by histological investigations in the future. Last, results from an animal model cannot be directly transferred to humans.

**Conclusion**

In summary, the impact of FiO2 on cardiac function during V-A ECMO with femoral cannulation was investigated. Pulmonary hypoxia led to hypotension and severe cardiac dysfunction determined by a lactate-induced acidosis and severely impaired ScvO2. For this reason, apnea induced pulmonary hypoxia might endanger patients for cardiac ischemia during V-A ECMO with femoral cannulation.

**Abbreviations**

BE: Base excess; CO: Cardiac output; CPB: Cardiopulmonary bypass; ECMO: Extracorporeal membrane oxygenation; ELISA: Enzyme-linked immune sorbent assay; FiO2: Inspiratory oxygen fraction; HR: Heartrate; LDH: Lactate dehydrogenase; LVEDP: Left ventricular end diastolic pressure; LVEDV: Left ventricular end diastolic volume; LVEF: Left ventricular ejection fraction; pCO2: Carbon-dioxide partial pressure; pO2: Oxygen partial pressure; PV: Pressure
Volume, $S_O_2$: Central venous saturation; $SV$: Stroke volume; $V_A$: Venoarterial; VILI: Ventilator-induced lung injury.

**Supplementary Information**

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**Additional file 1. Supplementary Table S1:** Results of the blood gas analysis.

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**Author contributions**

FE, ES, MS and CK designed the study and wrote the manuscript. FE and CK performed the experiments. JS designed the study and contributed scientific advice. CS, MS, GS and ES analyzed the blood samples, collected the data and conducted statistical analysis. MS supervised this work and critically revised the manuscript. All authors have read and approved the final manuscript.

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**Availability of data and materials**

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

**Declarations**

**Ethics approval and consent to participate**

All procedures involving animals were conducted in compliance with standards for animal care and the ARRIVE guidelines, and were approved by the responsible single local committee for animal care (animal welfare commission of the department of veterinary medicine at the regional council Gießen—Gl 20/26 G45/2018; Regierungspräsidium Gießen, Germany).

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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