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Feasibility and Stability of a Long Term “Awake” Extracorporeal Membrane Oxygenation Model in Large Animal

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Research Article

Keywords: extracorporeal membrane oxygenation, long-term support, awake animal model

Posted Date: May 11th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-475008/v1

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Abstract

Background: Extracorporeal membrane oxygenation (ECMO) is rapidly becoming a mainstream technology for lung or heart/lung support especially during the COVID-19 pandemic. “Awake ECMO”is frequently used to indicate an alternative approach of using ECMO without invasive mechanical ventilation, and it has the unique advantages of application. In this study, we explored the feasibility and stability of the establishment method and management strategy of long-term awake ECMO model in healthy sheep. As the sheep are healthy, according to the histopathological analysis , we explored the effect of ECMO circuit itself on organs and tissues. As a preclinical study in large animals, our study aims to provide clues for further research on application expansion, management strategy optimization, pathophysiology exploration, equipment development and subsequent establishment of the disease animal model.

Methods: Ten healthy sheep were treated with awake veno-arterial (V-A) or veno-venous (V-V) ECMO for 7 days. They were transferred into the monitoring cages after operation and were ambulatory after anesthesia recovery. ECMO configurations, hemodynamic and hematologic parameters were measured every day. Necropsy was conducted at the endpoint of the experiment to visualize the cannula position in vivo and to examine cannulation related injury and thrombus formation in blood vessels and major organs. Main organs and blood vessels were harvested for pathological investigation.

Results: All sheep survived to the end of the experiment (the 7th day). In the whole process of the experiment, the vital signs of which were stable, and no serious bleeding and coagulation events occurred. Hemoglobin concentration and platelet count were in normal reference range, plasma free hemoglobin concentration was maintained at a low level. ECMO flow was stable, and oxygenation performance of oxygenator was satisfied. There was no major adverse pathological injury occurred.

Conclusions: Long term awake extracorporeal membrane oxygenation model in large animal is feasible and stable. Perioperative management is the key to the success of this model. As a basic research, it can also provide an alternative strategy for mechanical circulatory support in patients with awake ECMO indications.

Introduction

Extracorporeal membrane oxygenation (ECMO), also called extracorporeal life support (ECLS), is rapidly becoming a mainstream technology especially during the COVID-19 pandemic[1]. ECMO can be cannulated via veno-venous (V-V) or veno-arterial (V-A). The former can support respiratory function, while the latter can support both respiratory and circulatory function [2,3]. As a long-term mechanical circulatory support, ECMO provides a bridge to recovery of the natural organs or transplantation.

“Awake ECMO”is frequently used to indicate an alternative approach of using ECMO without invasive mechanical ventilation (IMV)[4]. The awake ECMO aims at avoiding the unfavorable complication related to intubation and sedation, involving muscle deconditioning, neuromuscular complications, hospital-acquired infections, and even poor post-transplant outcomes [5]. Early mobilization of critically ill patients is increasingly being recognized as not only safe, but also as a potential means of optimizing outcomes in the intensive care unit (ICU) [6]. With the rapidly expanding use of extracorporeal life support (ECLS) for severe cardiopulmonary failure, there is a growing interest in the application of awake ECMO and early mobilization.

In previous studies, we noticed that there were few articles about long-term ECMO support in awake large animals [7-9], and they were all single ECMO mode (V-V or V-A ECMO). The number of experimental animals was limited, and the purpose and method of the experiment were focused on the description and verification of the equipment. There was a lack of standardization and optimization establishment method in long-term ECMO support model and perioperative management in awake large animals, and there was still a lack of research on the effect of long-term ECMO support on tissues and organs.

The purpose of this study is to explore the feasibility and stability of the establishment method and management strategy of long-term awake ECMO model in sheep. As the experimental sheep are healthy, according to the histopathological analysis after the endpoint, we are able to explore the effect of ECMO circuit itself on organs and tissues. As a preclinical study in large animals, our study can provide clues for further research on application expansion, management strategy optimization, pathophysiology exploration, equipment development and subsequent establishment of the disease animal model.

Materials And Methods

Animals and preparation

This experimental protocol was approved by the Ethics Committee for animal experimentation of Fuwai Hospital [0101-2-20-HX(X)], and all experimental procedures were in accordance with the guide for the care and use of laboratory animals published by the National Institute of

health (National Institutes of Health Publication No. 86-23, revised 1996). This animal experiment was completed at Beijing Key Laboratory of Pre-clinical Research and Evaluation for Cardiovascular Implant Materials (Animal Experimental Center of Fuwai Hospital). The experimental animals were all healthy sheep with qualified quarantine provided by the animal experimental center of Fuwai Hospital. During the whole process of the experiment, we strictly followed the ARRIVE guidelines for pre-clinical animal studies.

Ten healthy sheep survived for 7 days after ECMO implantation were included in the study. Two sheep died of pulmonary infection (caused by reflux aspiration) and hemorrhagic shock (bleeding around catheterization which used for hemodynamic monitoring) within 24-48 hours after ECMO implantation were excluded. 10 healthy adult male sheep (Small Tailed Han sheep, weight 54-63kg)were randomly divided into 2 groups: 5 supported by V-A ECMO and 5 supported by V-V ECMO (Table 1). The animals received 24-hour cage-side care with a veterinarian monitoring in adherence to the animal management protocol. Experimental sheep were routinely fasted for 48 hours and were given no water for 12 hours before the surgery. The experiment prepared blood donor sheep with preoperative cross matching, and the need for blood transfusion was decided according to intraoperative and postoperative blood loss (a maximum of 30% blood volume was drawn for each donor sheep).

Table 1 Detailed characteristics of ECMO support

Sheep number	Gender	Weight(kg)	Survival days	ECMO mode	Cannulation
S2020-013	M	56	7	V-V	right jugular vein (23Fr DLC)
S2020-014	M	58	7	V-A	Vjr(24Fr)-Ajr(18Fr)
S2020-017	M	60	7	V-V	right jugular vein (23Fr DLC)
S2020-036	M	63	7	V-V	right jugular vein (23Fr DLC)
S2020-037	M	59	7	V-V	right jugular vein (23Fr DLC)
S2020-035	M	60	7	V-V	right jugular vein (23Fr DLC)
S2020-040	M	54	7	V-A	Vjr(24Fr)-Ajr(18Fr)
S2020-041	M	55	7	V-A	Vjr(24Fr)-Ajr(18Fr)
S2020-043	M	56	7	V-A	Vjr(24Fr)-Ajr(18Fr)
S2020-038	M	57	7	V-A	Vjr(24Fr)-Ajr(18Fr)

M: male; DLC: dual lumen cannula; Vjr: right jugular vein; Ajr: right jugular artery.

Device

V-A ECMO circuit consisted of a arterial perfusion cannula (18Fr, OPTI18, Edwards Lifesciences LLC, USA), a venous cannula (24Fr, VFEM024, Edwards Lifesciences LLC, USA), a centrifugal pump drive and console (), a centrifugal pump (Hilite7000LT, V-V ECMO circuit consisted of a dual lumen cannula (23Fr,), a centrifugal pump drive and console (), a centrifugal pump (Hilite7000LT,

2%-3%8-10 mg/kg/h

V-A ECMO, the right jugular artery and right jugular vein were exposed. A 24-Fr outflow cannula was inserted from the right jugular vein to the right atrium (if necessary, ultrasound could help to confirm the position) after the activated clotting time (ACT) > 200s, and an 18-Fr inflow cannula was inserted into the right jugular artery (the depth of catheterization was about 10-15cm). For V-V ECMO, the right jugular vein was exposed. After ACT > 200s, a DLC (23-Fr) was inserted into the superior vena cava (SVC), traversing the right atrium (RA), with the tip positioned in the inferior vena cava (IVC). Correct positioning of the DLC was assured by intraoperative ultrasound and anatomic data from previous sheep necropsies.

The centrifugal pump and a membrane oxygenator were connected and primed. The outflow cannula and the DLC drainage outlet was connected to the pump inlet, while the inflow cannula and DLC infusion lumen outlet was connected to the oxygenator outlet. Pay attention to aseptic manipulation during connection and avoid air embolism. Turn on the pump, and O_2 sweep gas was connected to the oxygenator. Extracorporeal circulation was started with pump flow of 1.8–2.5 L/min. The neck incision was sutured consecutively, the cannula was fixed securely, and the line was half looped around the neck, avoiding displacement and kinking (Figure 1A).

After the operation was completed and vital signs of the experimental sheep were stable, the sheep were moved into a metabolic cage and properly restrained. In this process, special attention should be paid to the fixation of head and shoulder of the sheep to prevent the cannula from dislocation or kinking. Gradually reduced the depth of anesthesia, extubated the endotracheal tube when the sheep recovered to spontaneous respiration and blood gas analysis was stable.

Post-surgical care, monitoring, and data collection

Extracorporeal circulation was maintained with target pump flow of 2.0 L/min [30 ml/(kg·min)] and pump speed around 3500rpm during the experimental period. Oxygen flow to the oxygenator was 1.0-1.5 L/min at a concentration of 50–80%, the dynamic adjustment was made according to the blood gas analysis, venous oxygen saturation (SvO_2) and arterial oxygen saturation (SaO_2). Heparin was infused continuously to maintain ACT in the range of 220 to 250 seconds. In the first 24 hours after surgery, flurbiprofen axetil (1-2mg/kg) and dexmedetomidine (0.2-0.3ug/kg•h) were administered intravenously.

After the surgery, the sheep stayed awake and could eat and move in the monitoring cage (Figure 1B and Figure 1C). Intravenous infusion was adjusted according to their intake, urine volume, blood pressure and mental state. After operation, antibiotics were used to prevent incision infection every day (cefuroxime sodium 1.5g, iv, bid); the wound was disinfected daily, the infection and bleeding of the wound were also observed at the same time.

The basic vital signs of experimental sheep and ECMO parameters were monitored in real time. The blood gas analysis and ACT were tested every 6 hours. The complete blood count, blood chemistry and coagulation action test were monitored every day. After 168 hours (7 days), the ECMO system was weaned and the sheep were euthanized. Necropsy was conducted by two pathologists to visualize the cannula position in vivo and to examine cannulation related injury and thrombus formation in blood vessels and major organs. Heart, lung, kidney, liver, brain, intestine and blood vessels related to intubation were harvested for further histopathological evaluation.

Histological analysis

The obtained specimen was cut into small pieces, then fixed with 10% neutral fumarin fix solution (Yili Fine Chemical Co., Ltd, China). Then the fixed tissues were embedded in paraffin. Hematoxylin & eosin (H&E, Hematoxylin: Yili Fine Chemical Co., Ltd, China; eosin: Sigma-Aldrich Trading Co., Ltd, China) staining were performed on samples of 5 μm thickness. Histological analysis was performed under the light microscopy. Histological analysis were evaluated by two excellent pathologists.

Data analysis

Data were analyzed using SPSS software (version 26.0, IBM Corp, Chicago, IL, USA) and GraphPad Prism 8 (version 8.4.0, San Diego, California, USA). Continuous variables were presented as means ± standard deviation (SD), and significant difference at various time points was determined with 1-way analysis of variance with multiple comparisons. Post Hoc LSD test was used to compare changes in pre-surgical baselines and post-surgical hematology data. Values of $p < 0.05$ were considered statistically significant.

Results

All 10 sheep survived the surgery and recovered from anesthesia, and also survived without any major adverse events during the planned experimental period. In the whole process of the experiment, heart rate fluctuated slightly (p□0.001, n=5 for each group, Figure 2A), mean arterial pressure (MAP) was stable (p□0.05, n=5 for each group, Figure 2B). All experimental sheep survived 7 days after operation and successfully weaned from ECMO.

As the trend of changes in hematology and coagulant data were consistent in both groups, 10 sheep were summarized in Table 2 together. The hemoglobin (Hb) levels decreased after surgery, but still exceeded 96g/L throughout the experiment. No blood transfusion was needed. Compared with a pre-surgery baseline level of 120±24g/L, hemoglobin decreased immediately after surgery but remained at stable and satisfactory levels. White blood cell (WBC) counts increased at 24h, 48h, 144h and 168h after surgery but high sensitivity C-reactive protein (hsCRP) was still within normal ranges [10].Platelet (PLT) counts were within the physiologic range throughout the experiment [10], although

they were slightly decreased after surgery (p□0.05). The plasma free hemoglobin (fHb) of all experimental sheep kept at a low level after surgery (p□0.05). ACT was maintained at the target level by dynamically adjusting the dosage of heparin.

Table 2□Hematology and anticoagulant activation data

Variable*	Pre-surgical	Hours after surgery							
		6h	24h	48h	72h	96h	120h	144h	168h
Hb (g/L)	120±24	101±18[a]	108±12	102±10[a]	98±11[a]	105±12[a]	100±10[a]	100±8[a]	96±12[a]
WBC ($\times10^3$/L)	6.58±0.90	5.95±3.34	11.64±5.29[a]	10.59±2.44[a]	8.18±1.66	7.28±2.26	8.44±1.95	9.79±2.66[a]	12.01±5.19[a]
hsCRP (mg/L)	0.38±0.32	0.20±0.16	0.21±0.12	0.18±0.18	0.21±0.13	0.21±0.12	0.18±0.13	0.21±0.16	0.21±0.19
PLT ($\times10^9$/L)	227±107	204±72	176±68	150±51	157±50	187±93	256±162	304±159	347±160[a]
fHb (g/L)	0.5±0.4	0.1±0.1	0.1±0.0	0.1±0.0	0.1±0.1	0.1±0.0	0.2±0.0	0.2±0.1	0.1±0.1
ACT(s)	187±45	250±44	251±29	238±41	221±25	232±27	239±24	253±25	245±44

Hb: hemoglobin; WBC: white blood cells; hsCRP: high sensitivity C-reactive protein; fHb: plasma free hemoglobin; ACT: activated clotting time.

*: Data were shown as mean±standard deviation

[a] : p□0.05 (comparing with pre-surgical baseline)

During the experiment, the flow rate of ECMO was stable (p□0.05, n=5 for each group, Figure 3A), and the oxygenation performance of the oxygenator was good (p□0.05, n=5 for each group, Figure 3B, 3C). According to the necropsy , the position of the cannula was correct and there were no subcutaneous hematoma or other bleeding signs. Thrombosis around the cannulation in the V-V group were more than that in V-A group, but there was no vascular occlusion or stenosis. According to the histopathological evaluation, organ infarction rate was low (2/10 cases), and the infarct focal size was small (□5% surface area, with no obvious clinical effect). Focal lymphocytic myocarditis near epicardium was observed in one sheep under light microscope, but the size of the lesion was small (□5% surface area), and there was no obvious clinical effect (Table 3 and Figure 4).

Table 3: Main pathological findings of experimental sheep.

Serial number	Sheep number	Experiment duration	Cannulation position	Vascular injury around cannulation	Cardiac findings	Pulmonary changes	Other changes
V-A ECMO mode							
1	S2020-014	7days	Correct	None	Myocarditis	None	None
2	S2020-038	7days	Correct	Thrombosis	None	None	Small focal renal infarction
3	S2020-040	7days	Correct	None	None	None	Hepatic cyst
4	S2020-041	7days	Correct	Thrombosis	Small calcification	None	None
5	S2020-043	7days	Correct	Thrombosis	None	None	None
V-V ECMO mode							
1	S2020-013	7days	Correct	Thrombosis	None	None	None
2	S2020-017	7days	Correct	Thrombosis	None	None	None
3	S2020-035	7days	Correct	Thrombosis	Small infarction	None	Small focal renal infarction Calcified nodule of liver
4	S2020-036	7days	Correct	None	None	None	None
5	S2020-037	7days	A little bit shallow	Thrombosis	None	None	None

Discussion

In our study, sheep were used as experimental animals. The heart structure, size and hemodynamics of sheep are similar to those of human. Using sheep as experimental animals, the experimental procedures are very close to clinical, and the experimental results have more guiding significance for clinical application. The character of sheep is docile, which is conducive to perioperative management. We established a long term “awake” extracorporeal membrane oxygenation model in both V-A and V-V ECMO mode in sheep. This system achieved long-term respiratory support or both respiratory and circulatory support. In our study, the ambulatory sheep obtained adequate nutrition from normal eating and maintained a satisfactory hemoglobin level, with no need of additional artificial nutritional support or blood transfusion.

V-V ECMO model was established cannulated by single site percutaneous DLC. The DLC withdraw total venous blood from both IVC and SVC through a drainage lumen for oxygenation, then, the oxygenated blood can be infused back to the RA. The drainage lumen openings in the SVC and IVC are spatially separated from the infusion lumen opening in the RA, which maximally reduces recirculation and enhances blood transfer efficiency [7,11,12]. V-V ECMO by DLC can provide long-term respiratory support with less invasive procedure and bleeding events while reducing the IMV-related complications.

Awake ECMO management is crucial for successful weaning and perioperative management is the key to the success of this model. Necropsy and pathological results also showed that the perioperative management strategies were effective. The anticoagulant management in the pilot study (3 sheep with V-V ECMO) referred to previous studies [7,8,13-15], ACT value was set at 180-220s. However, the fibrinogen and thrombosis formed in the oxygenator within 48 hours, which indicated that the sheep needed higher anticoagulant conditions. Meanwhile, bleeding signs were not observed, hemoglobin concentration and platelet count were relatively stable, so we adjusted the target ACT value to 220-250s in the formal experiment. Fibrinogen deposition and thrombosis formation in the oxygenator were reduced, oxygenation performances were stable, and no serious coagulation events occurred. However, it should be emphasized that the catheter should be placed cautiously during the operation, and the hemostasis and suture should be done carefully to avoid postoperative bleeding caused by the operation.

Respiratory monitoring was a major challenge in postoperative management. Physicians were blinded to both airway pressures and tidal volumes, which were main respiratory monitoring in ventilated patients. We should pay attention to avoid regurgitation and aspiration after

spontaneous breathing recovery of the sheep. In our experiment, after the spontaneous breath recovered and the tracheal tube was removed, respiratory rate and blood gas analysis were monitored every 6 hours. The sweep gas flow and FiO_2 of the oxygenator were adjusted according to the PaO_2 and $PaCO_2$. Therefore, we maintained $PaCO_2$ at 35-40mmHg and PaO_2 more than 80mmHg in the whole process of the experiment.

Fluid management during awake ECMO support was imperative. Some retrospective studies reported that fluid overload (FO) occurred commonly in patients supported with ECMO [16,17]. Progressive FO during the ECMO is associated with acute kidney injury (AKI), higher mortality, prolonged mechanical ventilation and ECMO duration. We paid close attention to the fluid balance (positive balance 800-1000ml per day) of the experimental sheep every day and maintained central venous pressure (CVP) at 5-12 cmH_2O. No diuretic was used and the urine color was clear. The creatinine levels during ECMO support (115.04±19.83μmol/L) and after weaning (105.00±22.26μmol/L) were lower than the baseline level (134.44±15.20μmol/L), indicating that the renal tissue perfusion was good and the renal function was maintained in the normal range. In the temperature management of experimental sheep during ECMO support, according to the basic temperature of sheep (rectal temperature 39 ± 0.5℃), we set the temperature of the heat exchanger at 38.5 ℃. The rectal temperature of experimental sheep was maintained at 38-39.5℃.

Compared to the perioperative management of V-V ECMO, there are some key points in the management of V-A ECMO. Because V-A ECMO involves both arterial and venous systems, partly or completely replacing cardiopulmonary function, we paid more attention to anticoagulation and infection indexes. During the perioperative anticoagulation with heparin, the change of ACT was closely monitored, the dosage of heparin was adjusted in time, and the coagulation signs were closely observed. In terms of anti-infection management, in addition to the routine incision disinfection and application of antibiotics, we also closely monitored the changes of body temperature and infection indicators (such as white blood cell count and hsCRP).

The advantage of ambulation of the experimental sheep was obvious [4,6]. First of all, because the experimental sheep can breathe and cough spontaneously, the possibility of pulmonary infection was reduced. Second, no intubation allowed independent feed to get enough nutrition, contributing to normal hemoglobin level and no need for blood transfusion. In this study, consciousness and autonomous activities of sheep increased the difficulty of ECMO management. In order to ensure the stable flow and avoid cannula dislocation or kinking in awake animal, we restricted the sheep properly (Fig. 1C shows the details). No cannula kinking or displacement events appeared.

Patients receiving ECLS support may benefit from early mobilization. For instance, early mobilization may be particularly beneficial in patients awaiting heart or lung transplantation, as maintenance of physical conditioning may be an important component of a patient's transplant candidacy. The ability to engage critically ill patients in active physical therapy and early mobilization necessarily involves minimization of sedation and is often further facilitated by a strategy that favors endotracheal extubation [6]. Informed by reports of successful use, transplant centers are rapidly embracing awake ECMO as a means to maintain patient viability and survival to bridge-to-transplant (BTT). One center reported a six-month survival rate of 80% in patients supported on awake ECMO pre-transplant, compared to 50% of patients maintained on mechanical ventilation, which indicated ECMO support in patients who are awake and nonintubated represents a promising bridging strategy [18]. Another experienced center reported survival rates of 84% at two years post-transplant for patients supported with ECMO prior to surgery, which demonstrated favorable survival in patients receiving awake ECMO as a bridge to lung transplantation [19]. As a BTT strategy, awake ECMO can afford critically ill patients freedom from mechanical ventilation and can enable daily rehabilitation, including ambulation.

Previous studies have shown that the initiation of ECMO is associated with an immediate and complex inflammatory reaction, similar to that seen in systemic inflammatory response syndrome (SIRS). At that moment when the patient's blood first comes into contact with the foreign surface of the extracorporeal circuit, a variety of coagulative and inflammatory cascades are activated. Levels of pro-inflammatory cytokines rise rapidly [20-22], which, in association with activation of the complement and contact systems, results in leukocyte activation [23]. This innate immune response, if severe, persistent or unchecked by a compensatory anti-inflammatory response (CARS), may lead to endothelial injury, disrupted microcirculation, and end organ dysfunction [24,25]. Under the condition of good perfusion of main organs and tissues, according to the histopathological evaluation of our experiment, we found that the inflammatory reaction caused by ECMO circuit itself may be at a low level, it had little effect on the function of main organs and tissues in sheep. At the same time, it also showed that our perioperative anti-infection management was relatively successful.

To our knowledge, this study is the first awake large animal model to explore the long-term survival in both V-V and V-A ECMO simultaneously. According to our pre-experiment and previous clinical experience, we made a detailed management plan in advance and adjusted it dynamically during experimental progress. We optimized the perioperative management and made pathological analysis after the endpoint, focusing on the effect of long-term awake ECMO on tissues and organs. As the experimental sheep are healthy, according to the histopathological analysis after the endpoint, we were able to explore the effect of ECMO circuit itself on organs and tissues.

The establishment and management experience of long-term awake ECMO in big animal model will lay a solid foundation and provide a stable platform for further research. The establishment of long-term ECMO disease model (such as cardiogenic shock, heart failure and acute respiratory distress syndrome model) in large animals will be closer to the actual situation of clinical patients, but at the same time, the requirements of perioperative management are also higher. In previous studies, the disease models of ECMO in large animals were acute disease models, and the survival time of large animals was less than 24 hours [13-15,26-34]. Our study can provide technical support and management strategies for the establishment of long-term awake ECMO disease model in large animals in the future. For instance, in the future clinical practice, when the optimization or change of management strategy and concept is involved, but it is uncertain whether patients will get a profit, this model can be used for preclinical verification, and specific data such as pathophysiological changes of tissues and organs can be obtained. In addition, this study can also provide a stable platform for the development and optimization of extracorporeal life support equipment in the future. In general, our study has extensive clinical translational value.

Our study has several limitations. First, this research is still in the preliminary stage of long-term awake ECMO model in big animals and the number of experimental sheep was relatively small. Second, in order to keep the blood volume and hemoglobin concentration in the normal range, except for basic hematology and coagulation data, we did not dynamically monitor the levels and changes of inflammation related indicators. Third, there were two peaks in white blood cell count during the experiment, it is necessary to take blood culture and adjust the use of antibiotics. Further research is needed to standardize the establishment and management of long-term awake ECMO model, and focus on long-term ECMO support related application expansion, management strategy optimization, pathophysiology exploration, equipment development and subsequent establishment of the disease animal model.

Conclusions

In this study, a long-term awake ECMO model of healthy sheep was successfully established. There was no serious bleeding or coagulation event within 7 days. During the experiment, hemoglobin concentration and platelet count were relatively stable, free hemoglobin was maintained at a low level, ECMO flow was stable, oxygenator oxygenation performance was good. According to the necropsy and histopathological evaluation, there was no major adverse pathological injury occurred. Long term awake extracorporeal membrane oxygenation model in large animal is feasible and stable.

Abbreviations

ECMO: extracorporeal membrane oxygenation; ECLS: extracorporeal life support; V-A ECMO: veno-arterial extracorporeal membrane oxygenation; V-V ECMO: veno-venous extracorporeal membrane oxygenation; IMV: invasive mechanical ventilation; ICU: intensive care unit; DLC: dual lumen cannula; ECG: electrocardiogram; PaO_2: partial pressure of arterial oxygen; ACT: activated clotting time; SVC: superior vena cava; RA: right atrium; IVC: inferior vena cava; SvO_2: venous oxygen saturation; SaO_2: arterial oxygen saturation; MAP: mean arterial pressure; Hb: hemoglobin; WBC: white blood cells; hsCRP: high sensitivity C-reactive protein; fHb: plasma free hemoglobin; FO: fluid overload; AKI: acute kidney injury; CVP: central venous pressure; BTT: bridge-to-transplant; SIRS: systemic inflammatory response syndrome; CARS: compensatory anti-inflammatory response.

Declarations

Acknowledgments

The authors would like to thank he authors also would like to

Authors' contributions

Conception and design: B Ji, J Qi, S Gao, G Liu, S Yan; Administrative support: B Ji; Experimental implementation and animal management: J Qi, S Gao, G Liu, S Yan, M Zhang, W Yan, Q Zhang, Y Teng, J Wang, C Zhou, Q Wang; Collection and assembly of data: J Qi, S Gao, M Zhang; Data analysis and interpretation: J Qi; Manuscript writing: All authors; Final approval of manuscript: All authors.

Funding: None.

Availability of data and materials

All data and materials generated and analyzed during our study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All experimental procedures were approved by the Ethics Committee for animal experimentation of Fuwai Hospital [approval number: 0101-2-20-HX(X)] and were performed according to the National Institutes of Health in the Guide for the Care and Use of Laboratory Animals (NIH Publications no. 86-23, revised 1996).

Consent for publication

Not applicable.

Competing interests

STMed Technologies Co. provided financial and equipment (centrifugal pump drive and console/

centrifugal pump) support for this study. The authors have no other conflict of interest to declare.

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Figures

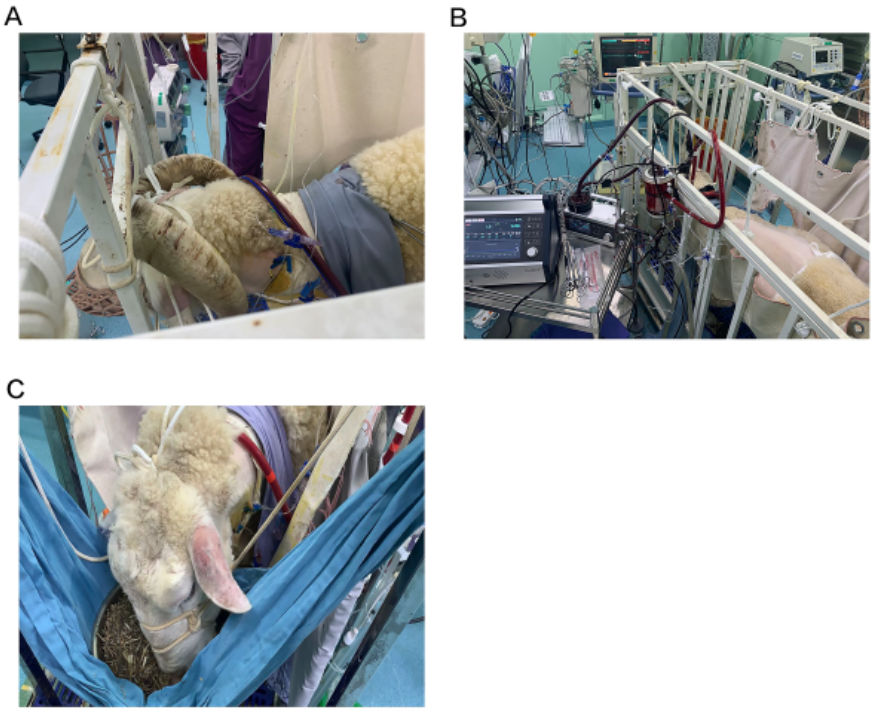

Figure 1

(A) Fixation of cannula around the neck (from right to left side); (B) The sheep could eat and move freely within a certain range in the monitoring cage; (C) The sheep were provided with appropriate amount of hay, feed and water every day.

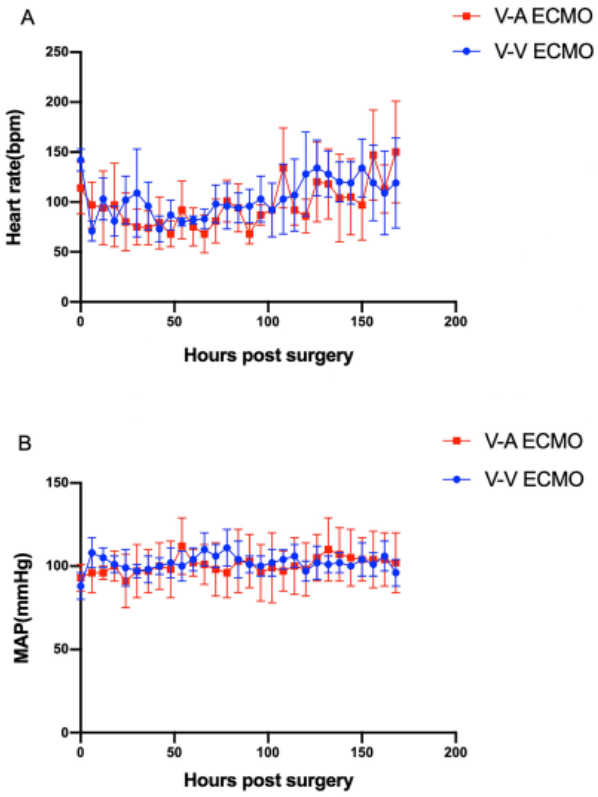

Figure 2

(A)Basic vital signs as assessed by heart rate (p□0.001, n=5 for each group)and (B) mean arterial pressure (MAP, p□0.05, n=5 for each group) during the experimental period . The error bars show the standard deviation.

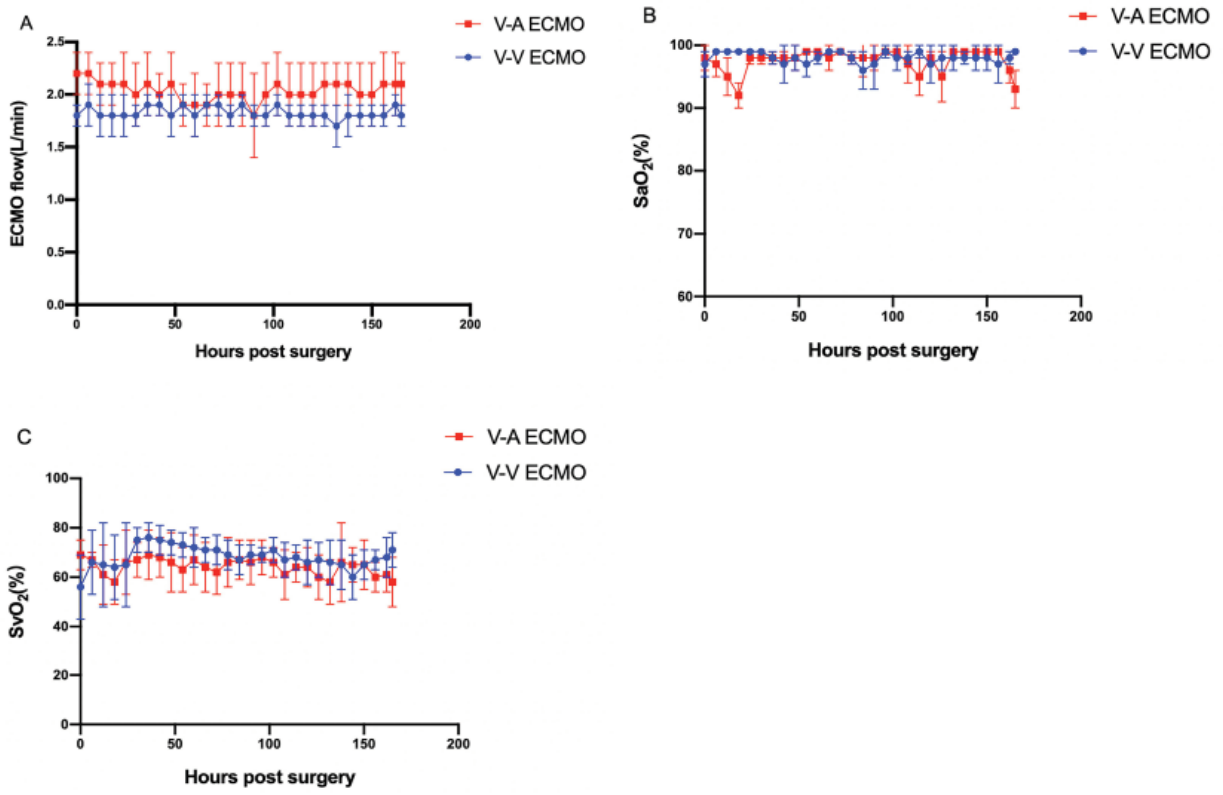

Figure 3

(A) Circuit blood flow and (B)(C)oxygenation performance of the oxygenators (SaO2: post-oxygenator oxygen saturation; SvO2: pre-oxygenator oxygen saturation) was good (p□0.05, n=5 for each group, respectively). The error bars show the standard deviation.

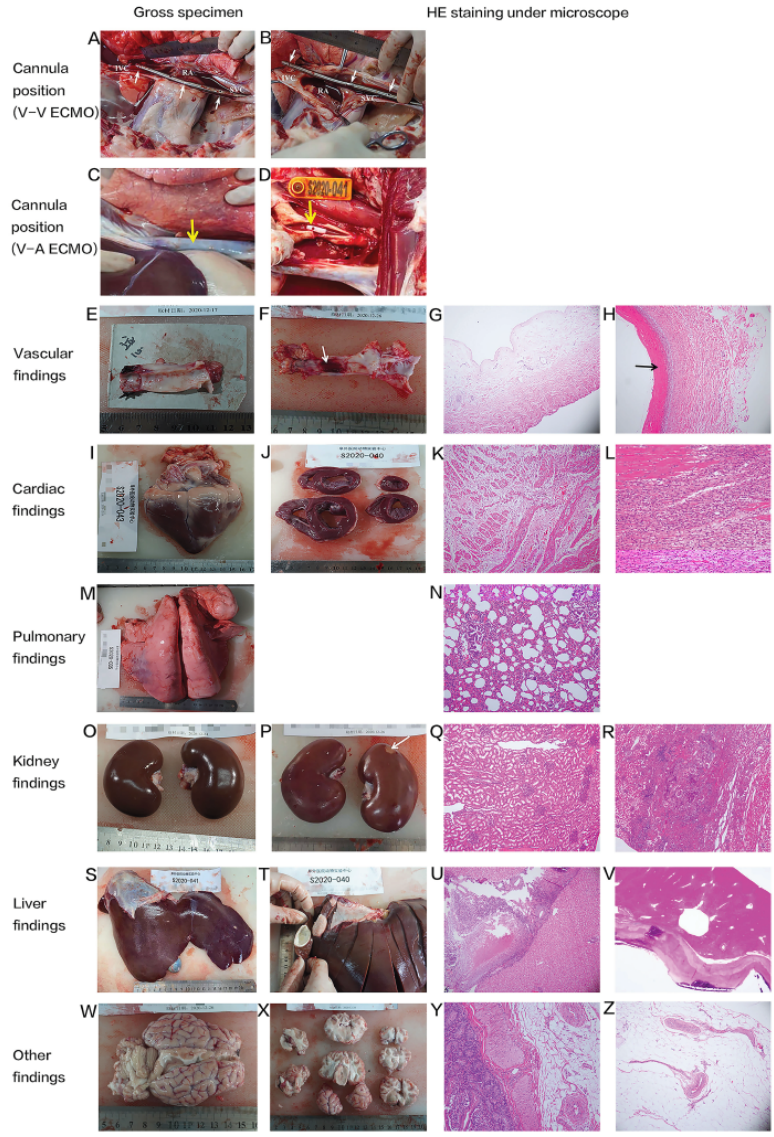

Figure 4

Representative pathological pictures. A: In the V-V ECMO group, the jugular vein cannula was observed in situ, the implantation position was accurate, and there was no cannulation displacement (arrow shows the entrance and exit of dual lumen cannula), animal No. S2020-036. B: In the V-V ECMO group, the depth of jugular vein cannula was insufficient, and the outlet (arrow in the middle) was higher than right atrium and located at the entrance of superior vena cava, animal No. S2020-037. C: In the V-A ECMO group, the internal thoracic segment of jugular vein cannula was observed in situ. The superior vena cava was not dissected (as shown by the arrow), and there was no cannulation displacement, animal No. S2020-038. D: In the V-A ECMO group, the tip of cannula (as shown by the arrow) could be seen in the jugular artery, animal No. S2020-041. E: Normal gross specimen of right jugular intima, animal No. S2020-040. F: Thrombus (as shown by the arrow) was seen in the middle intima of right jugular vein in the V-A ECMO group, animal No. S2020-038. G: In the V-A ECMO group, the normal jugular vein was observed under light microscope (stained with hematoxylin and eosin, at 10×magnification), animal No. S2020-014. H: In the V-A ECMO group, there was a small amount of thrombosis in jugular vein around the cannulation(as shown by the arrow) (HE, ×100), animal No. S2020-038. I□J: Normal gross specimens of heart, animal numbers were S2020-043 and S2020-040. K: Right atrial normal myocardial tissue (HE, ×10), animal No. S2020-013. L: Small infarctions in the lateral wall of the left ventricle with granulation tissue ingrowth (HE ×200), animal No. S2020-035. M: Normal lung specimen, animal No. S2020-035. N: Normal lung tissue observed under microscope (HE, ×20), animal No. S2020-038. O: The gross specimen of normal kidney, animal No. S2020-043. P: The gross specimen of small renal infarction in the V-A ECMO group(as shown by the arrow) was No. s2020-038. Q: Normal kidney observed under microscope (HE, ×10), animal No. S2020-043. R: In the V-V ECMO group, small infarct (HE, ×40) was observed in the kidney, animal No. S2020-035. S: Normal liver specimen, animal No. S2020-041. T: In V-A ECMO group, cyst was found in the liver, animal No. S2020-040. U: Normal liver observed under microscope (HE, ×10), animal No. S2020-035. V: Spontaneous cyst with calcification (HE, ×20) was found in the liver in the V-A ECMO group, animal No. S2020-040. W□X: Gross specimens of

brain, there were no hemorrhage or infarction, animal No. S2020-038. Y: Normal intestine tissue observed under microscope (HE, ×20), animal No. S2020-043. Z: Normal mesentery tissue observed under microscope (HE, ×10), animal No. S2020-014.