SUPPLEMENTAL METHODOLOGY STATEMENT

Fetal Instrumentation

The fetus was partially exteriorised (head and chest) from the uterus and flow probes were placed around the left main pulmonary artery and carotid artery (Transonic Systems, Ithaca, NY, USA). Heparinised saline-filled catheters were inserted into a carotid artery, brachial artery and jugular vein as described previously.(14) Saline-filled balloon-tipped catheters were placed in an incision in the abdomen and pleura to measure intra-abdominal and intra-pleural pressures respectively. All pressures and blood flows were digitally recorded (1kHz, Powerlab; ADInstruments, Castle Hill, NSW, Australia). The fetus was intubated with a 4.5mm cuffed endotracheal tube, lung liquid was drained passively, and the endotracheal tube clamped. SpO_2 monitoring (Masimo, Radical 4, CA, USA) was placed on the right forelimb, and a Near Infrared Spectroscopy (NIRS) Optode (Casmed Foresight, CAS Medical Systems Inc, Branford, CT, USA) placed over the left frontal cortex for measurement of cerebral tissue oxygen saturation ($SctO_2$).

Resuscitation of the Lamb

The lamb was transferred to an infant warmer (Fisher and Paykel Healthcare, Auckland, New Zealand) for temperature maintenance. Resuscitation was initiated with positive pressure ventilation, in air, by T-piece device (Neopuff; Fisher and Paykel Healthcare, Auckland, New Zealand) with peak inflation pressure 30 cmH_2O and end-expiratory pressure 5 cmH_2O, targeting 60 breaths per minute.

After one minute, chest compressions were commenced with a target of 90 compressions and 30 ventilation breaths per minute, and the fraction of inspired oxygen was increased to 1.00 as per resuscitation guidelines. For groups 3 and 4, AC was applied at commencement

of chest compressions, and maintained until achievement of ROSC or cessation of resuscitation. AC was achieved by flexing the lower limbs onto the abdomen. An oval pressure pad held in place by a polypropylene strap encircling the abdomen was used to hold the limbs in the flexed position, with additional downward pressure applied manually by a member of the research team, with the aim of providing pressure continuously through the resuscitation.

The first treatment dose of either intravenous epinephrine or saline was administered via the jugular vein catheter after one minute of chest compressions, and every three minutes thereafter, if ROSC had not been achieved. Lambs allocated to epinephrine could receive a maximum of five doses. Lambs allocated to saline could receive three doses, after which two 'rescue' epinephrine doses (20 micrograms/kg) could be administered. Resuscitation was ceased at 15 minutes after ventilation onset if ROSC had not been achieved. At achievement of ROSC, AC was ceased immediately.

Support After Return of Circulation

After ROSC, lambs received pressure-limited volume guarantee ventilation at a tidal volume of 7ml/kg (Dräeger Babylog 8000+, Dräeger, Lübeck, Germany) using heated humidified gas (F&P 950 System, Fisher and Paykel, Auckland New Zealand). Ventilation parameters were digitally recorded. Lambs were sedated for comfort (Alfaxan IV 5-15mg/kg/h in 5% dextrose). Blood gas samples (ABL30, Radiometer, Copenhagen, Denmark) were collected every three minutes until 15 minutes after ROSC, then at 20, 25, 30, 40, 50, and at study completion at 60 minutes. Ventilation settings were adjusted to target SpO_2 90-95% and $PaCO_2$ 35-45 mmHg. At study completion, ewes and lambs were euthanized using an

intravenous overdose of sodium pentobarbitone (>100 mg/kg, Lethobarb, Virbac, Australia, Pty LTD).

Brain Histology

Immediately after euthanasia, the lamb brain was removed, weighed and the hemispheres separated along the midline. The cerebellum was removed from the brain at the level of the cerebellar peduncles. The whole brain was immersion-fixed in 10% formalin for ~5 days at 4°C. After fixation, the right hemisphere was cut into coronal slices (5mm in thickness), processed and wax embedded, then sectioned at the level of the parietal lobe (10-micron sections). Sections were mounted on Superfrost microscope slides for histological staining with a 0.5% Cresyl Violet and 1% Acid Fuchsin solution. For each lamb, two sections were assessed.

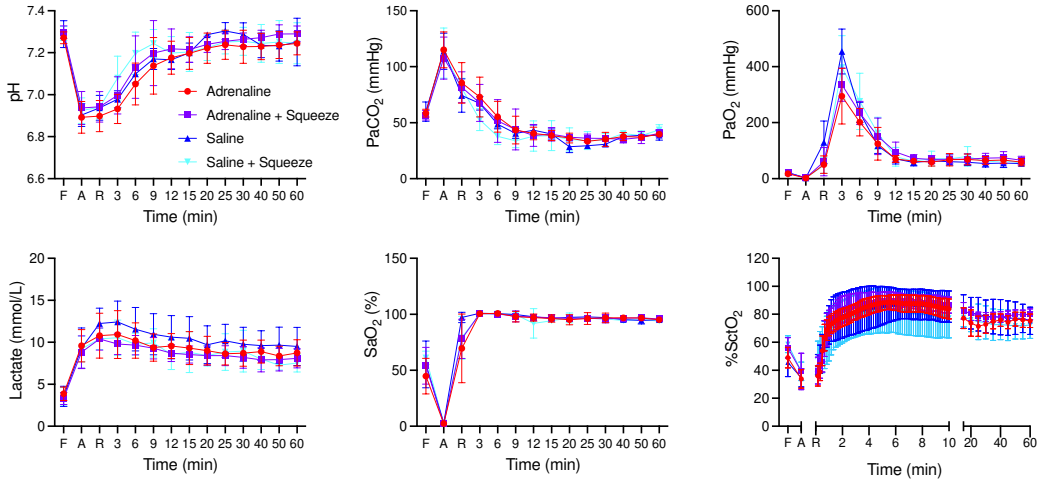
Slides were scanned at 40x magnification using Aperio Scanscope AT Turbo (Leica Biosystems, Germany). The presence or absence of microbleeds was assessed within four brain regions: cortical grey matter, white matter (subcortical white matter and periventricular white matter combined), deep grey matter (including the thalamus) and hippocampus (including both the dentate gyrus and cornu ammonis). A total of 8 field of views (FOV; 600 µm x 600 µm) were randomly placed within each region. FOV placements was kept consistent across all sections from all animals and conducted by an assessor blinded to the groups.

Quantitative analysis of microbleeds were assessed within FOVs and was performed on coded slides (observer blinded to group) using image analysis software (ImageScope, Aperio technologies, Vista, CA, USA).
Micro-bleeds were identified according to the following criteria:
1. Indistinguishable basement membrane,
2. Random dispersion of erythrocytes and
3. Irregular shape of perimeter.

These criteria are characteristic of blood extravasation. The total number of microbleeds were averaged between the two sections per lamb and the incidence of lambs with a presence of microbleeds within each group was calculated.

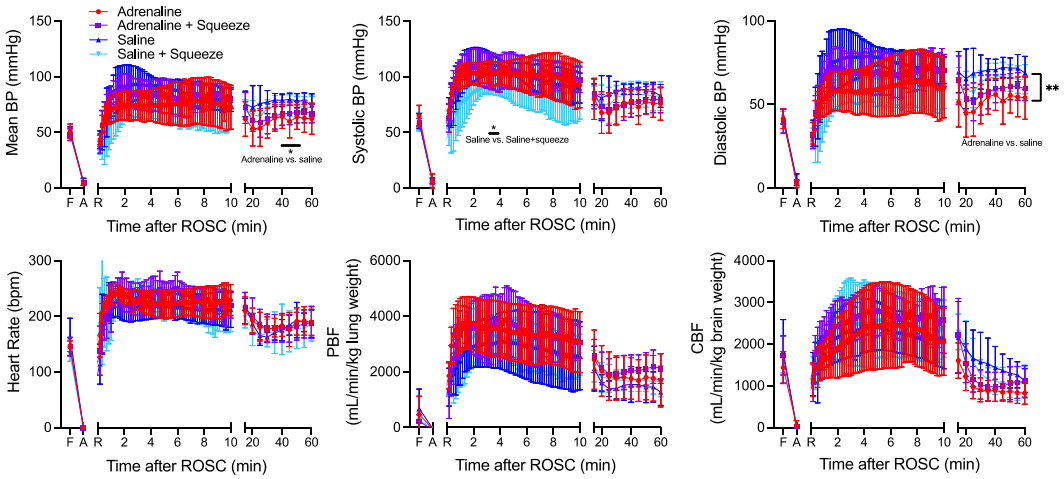
Supplemental Figure 1: Blood gas parameters following ROSC

A) pH, B) $PaCO_2$, C) PaO2, D) Lactate, E) SaO2, and F) SctO2 in Epinephrine (red), Epinephrine+AC (purple), Saline (dark blue) and Saline+AC (light blue) lambs. Data are mean ± SD. No statistically significant differences were identified.



Supplemental Figure 2: Physiological parameters following ROSC

A) Mean, B) Systolic and C) diastolic blood pressure (BP) measured form the carotid artery, D) Heart rate, E) pulmonary blood flow (PBF) and F) carotid arterial blood flow (CBF) in Epinephrine (red), Epinephrine+AC (purple), Saline (dark blue) and Saline+AC (light blue) lambs. Data are mean ± SD. * indicates $p<0.05$, ** indicates $p<0.0025$.


Adrenaline
Adrenaline + Squeeze
Saline
Saline + Squeeze
Mean BP (mmHg)
Systolic BP (mmHg)
Diastolic BP (mmHg)
Heart Rate (bpm)
PBF (mL/min/kg lung weight)
CBF (mL/min/kg brain weight)
Adrenaline vs. saline
Saline vs. Saline+squeeze
**
F A R 2 4 6 8 10 20 40 60
Time after ROSC (min)