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Application of a novel extracorporeal membrane oxygenation system in awake Hu sheep under various durations

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Abstract

Background Extracorporeal membrane oxygenation (ECMO) provides effective support for respiratory and circulatory functions and stands as an essential means in the management of life-threatening conditions. This study aimed to evaluate the safety and efficacy of a novel ECMO system in awake sheep models across various support durations.

Methods Ten healthy Hu sheep were divided into venovenous (VV) and venoarterial (VA) groups, further categorized into short, medium, and long-term observation subgroups. Vital signs and blood indices were meticulously monitored. The integrity of heparin coatings and thrombosis in the ECMO system were assessed at indicated time points, followed by histopathological analysis.

Results All ten sheep survived to their planned endpoints under awake ECMO, exhibiting stable vital signs without notable reductions in blood cells. With the support of ECMO, all sheep demonstrated a significant increase in partial pressure of oxygen (PO_2) and maintained oxygen saturation (SO_2) between 99.8 and 100%. The heparin coating remained relatively intact even after prolonged ECMO operation. Anatomical observations and pathological histology analyses of major organs revealed no apparent abnormalities.

Conclusions In healthy Hu sheep models with different maintenance periods, the novel ECMO support in awake state demonstrated high safety and feasibility.

Keywords Extracorporeal membrane oxygenation, ECMO, Awake ECMO, Ovine model, Cardiopulmonary support, Preclinical evaluation

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Introduction

Extracorporeal Membrane Oxygenation (ECMO) is a vital life-support technology primarily used for critically ill patients unresponsive to conventional treatment due to severe cardiac and/or respiratory failure. It offers respiratory and circulatory support, buying time for spontaneous cardiac or pulmonary recovery or further treatment [1]. The main structure of ECMO usually includes the system unit, blood drive pump, special disposable consumables (mainly including membrane oxygenator, pump head, the arterial and venous cannula, blood circuits and various connectors), temperature control system, air-oxygen blender and monitoring equipment such as pressure, temperature and blood oxygen saturation sensors.

Since the first successful ECMO treatment in the 1970s, its global application has surged, especially during the COVID-19 pandemic. The effective cardiopulmonary support function of ECMO has made it stand out among a number of emergency and critical treatment methods [2]. Nevertheless, the expanding clinical demand clashes with insufficient market supply and high costs. The widespread promotion of the technology has encountered huge obstacles.

Traditional ECMO centrifugal pumps typically use a magnetic coupling structure, combined with a contact-bearing design for the impeller, which often leads to complications such as friction-induced heat and hemolysis. Besides, research on high-durability membrane oxygenators and anticoagulant coatings has become another key focus in ECMO technology. Furthermore, most current ECMO-related animal studies focus on short-term durations of 1 to 7 days. Establishing long-term large animal awake ECMO models would provide greater reference value for clinical practice.

Hengrui Company developed a novel ECMO system —— Hengrui Hongyuan extracorporeal cardiopulmonary support system (HR-ECMO, Suzhou Hengrui Medical Devices Co., LTD, Suzhou, China), which has undergone preliminary extracorporeal hemodynamics and hemolysis evaluation tests. The main purpose of this study is to evaluate the safety and effectiveness of the novel ECMO support system in awake state by constructing short, medium, and long-term healthy Hu sheep models of ECMO.

Materials and methods

In vitro tests

In vitro tests were performed with heparinized bovine, porcine, and ovine blood (4500 units of heparin per liter) at a blood flow rate of 7 L/min and a gas flow rate of 14 L/min for 342 h. Heparinized blood was used for circulation during the first and last 6 h, with saline in between. Blood conditions: hemoglobin (120 ± 10)g/L, CO_2 partial pressure (5.3 ± 0.7)kPa, base excess (0 ± 5)mmol/L, blood glucose (10 ± 5)mmol/L, and temperature 37 ± 1℃. Parameters such as plasma free hemoglobin, blood cell counts, and blood gas analysis were measured at 10, 30, 180, and 360 min during the first and last 6 h. For the hydraulic performance testing of the centrifugal pump head, simulated blood was used, prepared by mixing 400 g glycerol (purity ≥ 98%) with 600 g saline to create a 1000 g blood simulant solution.

Animal selection and grouping

Ten adult female Hu sheep, weighing between 50 and 70 kg, were included in the experiment. They were adaptively fed for 7 days before random grouping. Randomization was performed using a random number table method. As shown in Table 1, two sheep were included in the short-term group (1 day post-operation), four in the medium-term group (4–9 days post-operation), and four in the long-term group (10–15 days post-operation). All the animals were purchased from Zhejiang Sainuo Ecological Agriculture Co., LTD. and raised in the general feeding room of Hangzhou Lifutai Biotechnology Co., LTD. Animal feeding and experimental procedures were

Table 1 Detailed characteristics of experimental sheep

Group	No.	Sex	Weight (kg)	Duration (d)	Termination	Cannulation Site
VV ECMO	Sheep-08	Female	64.7	1	Scheduled	Vjr (20 F) - Vjl (16 F)
	Sheep-07	Female	65.6	7	Scheduled	Vjr (20 F) - Vjl (16 F)
	Sheep-03	Female	52.2	9	Scheduled	Vjr (20 F) - Vjl (16 F)
	Sheep-01	Female	57.1	15	Scheduled	Vjr (20 F) - Vjl (16 F)
	Sheep-06	Female	52.8	15	Scheduled	Vjr (20 F) - Vjl (16 F)
VA ECMO	Sheep-09	Female	58.7	1	Scheduled	Vjr (20 F) - Acl (16 F)
	Sheep-04	Female	61.7	4	Scheduled	Vjr (20 F) - Acl (16 F)
	Sheep-05	Female	50.3	9	Scheduled	Vjr (20 F) - Acl (16 F)
	Sheep-02	Female	52.5	10	Scheduled	Vjr (20 F) - Acl (16 F)
	Sheep-11	Female	63.1	15	Scheduled	Vjr (20 F) - Acl (16 F)
	Sheep-10	Female	56.8	2	Unscheduled	Vjr (20 F) - Acl (16 F)

VV ECMO, venovenous extracorporeal membrane oxygenation; VA ECMO, venoarterial extracorporeal membrane oxygenation; Vjr, right jugular vein; Vjl, left jugular vein; Acl, left carotid artery

approved by the Animal Ethics Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine and the Laboratory Animal Management and Use Committee of Hangzhou Lifutai Biotechnology Co., LTD (IACUC approval number: 20221221-01), and complied with the 3R principles of animal welfare.

Novel ECMO system

The HR-ECMO system used in this study covers maglev-type centrifugal pump, the membrane oxygenator, disposable arteriovenous cannulas, the system unit and temperature control system (Fig. 1).

Maglev-type centrifugal pump

The HR-ECMO centrifugal pump utilizes magnetic levitation technology, optimizing the impeller, volute structure, and internal flow field to increase the fluid gap between the impeller and volute. Extensive multi-phase flow field simulations and hemolysis tests have been conducted. The pump operates at speeds of 0-5000 rpm with a speed control accuracy of ± 2%, and a maximum flow rate of 9.99 L/min. The priming volume of the pump head is approximately 32mL, and the NIH value is ≤ 0.1 g/100L at the flow rate of 5 L/min (see Fig. 2).

High efficiency PMP membrane oxygenator

The HR-ECMO oxygenator uses a third-generation PMP (Poly 4-Methyl-1-Pentene) hollow fiber membrane, optimized for surface quality, gas exchange efficiency, and anticoagulant performance by adjusting parameters like melting temperature, winding speed, annealing temperature, annealing time and tensile ratio. Its internal fiber

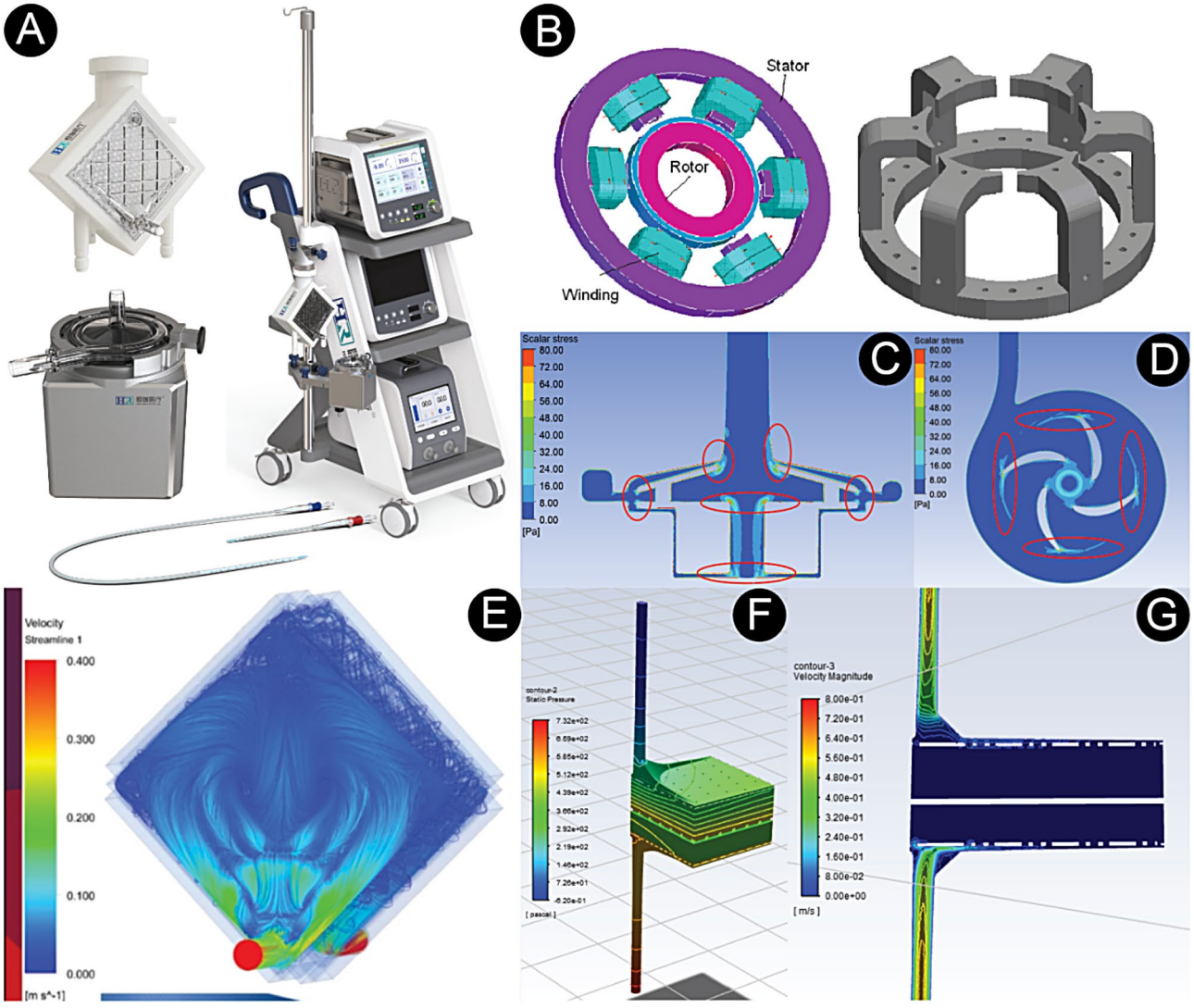

Fig. 1 Hengrui Hongyuan Extracorporeal Cardiopulmonary Support System: (**A**) Appearance of the HR-ECMO system; (**B**) The basic internal structure of the pump; (**C**) The distribution of scalar stress in the central plane; (**D**) The distribution of scalar stress in the XY-plane; (**E**) The particle residence, (**F**) pressure distribution and (**G**) velocity distribution inside the membrane oxygenator

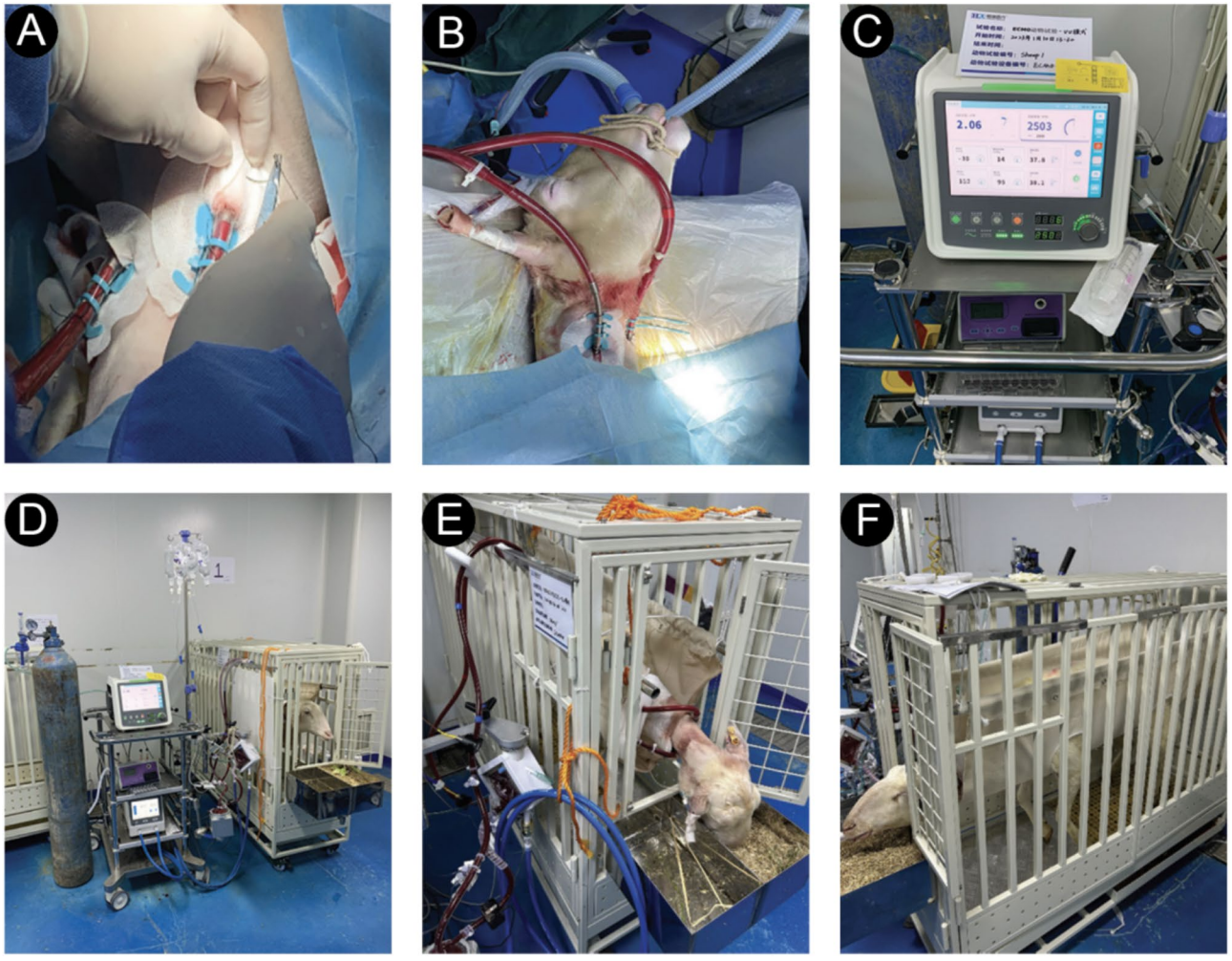

Fig. 2 Surgical procedure and postoperative care: (**A**, **B**) Cervical vascular catheterization and pipeline suture fixation; (**C**, **D**) ECMO operation and overall status; (**E**, **F**) The sheep was immobilized by a suspended cloth bag, it remained awake during the experiment and had free access to forage and water

arrangement is more uniform than traditional ECMO devices. Design improvements include a larger baffle mesh, minimized sharp edges, and expanded blood inlet/outlet tapers for more uniform blood diffusion. Internal flow field analysis also demonstrated that the novel ECMO oxygenator features a smaller pressure gradient, uniform velocity distribution, and relatively consistent particle residence time. These characteristics help reduce the risk of blood stagnation and thrombus formation to some extent. The oxygenator has a priming volume of 215mL and membrane area of 1.8m^2. The maximum flow rate is 7 L/min, under which the pressure loss of the oxygenator is ≤ 75mmHg, the amount of oxygen bound is ≥ 45mL O_2/L blood and the amount of carbon dioxide discharged is ≥ 38mL CO_2/L blood.

Anti-scouring anticoagulant coating

A long-acting, scour-resistant anticoagulant coating was developed by optimizing surface activation, heparin grafting, and introducing active groups. The ECMO circuit tubing is made of polyvinyl chloride (PVC) material. The main components of the coating are high molecular weight heparin and protein molecules (bovine serum albumin). Protein molecules are uniformly distributed on the surface of the material, supporting the physiological action of heparin molecules and serving as a bridge for the binding of heparin molecules to the material surface. Covalent and ionic bonds exist simultaneously between proteins and heparin, ensuring both the stability of heparin molecule attachment and the protection of heparin's active groups. The continuous surface created by proteins, resembling the natural endothelial surface, effectively prevents thrombus formation.

Temperature control system

The warming instrument for extracorporeal cardiopulmonary support (product model: ECW-100, Suzhou Hengrui Medical Devices Co., LTD, Suzhou, China) is

used to exchange heat with the patient's blood flow via the oxygenator to maintain the patient's body temperature. The control accuracy of the temperature control unit is ± 0.1℃.

Surgical Procedure and Postoperative Care

All animals fasted for 48 h and were water-deprived for 8 h before surgery. A 25 mg intramuscular injection of Xylazine was given, followed by 2 mg/kg Propofol through the ear vein to induce anesthesia, with the dose adjusted as needed. An endotracheal tube (8–10#) was inserted under laryngoscopic guidance, and anesthesia was maintained with 3% isoflurane. Systemic anticoagulation was achieved with 120IU/kg heparin. Experienced surgeons performed catheterization to establish VV ECMO and VA ECMO pathways. For VV ECMO, a 16Fr arterial cannula (DM-16-150, Suzhou Hengrui Medical Devices Co., LTD, Suzhou, China) was inserted into the left jugular vein for reinfusion, with the tip about 15 cm into the vessel. A 20Fr venous cannula (JM-20-550, Suzhou Hengrui Medical Devices Co., LTD, Suzhou, China) was inserted into the right jugular vein, passing through the right atrium into the inferior vena cava for drainage. For VA ECMO, an arterial cannula was placed in the left carotid artery to transfuse oxygenated blood, while a venous cannula was inserted into the right jugular vein to drain blood from the inferior vena cava. After confirming the catheter position with X-ray, it was secured by suturing to the sheep's neck and preventing displacement with gauze and an elastic bandage. In VA mode, the pump speed was set to 3200 rpm for maintaining 2.00 L/min blood flow, while in VV mode, it was set to 2500 rpm for maintaining the same flow rate. The ECMO oxygenator was connected to an oxygen cylinder with a gas flow rate of 4 L/min for VA ECMO and 2 L/min for VV ECMO. The warming device target temperature was 38.5 °C. After surgery, the endotracheal tube was removed. The animal was placed in a cage, immobilized in a suspended cloth bag to limit frequent head movement. The animals sustained themselves by autonomous ingestion of forage and water throughout the experiment.

Observational indicators

During the experiment, the general condition of each Hu sheep, including mental state, behavior, and diet, was monitored. The ECMO system's parameters were tracked, and every 6 h, ACT levels and vital signs like blood pressure and heart rate were assessed. Heparin was administered to maintain ACT between 180 and 220 s, starting at 20-30IU/kg/hr with adjustments based on ACT [3]. Preoperatively, during post-anesthetic recovery, and daily postoperatively, blood gas analyses were performed both pre- and post-membrane. Additional measurements included WBC, RBC, reticulocyte, PLT counts, liver and kidney function, myocardial enzymes, electrolytes, coagulation indicators, and plasma free hemoglobin. At set time points, euthanasia was performed under sedation with propofol via injecting 30mL of 10% potassium chloride. After tube disconnection and machine withdrawal, X-rays were was performed to reconfirm the positioning of the arterial and venous catheters. The circulatory tubing and centrifugal pump head were flushed with saline and checked for thrombus. A 2% toluidine blue solution stained the circuits and membrane oxygenator to confirm the heparin coating stability. Anatomical dissection of major organs was done, followed by H&E staining for histopathological analysis.

Statistical analysis

The Shapiro-Wilk test was used to assess the normality of the distribution of continuous variables. Normally distributed variables are presented as mean ± standard deviation (SD) and compared using One-way ANOVA. Oxygenation status pre- and post-membrane oxygenator were compared using the paired t-test. Non-normally distributed variables are presented as median and interquartile range (IQR) and compared using the Kruskal-Wallis test and Bonferroni-adjusted pairwise comparison. $P < 0.05$ was considered statistically significant. Statistical analyses were conducted using IBM SPSS Statistics Version 25.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism Version 8.0.1 (GraphPad Software Inc., San Diego, CA, USA).

Results

Preliminary in vitro tests

In vitro hemolysis tests showed that, in 100mL of blood, the increase in free hemoglobin concentration is ≤ 20 mg/h, and the hourly decrease rate of white blood cells and platelets is ≤ 20% (Supplemental Table 1). Measured data under the condition of 2.0cp, the computational fluid dynamics (CFD) simulation data and the overall deviation are listed in Supplemental Tables 2–4. The simulation model is relatively accurate, and based on this, we further investigated the magnitude and distribution of scalar stress within the maglev pump (Fig. 1C, D). Most regions on the cross-section exhibit low scalar stress, with areas of extremely high scalar stress (greater than or equal to 80 Pa) primarily distributed along the walls (including the casing, blade surfaces, and impeller walls). Areas of slightly higher scalar stress (ranging from 8 Pa to 40 Pa) are mainly found in regions where flow separation occurs (highlighted by red circles).The equipment also showed good hydraulic properties. It can achieve a balance between stable blood drive efficiency and low hemolysis even during long-term operation.

General condition of animals and overall performance of the ECMO device

Under a well-established perioperative treatment and care system, all 10 Hu sheep survived to the planned time during awake ECMO operation, with no evident kinking or displacement of the tubing. On the second day post-surgery, the reinfusion tube of sheep number 10 in VA mode was bitten off due to inappropriate fixation, necessitating its exclusion and replacement with sheep number 11. Details of the groupings are presented in Table 1. Throughout the ECMO maintenance period, no apparent abnormalities were observed in the animals. Vital signs remained relatively stable, with normal food and water intake, regular excretion, and overall good general condition.

The novel ECMO system operated stably throughout the experimental process, and no equipment-related malfunctions occurred until the decannulation. The total priming volume of the ECMO device is approximately 585mL, which includes 32mL from the pump head and 215mL from the oxygenator. For VV group, the rotational speed ranged from 2499.82 ± 1.74 to 2500.28 ± 6.54 rpm, and for VA group, the speed ranged from 2614.16 ± 259.01 to 3199.50 ± 2.66 rpm. The ECMO flow rate of VV group ranged from 1.95 ± 0.05 to 2.04 ± 0.05 L/min, while 1.10 ± 0.35 to 1.94 ± 0.19 L/min in VA group. Sheep number 2, 5, and 11 in VA group had adjustments made to operational parameters during the maintenance period as the blood flow indicators did not reach the expected levels. Especially in Sheep 11, from the third day post-operation, the speed decreased to 2500 rpm, with the target flow rate adjusted to around 1.00 L/min. The pre-pump pressures for all groups remained relatively stable during the experiment, and the average transmembrane pressure was maintained at a low level close to the natural pressure drop across the body's lungs, as shown in Fig. 3. Blood gas analyses pre- and post-membrane oxygenator revealed a significant improvement in oxygenation status for all Hu sheep. There was a substantial increase in partial pressure of oxygen [54.67 ± 16.97mmHg vs. 412.42 ± 73.64mmHg, $p < 0.001$ (paired t-test)], and the blood oxygen saturation could reach 99.8-100% (see Table, Supplemental Table 5, which illustrates the blood gas analyses before and after the oxygenator). During extracorporeal life support, the warming function of the temperature control system maintained stable blood temperatures in the circuit. In

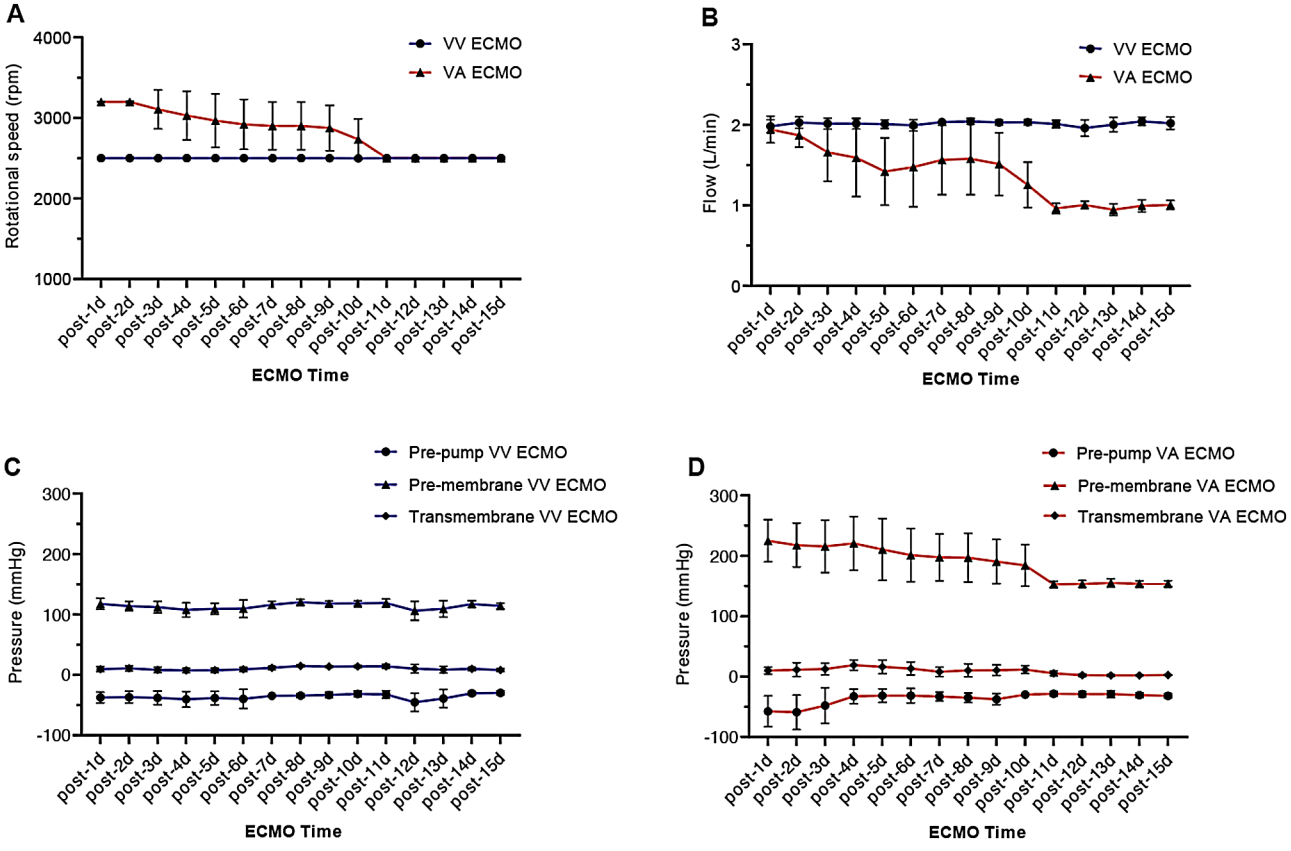

Fig. 3 Specific Operating Parameters of the ECMO Device: (**A**) The rotational speed of the HR-ECMO system for all sheep; (**B**) The flow of the HR-ECMO system; The pre-pump pressure, pre-membrane pressure and transmembrane pressure of the HR-ECMO system for (**C**) VV ECMO group and (**D**) VA ECMO group; post-1d, the first day post the operation

this trial, the temperature difference did not exceed 0.5℃ (see Table, Supplemental Table 6, which illustrates the circuit temperature throughout the experiment).

Hematological and biochemical indicators

On the ninth day post-operation, there was a significant increase in WBC compared to the baseline values at post-anesthesia awakening [11.83 × 10^9/L (IQR, 8.34–19.42 × 10^9/L) vs. 4.95 × 10^9/L (IQR, 3.21–5.87 × 10^9/L), $p = 0.009$ (Kruskal-Wallis test)]. As time progressed, there were no significant statistical differences observed in RBC levels [H = 12.215, $p = 0.729$ (Kruskal-Wallis test)]. fHb levels remained stable [H = 15.530, $p = 0.486$ (Kruskal-Wallis test)]. Only on the third day post-operation, PLT levels were lower than the baseline at post-anesthesia awakening [317 × 10^9/L (IQR, 251–366 × 10^9/L) vs. 452 × 10^9/L (IQR, 392–584 × 10^9/L), $p = 0.046$ (Kruskal-Wallis test)], and this difference was transient (Fig. 4). Details of complete blood count (CBC) and fHb levels in the short, medium, and long-term observation subgroups can be found in Supplemental Fig. 1.

During the experiment, there were no significant statistical differences observed in alanine aminotransferase (ALT) levels [H = 21.413, $p = 0.163$ (Kruskal-Wallis test)]. Bonferroni-adjusted pairwise comparisons following Kruskal-Wallis test revealed no significant increase or decrease in aspartate aminotransferase (AST) levels. Compared to the levels post-anesthesia awakening, blood urea nitrogen (BUN) levels exhibited varying degrees of decrease on the sixth [2.91mmol/L (IQR, 2.23-3.05mmol/L) vs. 6.45mmol/L (IQR, 5.54-7.56mmol/L), $p = 0.005$ (Kruskal-Wallis test)], seventh [2.46mmol/L (IQR, 2.02-3.74mmol/L) vs. 6.45mmol/L (IQR, 5.54-7.56mmol/L), $p = 0.012$ (Kruskal-Wallis test)], and eighth [2.83mmol/L (IQR, 1.97-3.70mmol/L) vs. 6.45mmol/L (IQR, 5.54-7.56mmol/L), $p = 0.015$ (Kruskal-Wallis test)] days post-operation. After adjustment with the Bonferroni correction, there were also no significant difference observed in creatinine (CREA) levels. Details of each sheep can be found in Supplemental Fig. 2.

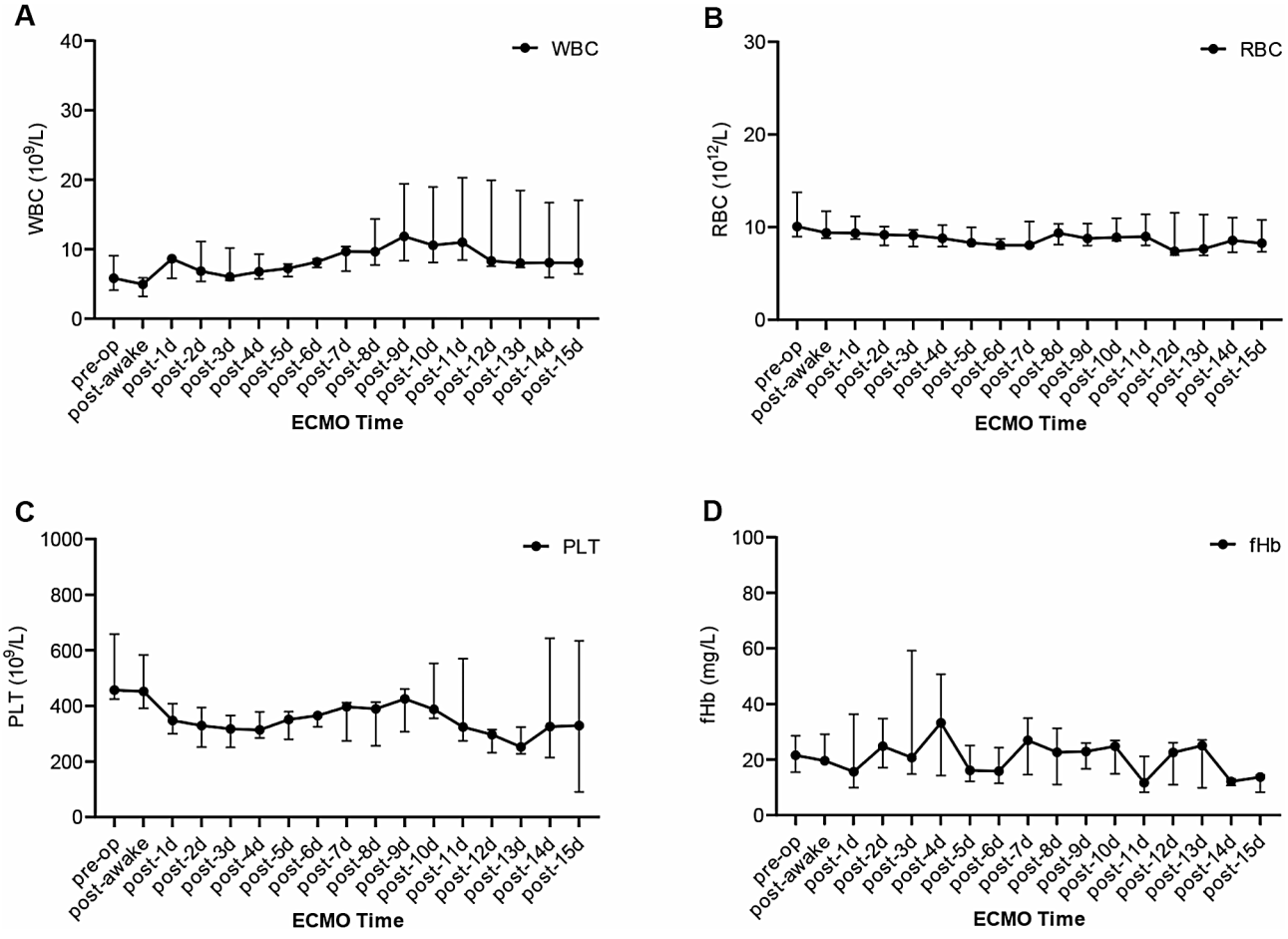

Fig. 4 Complete Blood Count (CBC) and Plasma Free Hemoglobin (fHb): (**A**) The white blood cell (WBC) levels of all sheep; (**B**) The red blood cell (RBC) levels; (**C**) The platelet (PLT) levels; (**D**) Plasma free hemoglobin (fHb) levels. pre-op, before the operation; post-awake, postoperative anesthesia recovery

Observation of ECMO equipment after withdrawal and heparin coating staining

Following the withdrawal of the ECMO system and subsequent constant-flow saline flushing, no visible thrombus formation was observed in the circulatory tubing or pump head. Despite adequate systemic anticoagulation and frequent monitoring of coagulation parameters, small amounts of blood clots, varying in degree, were still observed in some membrane oxygenators, primarily in the long-term group, including sheep 1, 6, and 11. However, it is unclear whether these clots formed during the withdrawal process or while the ECMO was in operation. After staining with toluidine blue, uniform blue-violet coloration was observed in the tubing and hollow fiber membrane, indicating the integrity of the heparin coating. Notably, after a prolonged use of 9 days and 15 days, the blue-violet color appeared slightly lighter (Fig. 5).

Anatomical and histopathological observation

Upon gross anatomical observation, the intestines, stomach, heart, liver, spleen, lungs, kidneys, and brain of all 10 Hu sheep showed no apparent abnormalities. Pathological histology analyses of major organs revealed no evident abnormalities. The cardiac structure of all 10 sheep were clear, with well-arranged and orderly myocardial cells showing homogeneous red staining. The liver exhibited distinct lobular structures, rich cytoplasm, and no apparent degeneration or necrosis. The spleen showed clear white and red pulp structures with normal color and reasonable distribution. The lungs showed no signs of bleeding or inflammation, with clear and intact structures such as the trachea and alveoli. The kidneys displayed clear structures, with intact glomeruli and renal tubules. The cortical neurons in the brain were orderly arranged without evident lesions (Fig. 6).

Discussion

Currently, most ECMO animal studies focus on short to medium durations, usually 1 to 7 days [4–7]. To address the clinical need for extended ECMO support, we created short, medium, and long-duration awake ECMO models in healthy Hu sheep to evaluate the novel ECMO system's practical use and provide insights for future clinical research. The novel HR-ECMO in study incorporates a magnetic levitation centrifugal pump head with a non-contact bearing design, replacing the mechanical rotating bearings of the impeller rotor. This design effectively minimized friction and wear at the contact parts. It enhances equipment durability, reduces shear stress, and mitigates the risks of red blood cell destruction and thrombus formation. The long-acting, scour-resistant anticoagulant coating in ECMO circuit and oxygenator is highly biocompatible, reducing impact on the coagulation system. Toluidine blue staining shows the coating stays intact with extended use. The high-durability PMP membrane oxygenator and anti-scouring coating lower risks from frequent kit replacements, like infections, embolism, bleeding, and support interruptions.

In our study, we did not observe significant hemodilatory effects as seen in other animal experiments, which

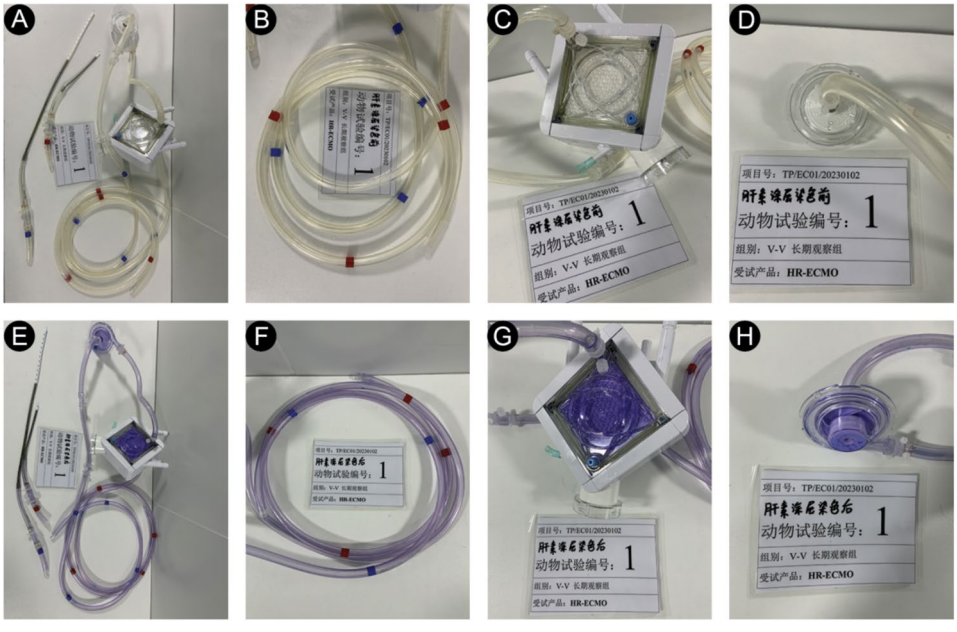

Fig. 5 Observation of ECMO equipment after withdrawal and heparin coating staining: (**A**, **B**, **C**, **D**) Circuit tubing, hollow fiber membrane and pump head before staining; (**E**, **F**, **G**, **H**) Circuit tubing, hollow fiber membrane and pump head exhibited a fairly uniform bluish-purple coloration after staining with toluidine blue, indicating that the heparin coating was relatively intact

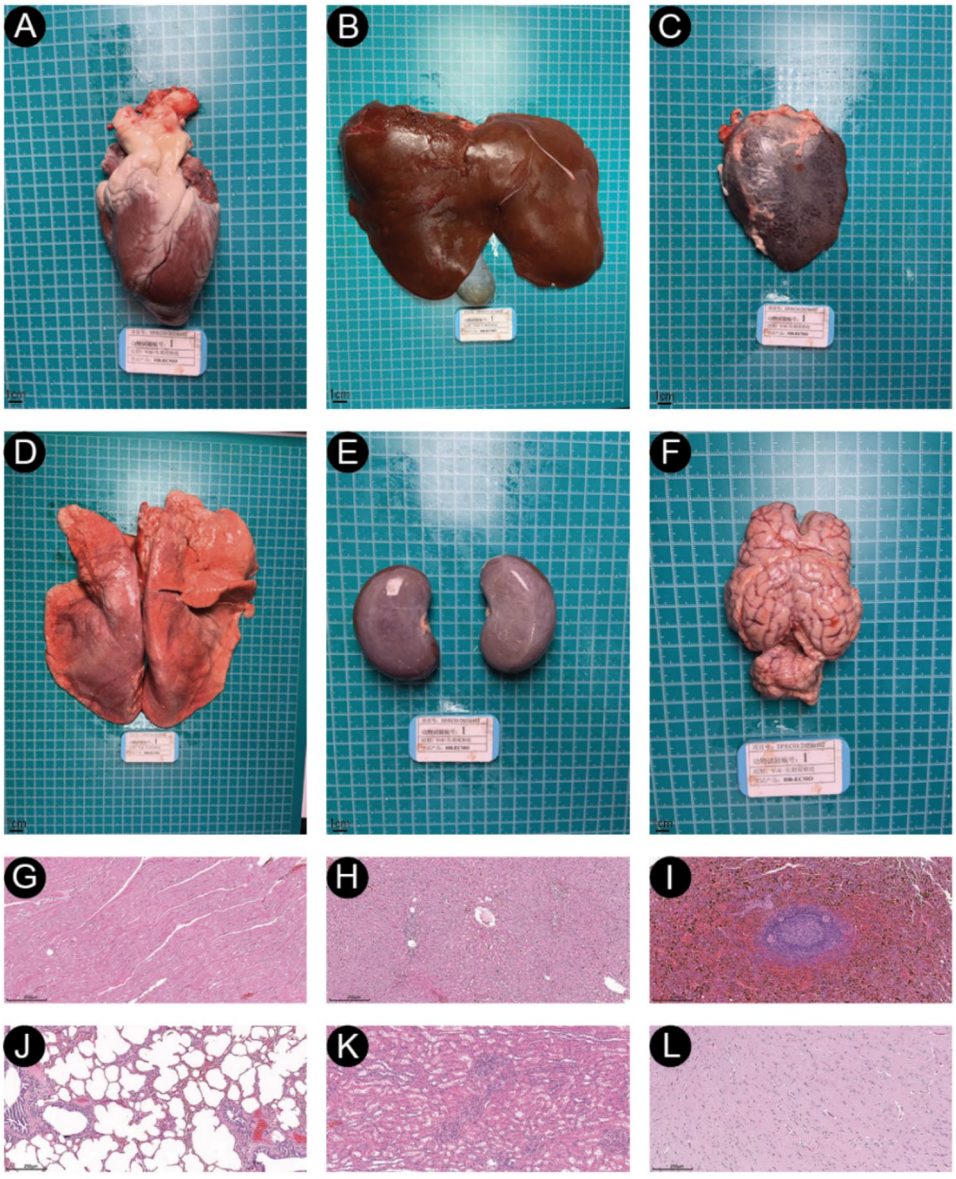

Fig. 6 Anatomical and histopathological observation: The representative images of anatomical observation in sheep, including (**A**) the heart, (**B**) liver, (**C**) spleen, (**D**) lungs, (**E**) kidneys, and (**F**) brain. Histopathological analysis of the (**G**) myocardial structure, (**H**) liver lobular structure, (**I**) white and red pulp structure in spleen, (**J**) trachea and alveoli, (**K**) glomeruli and renal tubules, (**L**) cortical neurons of the brain

may be attributed to the smaller priming volume of the ECMO device [8]. Hematological tests showed no significant reduction in blood cell counts in all Hu sheep, indicating no severe blood cell damage or compensable loss. This may reduce or avoid blood product transfusions in clinical practice [9, 10]. The normal platelet range for adult Hu sheep is approximately 180–680 × 10^9/L, varying slightly by altitude. Platelet counts in VA group gradually declined with prolonged ECMO time. Specifically, sheep 5 in the medium-term group and sheep 11 in the long-term group both dropped below 100 × 10^9/L on the 9th and 15th days post-surgery, respectively. Given the prolonged use of intravenous heparin and frequent heparin flushing at the blood sampling site, we considered the possibility of Heparin-Induced Thrombocytopenia (HIT). Several studies have highlighted factors that increase the risk of HIT, such as heparin exposure time, type of heparin (regular heparin > low molecular weight heparin), exposure route (intravenous > subcutaneous), various extracorporeal circulation devices, and surgical procedures [11, 12]. Additionally, compared to VV ECMO, VA ECMO is more likely to cause severe thrombocytopenia and arterial thromboembolism [13]. However, this experiment lacks definitive serological confirmation of HIT and cannot rule out secondary platelet level decline due to other factors such as

potential infection. Since no severe thrombotic or bleeding events occurred during the experiment, we refrained from special interventions, and heparin therapy was not interrupted. The post-removal organ histopathological analysis also revealed no severe bleeding or coagulation events.

In this study, the WBC count in both the medium-term and long-term groups gradually increased with the extension of the experimental time. Especially in the later phases of long-term group, it became particularly evident. This trend is consistent with the elevation of inflammatory markers such as C-reactive protein, suggesting the potiental ECMO-related inflammatory reactions, either infectious or non-infectious. The inflammatory response related to extracorporeal circulation is recognized as one of the common complications of ECMO, mainly due to the blood exposure to the non-endothelial surface of the ECMO circuit. This triggers the innate immune system and the coagulation pathway, causing widespread endothelial damage, WBC activation, pro-inflammatory mediators production, and potential systemic inflammatory response and organs damage [14].

In our experiment, ALT and AST levels in the medium-term and long-term groups of sheep briefly peaked around 3–4 days of ECMO, then slowly declined. Anatomical and histological analyses showed no significant liver injury. This temporary elevation might be due to liver function impairment from surgical procedures, postoperative stress, or potential drug effects. A similar trend was observed in a 14-day in vivo artificial lung study by Skoog et al., [8] where liver enzymes in sheep transiently increased post-operation but then returned to normal. Blandino et al. [15] also found that 65% of patients receiving VA ECMO had early liver enzyme elevation, which typically resolved within a few days without affecting patient outcomes.

In our study, target flows were not achieved for sheep 2, 5 and 11. It may be due to the movement of the catheter or its adherence to the vessel wall caused by animal activity, leading to inadequate drainage and subsequent decrease in flow rate. Alternatively, increased resistance in the circuit, such as thrombus formation within the membrane lung, could also contribute. Sheep 2 exhibited obviously higher transmembrane pressure during the early and middle stages of the experiment, but we did not find any visible thrombus formation in the circuit tubing or hollow fiber membrane. This could be attributed to temporary thrombus formation or deposition of other proteins, which subsequently dissolved due to the continuous use of heparin. Additionally, the long-term performance of the ECMO device under high head pressure conditions will also be one of the key focus of our future research.

Awake ECMO is recommend, with increasingly used in lung transplantation bridging therapy, chronic obstructive pulmonary disease, and refractory cardiogenic shock [16, 17]. For non-intubated patients, awake ECMO minimizes complications, helps preserve respiratory and diaphragmatic muscle tone, improves the ventilation-perfusion ratio, reduces the risk of atelectasis and pulmonary infections. On the other hand, it decreases the incidence of critical illness myopathy and polyneuropathy, and promotes early rehabilitation exercise for patients [18, 19]. It also facilitates the detection of possible complications such as intracranial hemorrhage and limb embolism, while reducing emotional consciousness disorders caused by stress-induced trauma, such as delirium [20]. A study by Fuehner et al. found that preoperative awake ECMO reduced postoperative ventilation time and shortened hospital stays in lung transplant patients [21]. Other studies also showed improved survival with awake ECMO [20, 22]. While awake ECMO better meets clinical needs, it increases challenges in perioperative management, including adequate pain control and monitoring. It also imposes higher requirements on ECMO mechanical performance and secure tubing fixation. In the awake ECMO model of this study, jugular venous cannulation was more conducive to the sheep's autonomous activity and subsequent monitoring. The design of restrictive cage and suspended cloth bag helped prevent tube kinking or dislocation.

The study had several limitations. It included 10 Hu sheep, with small sample sizes in each group, which might cause biases and lack generalizability. Larger studies are needed for confirmation. In addition, the serological evidence for HIT is insufficient. Given the risk of tube disconnection and infection, we did not retain arterial or central venous catheters. Additionally, we could not dynamically monitor systemic arterial oxygen levels due to the difficulty of frequent arterial blood sampling in awake sheep, which may affect the accuracy of assessing overall oxygenation. In summary, the novel HR-ECMO system demonstrated high feasibility and safety in healthy Hu sheep models over various support durations, effectively providing blood oxygenation without significant adverse effects on blood indices or major organs. The successful development and management of a long-term awake ECMO sheep model paves the way for future research into ECMO-related pathophysiological mechanisms.

Abbreviations

ECMO	Extracorporeal membrane oxygenation
VV	Venovenous
VA	Venoarterial
PO_2	Partial pressure of oxygen
SO_2	Oxygen saturation
PMP	Poly 4-Methyl-1-Pentene
PVC	Polyvinyl chloride

WBC	White blood cell
RBC	Red blood cell
PLT	Platelet
fHb	Plasma free hemoglobin
SD	Standard deviation
IQR	Interquartile range
CFD	Computational fluid dynamics
CBC	Complete blood count
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BUN	Blood urea nitrogen
CREA	Creatinine
HIT	Heparin-Induced Thrombocytopenia
AKI	Acute kidney injury

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12871-025-02930-5.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

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Author contributions
SD, JC, YW, HL and ZL conducted the experiment, QL and GT analyzed the data, SD and JC drafted the manuscript, MH originally designed the research scheme and supervised the research process, provided clinical reference, and revised the article. All authors read and approved the final manuscript.

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Data availability
The raw data of this article are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The care of animals and experimental procedures were reviewed and approved by the Animal Ethics Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine and the Laboratory Animal Management and Use Committee of Hangzhou Lifutai Biotechnology Co., LTD (IACUC approval number: 20221221-01). All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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