SUPPLEMENTAL DIGITAL CONTENT – 1

DESCRIPTION AND VALIDATION OF AN OVINE MODEL OF SEPTIC SHOCK

SUPPLEMENT INDEX

A) SHEEP PREPARATION

Approval was obtained from the Animal Ethics Committees of all institutions involved and studies were conducted according to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th Edition, 2004).

Mature non-lactating female sheep (*Ovis aries,* Merino) were randomly chosen from a herd of similarly aged (> 3 years old) paddock raised healthy agricultural stock. Sheep grazed freely until the day prior to each study when they were housed with other animals in pens with free access to food and water.

At least four weeks prior to the commencement of each study, animals had undergone a thoracotomy for ligation of their left hemiazygous vein to allow subsequent sampling of coronary venous blood (in sheep, the coronary sinus receives blood from the coronary veins and the left hemiazygous vein).

Following an overnight fast, a sheep was removed from their pen and had anaesthesia induced by intravenous (i.v.) injection of Thiopentone (1 g), the trachea was intubated and anaesthesia maintained with spontaneous ventilation of Isoflurane (2%) in oxygen.

An indwelling urethral catheter (Bard, 14Fr, Covington, GA, USA) was placed into the urinary bladder and attached to a burette allowing hourly measurements. Note that male sheep were not considered for this model as the tortuous anatomy of their urethra renders bladder catheterisation difficult.

The animal's neck was shaved and topical iodine applied to the skin. An incision was made to expose the carotid sheath for insertion of vascular catheters. Following dissection of the carotid artery, an arterial catheter (Angiocath 1.7 x 133 mm, Becton Dickinson Pty Ltd, Franklin Lake, NJ, USA) was placed in the right carotid artery. The catheter was secured by silk ligature around the artery and then connected to a pressure monitoring line fluid filled with heparinised saline.

A length of jugular vein was dissected and exposed for insertion of a range of venous catheters. A four lumen central venous catheter (CVC) (8.5 Fr x 20 cm, Arrow International Inc., Reading, PA, USA) and a large bore single lumen catheter (8.5 Fr x 10 cm, Arrow International Inc., Reading, PA, USA) were placed into the right jugular vein.

A pulmonary artery catheter that allowed continuous cardiac output monitoring (Swan Ganz CCOmbo 7.5 Fr x 110 cm, Edwards Lifesciences, Irvine, CA, USA) was inserted via the large bore single lumen catheter. Under real time radiology imaging, the catheter was advanced into the right ventricle and the flow directed balloon inflated. The catheter was then floated and wedged into a branch of the pulmonary artery with confirmatory pressure waveforms. The balloon was deflated, catheter secured and connected to a pressure monitoring line containing heparinised saline to monitor pulmonary artery and central venous pressures.

Through the exposed length of right jugular vein, a wire (Glidewire, 0.89 mm x 180 cm, Terumo, Somerset, NJ, USA) was passed with radiological guidance into the coronary sinus, renal, hepatic and iliac veins. An angulated venous catheter (Vista Brite Tip, 7 Fr x 100 cm, Cordis, Bridgewater, NJ, USA) was passed over the wire into each vein and the position confirmed radiologically using intravenous contrast (Ultravist-370, Iopromide 769 mg/ml, Bayer Healthcare, Leverkusen, Germany). Catheters were secured to the jugular vein by silk ligature and flushed every four hours with heparinised saline.

The proximal trachea was exposed through a separate incision and a tracheostomy tube inserted (Portex, ID 9.0, 24 cm, Smith's Medical, St Paul, MN, USA). A rumen tube (16 gauge, Bard Medical, Covington, GA, USA) was inserted via the nose.

B) SUPPORTIVE CARE PROTOCOL

Sheep were placed in a sling and supported upright for the duration of each study. Medical and nursing staff with intensive care unit (ICU) expertise provided continuous care and monitoring of each animal. Supplementary nutrition was not provided as ruminants have residual nutrition in their gut that provides energy for more than 24 hours of fasting. Drooled sheep saliva was collected and replaced via the rumen tube every hour. Ambient temperature was 22^{o}C and warming blankets were available if animal core temperature was less than 36^{o}C.

Sedation / Analgesia

Sedation and analgesia were provided to ensure comfort of the ventilated animal and to replicate ICU care. Animals received i.v. infusion of midazolam (0.1 – 0.5 mg/kg/h) and ketamine (1 – 5 mg/kg/h) with boluses as required. Depth of sedation was continuously monitored and sedation titrated to depress the eyelash blink reflex and abolish nasal flaring. Volatile anaesthesia and further thiopentone were immediately available if required.

Ventilation

Sheep received synchronised intermittent mandatory ventilation (SIMV) using tidal volume (V_T) of 10 mL/kg and positive end expiratory pressure (PEEP) of 5 cmH_2O (Puritan Bennett 7200, Boulder, CO, USA). Respiratory rate and inspiratory flows were adjusted to maintain end tidal CO_2 of 30 mmHg (Intellivue MP 50, Phillips, Best, The Netherlands) with an inspiratory to expiratory ratio of 1:2. Fraction of inspired oxygen (FiO_2) was adjusted to maintain pulse O_2 saturation (SpO_2) greater than 95%. The normal V_T of the sheep is 4 – 9 mL/kg with anatomical dead space of 100 – 200 mL (1). The slightly higher V_T used in these ventilated sheep was to overcome ventilation circuit dead space.

A heat moisture exchanger was used (Medical Filter BB 22-15, Pall Corporation, Port Washington, NY, USA) and tracheal suctioning was performed hourly using an inline suction catheter. Peak and plateau inspiratory pressures and pulmonary compliance

(V_T/P_{plat}) were recorded hourly. Note that the pulmonary compliance in this model was measured using the plateau airway pressure at end expiration in non-paralysed animals supported by a sling under their chest. This arrangement impairs chest wall compliance and hence the calculated pulmonary compliance is used as a guide only.

Haemodynamics

Heart rate (HR), rhythm, mean arterial pressure (MAP), central venous pressure (CVP) and pulmonary artery pressure (AP) were continuously monitored and recorded hourly (Intellivue MP 50, Phillips, Best, The Netherlands). Pressure transducers (Transpac, Hopsira, Lake Forest, IL, USA) were located at the sheep elbow. Cardiac output was continuously measured (Vigilance Monitor, Edwards Lifesciences, Irvine, SA, USA), indexed for sheep body surface area (weight $(kg)^{0.67}$ x 0.0842) (2) and recorded every 10 minutes. Derived haemodynamic parameters were calculated using standard formulas and recorded hourly.

Parenteral fluids (Hartmann's solution) were administered by peristaltic pump at 3 mL/kg/h (Baxter Colleague CXE, Deerfield, IL, USA), as this is the maintenance fluid requirement for sheep (1). Total fluid intake (maintenance fluids + fluid boluses + drugs) and fluid output (urine) were measured for each animal.

Blood pressure targets were MAP 75 mmHg and CVP 5 mmHg and are the normal circulatory pressures in sheep (1). CVP was measured at end expiration. If MAP was below 75 mmHg, a 250 mL fluid bolus (0.9% saline) was delivered until CVP ≥ 5 mmHg. If MAP remained below 75 mmHg, norepinephrine (NE) infusion was commenced and titrated according to a management protocol, with maximum dose 1 μg/kg/min (*Table S1.1*). The NE dose and MAP were recorded every 10 minutes.

Study Termination

Due to the level of intervention on the animals and the severity of illness induced, it was considered most ethical that at the completion of each study, animals were to receive a lethal dose of pentobarbitone i.v. (6.5 g).

Table S1.1: Ovine model haemodynamic management protocol.

If MAP < 75 mmHg and CVP < 5 mmHg → administer 250 mL 0.9% saline bolus If MAP < 75 mmHg and CVP ≥ 5 mmHg → NE infusion and titrate 2 minutely (maximum NE dose 1 μg/kg/min)	
MAP (mmHg)	NE dose adjustment
> 100	↓ 10 μg/min
96 – 100	↓ 5 μg/min
91 – 95	↓ 3 μg/min
86 – 90	↓ 2 μg/min
81 – 85	↓ 1 μg/min
75 – 80	No change
70 – 74	↑ 1 μg/min
65 – 69	↑ 2 μg/min
60 – 64	↑ 3 μg/min
55 – 59	↑ 5 μg/min
50 – 54	↑ 10 μg/min

MAP = mean arterial pressure, CVP = central venous pressure, NE = norepinephrine

C) BLOOD ANALYSIS

Arterial blood samples were collected at 0, 2, 12 and 26 hours and placed into clot activator tubes, K3-ethylene-diamin-etra-acetic acid (EDTA) and sodium citrate tubes. Blood biochemical analysis included serum electrolytes (Na^+, K^+, Cl^-, HCO_3^-), creatinine, urea, bilirubin, alkaline phosphatase (ALP) and alanine aminotransferase (ALT) (Olympus AU5400 chemistry analyser, Olympus America Inc., NY, USA). Haematology cell counts were obtained with a SYSMEX-XE-2100 haematology analyser (TOA Medical Electronics, Kobe, Japan) and clotting times using STA-R Evolution (Roche/Stago, Diagnostica Stago Pty Ltd, Doncaster, Australia).

Blood samples in EDTA tubes were rested for 30 minutes then centrifuged at 3000 rpm for 10 minutes with the supernatant (plasma) placed into Eppendorf vials and stored at minus 80^{o}C until ready for batch hormone and cytokine assay.

Samples were collected from systemic arterial, pulmonary arterial and each venous catheter at 0, 2, 4, 8, 12, 16, 20 and 26 hours. Blood for pH, gas analysis and haemoglobin-oxygen saturation (Hb-O_2 %) were collected into lithium heparin coated syringes (Siemens) and analysed on a RAPID-Point 405 Blood-gas Analyser (Siemens Healthcare Diagnostics Inc., Norwood, MA, USA). Blood for lactate analysis was collected into fluoride EDTA tubes and measured using an Olympus AU5400 chemistry analyser (Olympus America Inc., NY, USA).

Blood cultures were taken aseptically from the left internal jugular vein at baseline, repeated at 12 and 26 hours and analysed on the Bactec system (Becton, Dickinson and Company, USA).

Hormone / Cytokine Assays

Plasma free T3 concentrations were measured by Chemiluminescent Micro-particle Immuno-Assay (CMIA). A monoclonal antibody (MAb) conjugated with alkaline phosphatase competitively binds to free T3 in the sample and T3 attached to a paramagnetic particle. The paramagnetic particle-T3-MAb complexes are isolated and a phosphatase reaction emits light measured by a luminometer. This was performed as an automated assay (Beckman Coulter Unicel DXi 800) using commercially available reagents and calibration solutions ("Access", free T3, A13422, Beckman Coulter Inc., USA, 2005). Description of the type of MAb used is under patent protection, however correspondence from the company confirms the MAb is not raised in sheep (Personal communication, Ms Reshimi Sharma, Beckman Coulter Inc., May 2010). The analytical range of this assay is 1.4 – 46 pmol/L with intra-assay coefficient of variation (CV) 8.0 – 10.6% with T3 in the range of 3.4 – 14.7 pmol/L.

Free T4 concentrations were measured by CMIA with an analytical range of 1.9 – 77.2 pmol/L and intra-assay CV of 6 – 8% for samples measured between 11 – 61 pmol/L ("Access", free T4, A3307A, Beckman Coulter Inc., USA, 2006).

Radioimmunoassay (RIA) with rabbit MAb was used to determine plasma concentrations of rT3 (RIAZEN Reverse T3, R-EW-MZ-002, ZenTech, Liege, Belgium, 2006) and cortisol (3). Sensitivity of this rT3 RIA was 0.01 nmol/L with intra-assay CV < 10%. The cortisol assay had an analytical range of 0.5 – 69 nmol/L with intra-assay and inter-assay CV 8% and 16% respectively.

Plasma concentrations of Interleukin 6 (IL-6) and Tissue Necrosis Factor α (TNFα) were measured by Enzyme Linked Immunosorbent Assay (ELISA). These assays used rabbit polyclonal cytokine antibody then swine anti-rabbit horseradish peroxidase conjugated immunoglobulin. Recombinant molecules were used for standard curves and assays were read on EL_X80 Universal Microplate Reader (Bio-tek Instruments, VT, USA) (4).

D) *E. coli* PREPARATION

An intravenous infusion of *E.coli* was chosen as the infectious challenge as it was considered the most suitable combination of clinical relevance and reliability to undertake a complex large animal pre-clinical sepsis trial.

For each septic animal study, a fresh solution of *E. coli* was prepared (FDA Strain Seattle, 1946, ATCC 25922). A working culture of *E. coli* was subcultured onto a Columbia Horse Blood agar plate and incubated overnight at 35ºC ± 2ºC in CO_2 atmosphere. Colonies of bacteria were then emulsified into 100 mL 0.85% sterile saline and suspension adjusted to yield a spectrophotometric optical density of approximately 0.10 at 550 nm (Shimadzu UV-160A) which corresponded to a concentration of approximately 10^8 Colony Forming Units (CFU) / mL. The number of CFUs / mL in each suspension was determined by plating 100 μL of a 10^{-5} serial dilution onto three separate Columbia Horse Blood agar plates and counting viable colonies after overnight incubation.

E) VALIDATION OF THE OVINE MODEL OF SEPTIC SHOCK

Eleven sheep were studied individually with alternate sheep rendered septic by infusion of *E. coli* (10^8 CFU / kg) i.v. over one hour. All sheep received ongoing ICU supportive care, monitoring and blood sampling as described for 26 hours. To ensure persistence of bacteraemia and a suitable severity of sepsis, antibiotics were not administered.

Data from septic and non-septic sheep were compared using: 1) unpaired t-tests for outcomes measured at a single point in time, and 2) linear mixed effects models for outcomes measured at multiple time points. Data was stored in Excel (Microsoft, 2008, version 12.3.6), analysed with SPSS (IBM, 2012, version 21) and presented as means (± SD on tables, ± SEM on graphs).

Group Characteristics

Six sheep were given *E. coli* (mean dose 1.0 ± 0.1 x 10^8 CFUs / kg); five survived to 26 hours and one animal died at 21 hours (mortality rate 17%). All five non-septic sheep survived 26 hours.

All animals were suitably sedated throughout the study without need for further anaesthesia. There was no difference in the mean amount of midazolam (mean ± SD mg/kg/h; non septic 0.3 ± 0.1 vs. septic 0.3 ± 0.1, p = 0.88) or ketamine (mean ± SD mg/kg/h; non septic 5.7 ± 1.4 vs. septic 5.3 ± 1.4, p = 0.44).

Blood cultures were sterile at baseline in all animals. Sheep inoculated with *E.coli* had this same species in blood cultures at 12 and 26 hours while all non-septic animals maintained sterile blood cultures. Temperature increased over time but there was no difference between septic and non-septic groups (*Figure S1.1*).

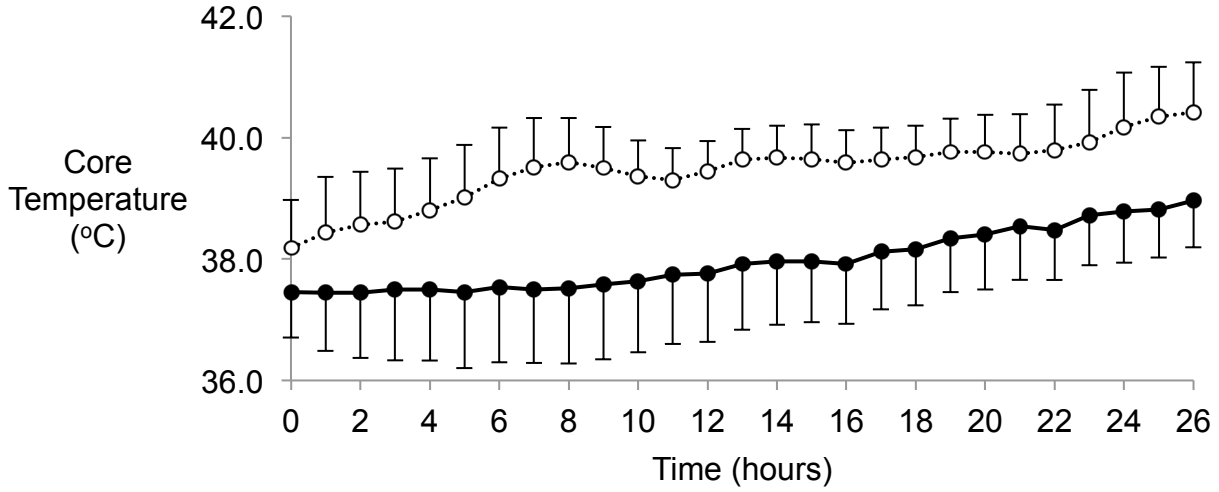

Figure S1.1: Temperature in non-septic (●, n=5) and septic (○, n=6) sheep managed with intensive care support for 26 hours.
Group x Time p = 0.39, Group p = 0.34, Time p = 0.01

Haemodynamic Parameters (*Figures S1.2, S1.3, S1.4*)

Septic animals developed a hyperdynamic cardiovascular response with a significant increase in cardiac index (CI), reduction of MAP and a requirement for NE. Non-septic animals maintained stable CI and MAP and did not require NE. HR was stable over time and did not differ between groups.

There was no significant difference in the amount of parenteral fluids administered (mean ± SD mL/kg; non septic 97 ± 13 vs. septic 115 ± 33 mL/kg, p = 0.27). CVP did not change over time and there was no difference between groups. PAP promptly increased following *E. coli* infusion and remained higher than non-septic animals.

SVRI changes were biphasic early after *E. coli* administration but after six hours steadily declined becoming markedly lower in septic sheep (*Figure S1.3*). There was a slight increase in RVSWI in septic sheep but other derived haemodynamic indices did not differ between groups (*Figure S1.3*).

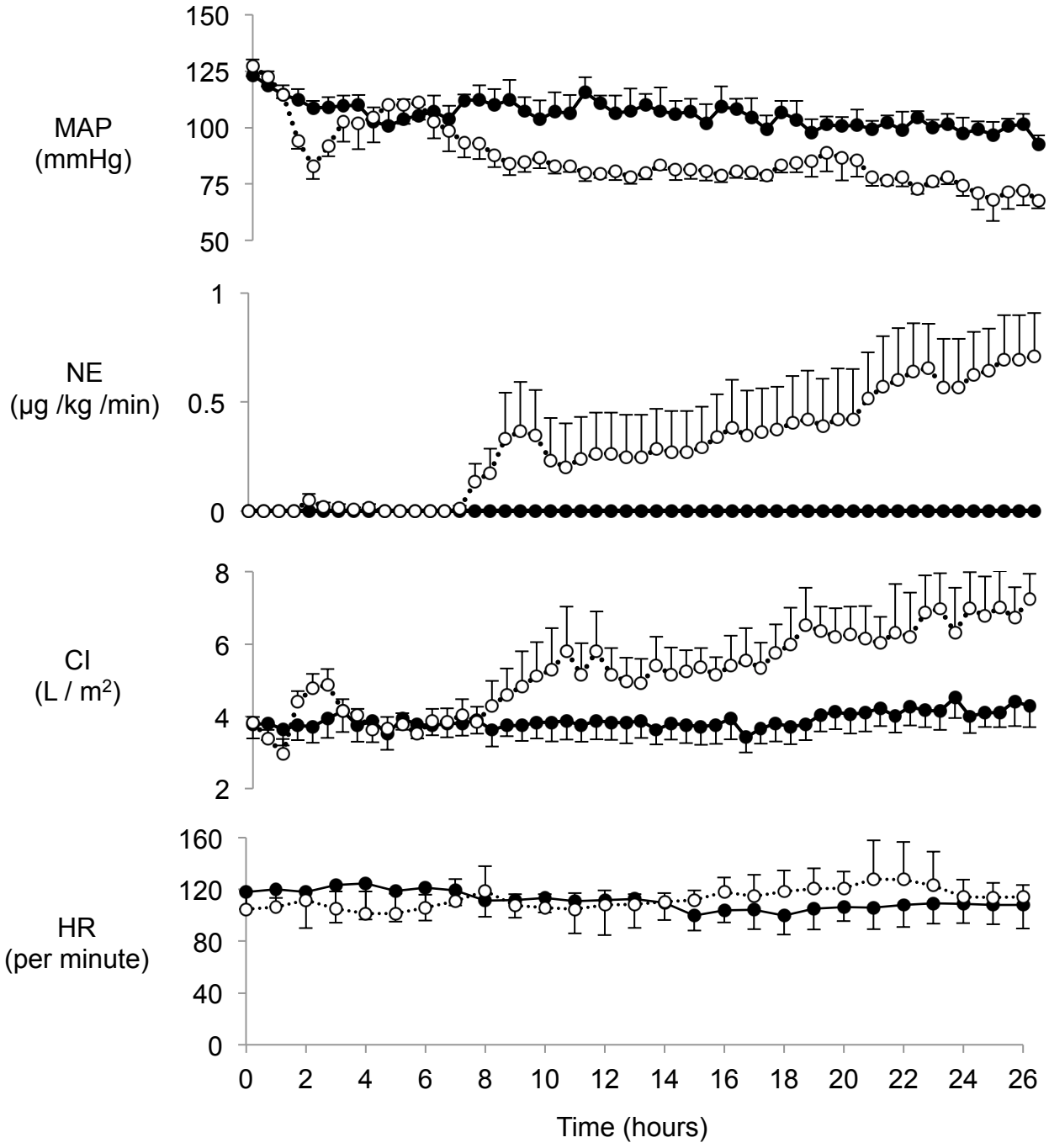

Figure S1.2: Mean arterial pressure (MAP), cardiac index (CI), norepinephrine (NE) dose and heart rate (HR) in non-septic (●, n=5) and septic (○, n=6) sheep managed with intensive care support for 26 hours. The non-septic sheep did not require NE.

MAP: Group x Time $p = 0.10$, Group $p < 0.01$, Time $p < 0.01$

CI: Group x Time $p < 0.01$

HR: Group x Time $p = 0.48$, Group $p = 0.97$, Time $p = 0.97$

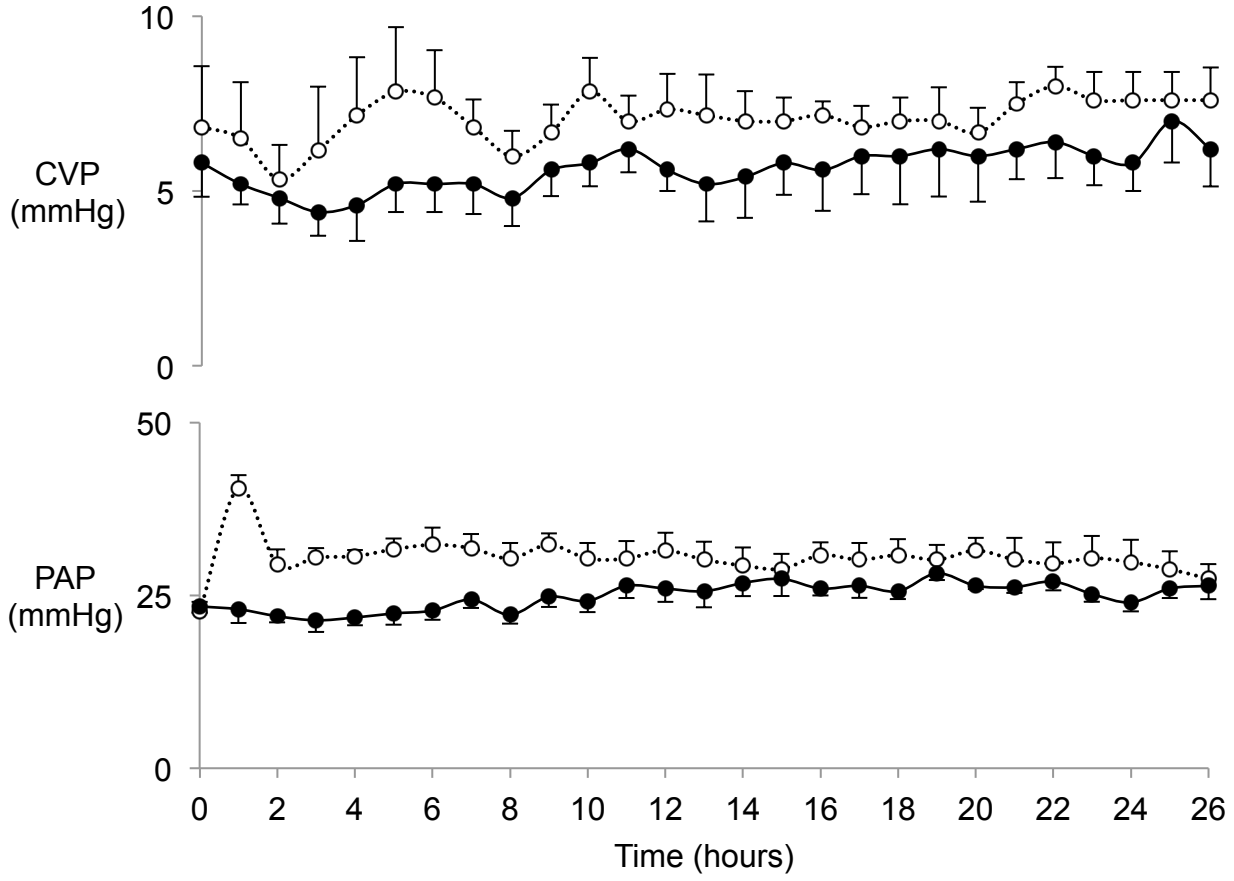

Figure S1.3: Central venous pressure (CVP) and mean pulmonary artery pressure (PAP) in non-septic (●, n=5) and septic (○, n=6) sheep managed with intensive care support for 26 hours.

CVP: Group x Time p = 0.70, Group p = 0.17, Time p = 0.12

PAP: Group x Time p < 0.01

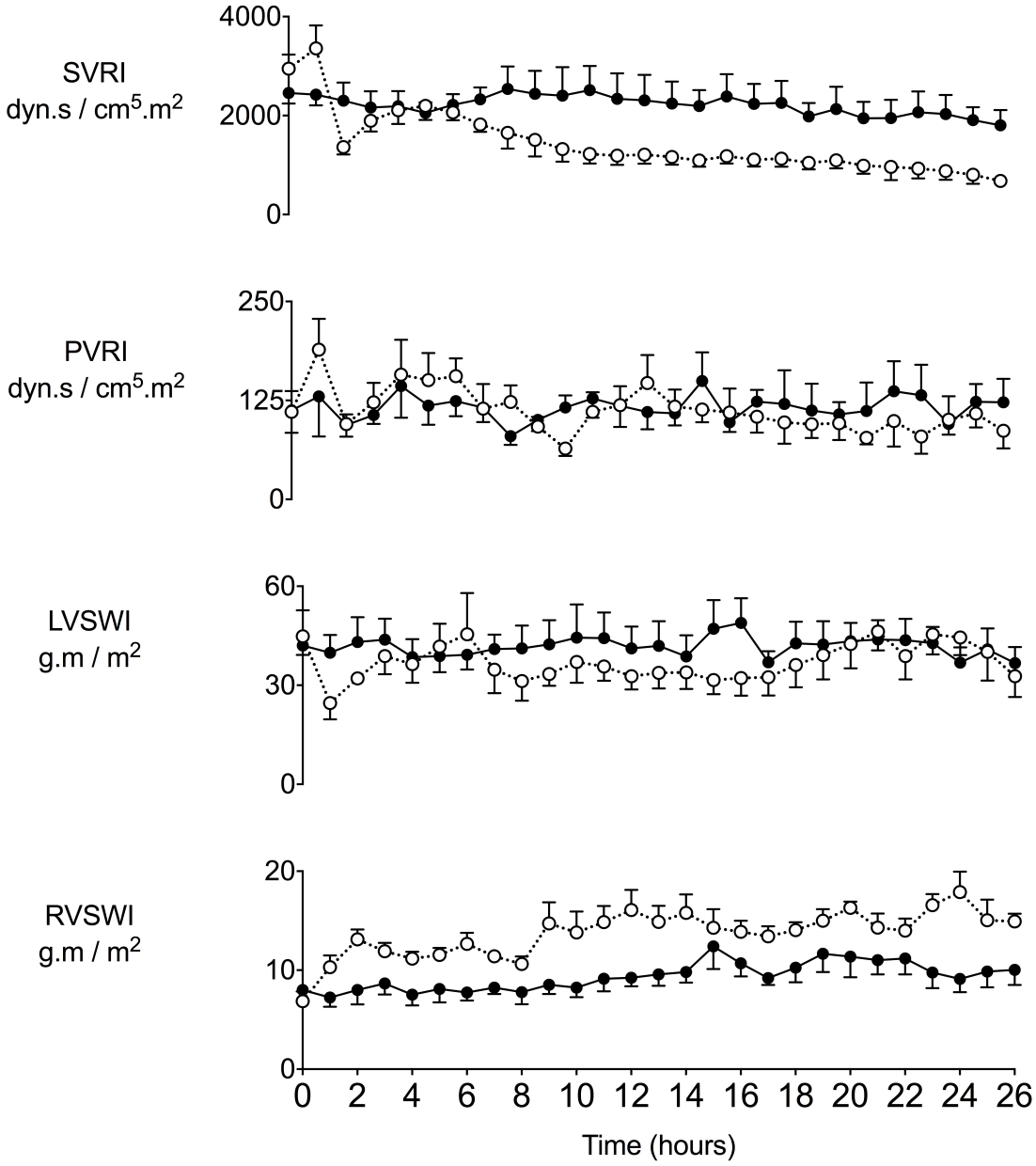

Figure S1.4: Systemic vascular resistance index (SVRI), pulmonary vascular resistance index (PVRI), left ventricular stroke work index (LVSWI) and right ventricular stroke work index (RVSWI) in non-septic (●, n=5) and septic (○, n=6) sheep managed with intensive care support for 26 hours.

SVRI: Group x Time $p < 0.01$

PVRI: Group x Time $p = 0.85$, Group $p = 0.39$, Time $p < 0.01$

LVSWI: Group x Time $p = 0.21$, Group $p = 0.67$, Time $p = 0.07$

RVSWI: Group x Time $p = 0.05$

Respiratory Parameters (*Figure S1.5*)

The ventilation algorithm maintained equivalent $PaCO_2$ in both groups. Oxygenation was not impaired, with P:F ratios remaining high throughout the model and no difference between septic and non-septic sheep. Pulmonary compliance was stable for the duration of the model and there was no difference between groups over time.

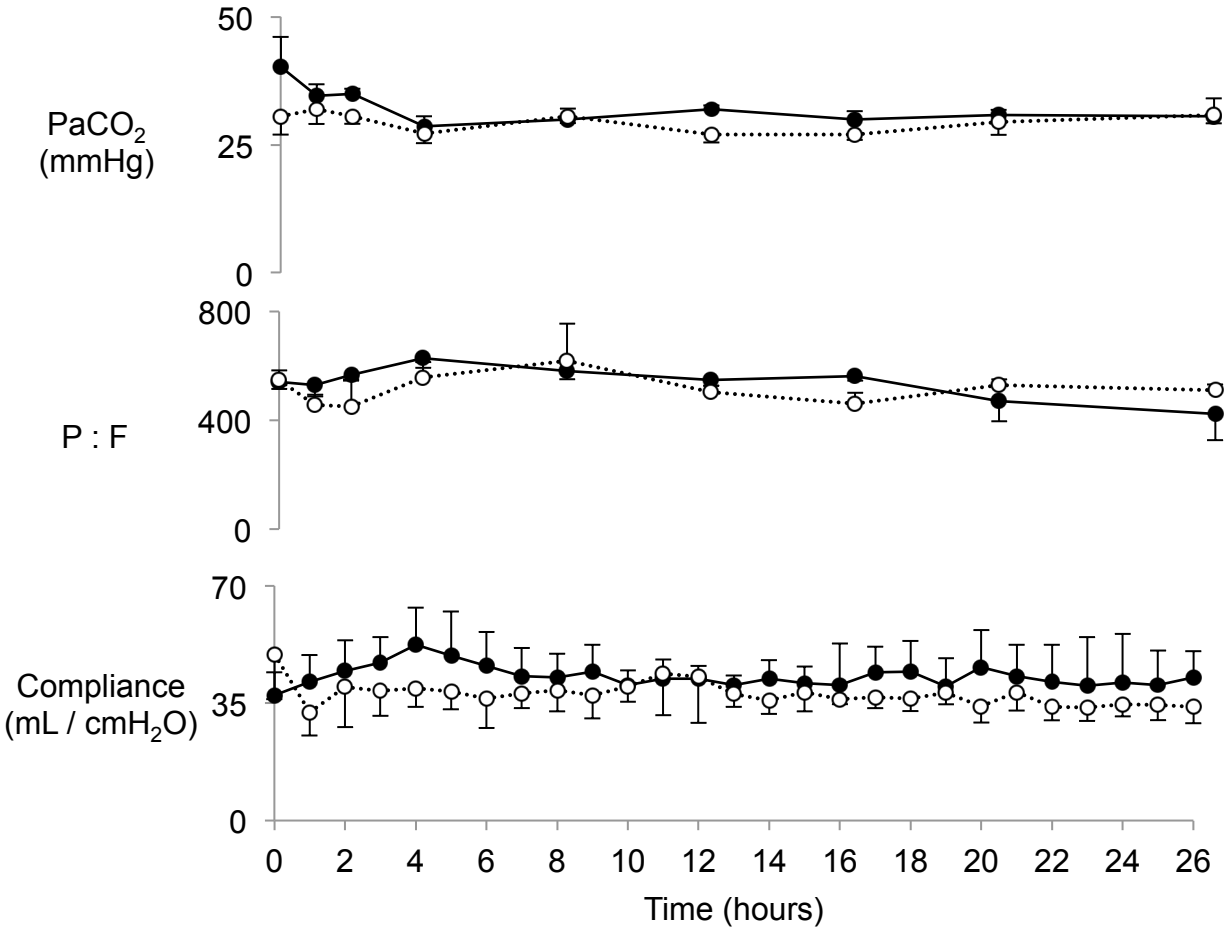

Figure S.5: $PaCO_2$, PaO_2:FiO_2 (P:F) and pulmonary compliance in non-septic (●, n=5) and septic (○, n=6) sheep managed with intensive care support for 26 hours.

$PaCO_2$: Group x Time p = 0.28, Group p = 0.15, Time p = 0.07

P:F: Group x Time p = 0.55, Group p = 0.33, Time p = 0.38

Compliance: Group x Time p = 0.10, Group p = 0.12, Time p = 0.26

Renal Parameters *(Figure S1.6, Table S1.2)*

Serum electrolytes, urea and creatinine concentration at baseline were within the normal range for sheep (5, 6). Both groups of sheep received similar amounts of radiological contrast (mean ± SD mL; non septic 74 ± 13 vs. septic 50 ± 13, p = 0.34). Hourly urine output declined over time but was not significantly different between septic and non-septic sheep. After 26 hours, total urine output was similar between groups (mean ± SD mL/kg; non septic 24 ± 2 vs. septic 31 ± 18, p = 0.42). Over the course of the study, septic sheep developed significantly increased serum creatinine, urea and K^+.

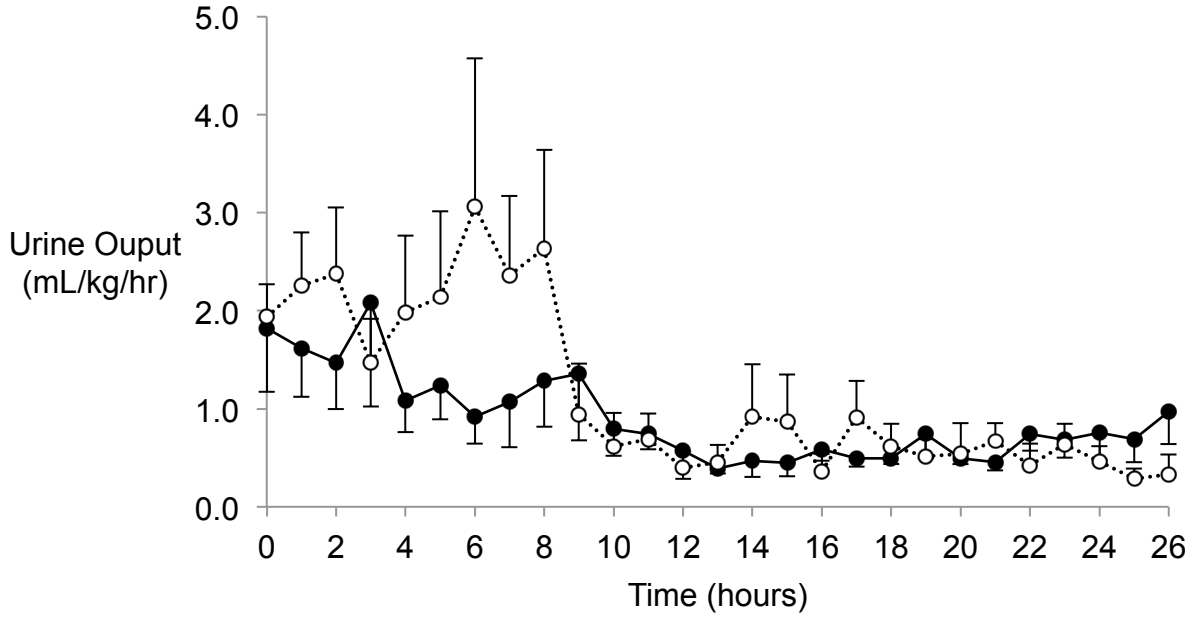

Figure S1.6: Hourly urine output in non-septic (●, n=5) and septic (○, n=6) sheep managed with intensive care support for 26 hours.

Group x Time p = 0.26, Group p = 0.51, Time p < 0.01

Table S1.2: Biochemical parameters in non-septic (n = 5) and septic sheep (n = 6) managed with intensive care support for 26 hours. * $p < 0.05$ for differences of adjusted means in non septic vs. septic sheep.

		0 hrs	**2 hrs**	**12 hrs**	**26 hrs**	Group x Time	Time	Group
Na^+ (mmol/L)	Non septic	146 ± 1	147 ± 1	147 ± 2	148 ± 1	p=0.44	p=0.06	p=0.98
	Septic	145 ± 2	146 ± 1	149 ± 5	148 ± 3			
K^+ (mmol/L)	Non septic	3.8 ± 0.3	3.3 ± 0.4	3.7 ± 0.5	3.8 ± 0.2	p<0.01		
	Septic	3.8 ± 0.3	3.1 ± 0.2	4.1 ± 0.5 *	5.9 ± 1.2 *			
Cl^- (mmol/L)	Non septic	107 ± 3	110 ± 2	118 ± 2	121 ± 3	p=0.01		
	Septic	109 ± 2	112 ± 2	113 ± 3 *	116 ± 6			
HCO_3^- (mmol/L)	Non septic	29 ± 3	24 ± 2	18 ± 1	17 ± 2	p=0.75	p<0.01	p=0.03
	Septic	27 ± 4	20 ± 2	13 ± 5	12 ± 4			
Anion Gap (mmol/L)	Non septic	14 ± 3	16 ± 3	15 ± 2	13 ± 1	p<0.01		
	Septic	13 ± 4	17 ± 3	27 ± 10 *	26 ± 8 *			
Urea (mmol/L)	Non septic	9 ± 2	8 ± 2	7 ± 2	7 ± 2	p<0.01		
	Septic	9 ± 1	8 ± 1	10 ± 1 *	12 ± 2 *			
Creatinine (μmol/L)	Non septic	97 ± 19	84 ± 10	75 ± 14	76 ± 16	p<0.01		
	Septic	91 ± 12	87 ± 30	197 ± 79 *	302 ± 156 *			

Hepatic Parameters (*Table S1.3*)

ALP, ALT and bilirubin remained within the normal range for all sheep throughout the study (5, 6). Serum ALP levels differed between groups over time, but this statistical difference reflects baseline variability. Bilirubin and ALT slightly increased over time but there was no difference between septic and non-septic sheep.

		0 hrs	**2 hrs**	**12 hrs**	**26 hrs**	Group x Time	Time	Group
ALP (U/L)	Non septic	85 ± 29 *	64 ± 16	51 ± 16	42 ± 10	p<0.01		
	Septic	45 ± 14	53 ± 21	72 ± 43	64 ± 31			
Bilirubin (μmol/L)	Non septic	4 ± 2	7 ± 1	11 ± 5	10 ± 3	p=0.27	p<0.01	p=0.12
	Septic	5 ± 1	5 ± 2	8 ± 2	7 ± 2			
ALT (U/L)	Non septic	11 ± 3	12 ± 4	19 ± 6	25 ± 7	p=0.07	p<0.01	p=0.20
	Septic	10 ± 2	11 ± 3	24 ± 11	37 ± 8			

Table S1.3: Hepatic biomarkers in non-septic (n = 5) and septic sheep (n = 6).

* p <0.05 for differences of adjusted means in non septic vs. septic sheep.

Haematological Parameters (*Figure S1.7, Table S1.4*)

Haematological parameters at baseline were within the normal range for sheep (5, 7). Following administration of *E. coli*, haemoglobin (Hb) concentration and packed cell volume (PCV) significantly increased and there was a transient reduction of white cell count (WCC). Septic animals developed a coagulopathy with increased clotting times (aPTT and PT) and low fibrinogen concentration. Platelet (PLT) counts declined over time but were not significantly different between septic and non-septic sheep.

TNFα sharply increased two hours after *E. coli* infusion and returned toward baseline by six hours. IL-6 concentrations increased steadily over the 26 hours following *E. coli*. TNFα and IL-6 levels in non-septic animals did not change from baseline.

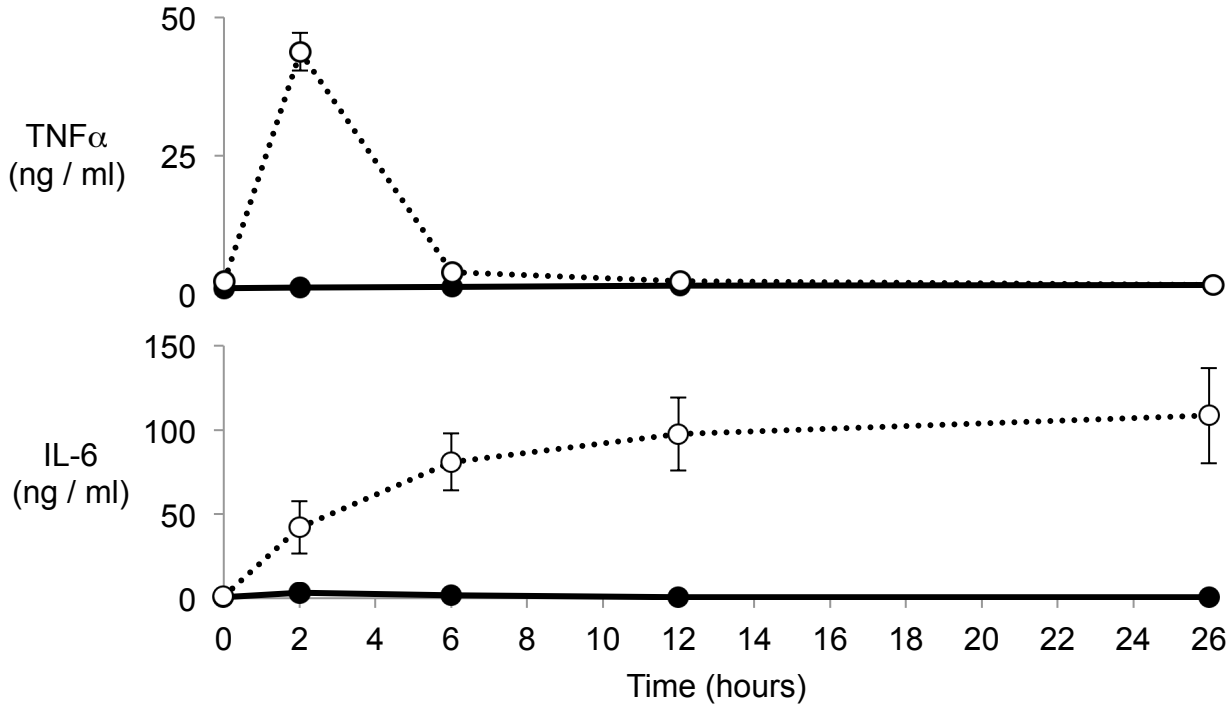

Figure S1.7: TNFα and IL-6 concentrations in non-septic (●, n=5) and septic sheep (○, n=6).

TNFα: Group x Time $p < 0.01$

IL-6: Group x Time $p < 0.01$

Table S1.4: Haematological parameters in non-septic (n = 5) and septic sheep (n = 6).

* p <0.05 for differences of adjusted means in non septic vs. septic sheep.

		0 hrs	**2 hrs**	**12 hrs**	**26 hrs**	Group x Time	Time	Group
aPTT (secs)	Non septic	33 ± 9	33 ± 4	34 ± 6	39 ± 5	p=0.03		
	Septic	29 ± 3	32 ± 4	42 ± 10	58 ± 18 *			
PT (secs)	Non septic	22 ± 1	22 ± 1	23 ± 1	24 ± 2	p<0.01		
	Septic	21 ± 1	24 ± 2	37 ± 9 *	68 ± 33 *			
Fibrinogen (g/L)	Non septic	1.6 ± 0.3	1.5 ± 0.3	1.9 ± 0.5	2.7 ± 0.7	p<0.01		
	Septic	2.0 ± 0.5	1.9 ± 0.3	0.9 ± 0.4 *	1.0 ± 0.6 *			
Hb (g/L)	Non septic	99 ± 3	106 ± 8	95 ± 14	92 ± 7	p<0.01		
	Septic	92 ± 9	132 ± 9 *	119 ± 14 *	108 ± 11 *			
PCV (L/L)	Non septic	0.35 ± 0.03	0.36 ± 0.02	0.34 ± 0.07	0.33 ± 0.05	p<0.01		
	Septic	0.32 ± 0.03	0.47 ± 0.04 *	0.43 ± 0.04 *	0.38 ± 0.05 *			
WCC ($x10^9$/L)	Non septic	6.5 ± 3.0	7.2 ± 3.6	9.1 ± 3.8	5.6 ± 3.5	p<0.01		
	Septic	4.8 ± 0.9	1.1 ± 0.1 *	3.6 ± 1.6 *	4.0 ± 1.1			
PLTs ($x10^9$/L)	Non septic	163 ± 79	160 ± 61	115 ± 36	106 ± 27	p=0.19	p=0.01	p=0.68
	Septic	166 ± 67	121 ± 90	110 ± 52	76 ± 51			

Metabolic Parameters (*Figures S1.8, S1.9*)

Both groups of sheep had a progressive fall in blood pH but there was no difference between groups over time. Serum lactate levels were significantly higher in septic sheep over time and this corresponded with a lower oxygen extraction ratio. Venous O_2 saturation in the pulmonary artery and coronary sinus was higher in septic sheep over time. Hb-O_2 saturation in blood from iliac, renal and hepatic veins did not significantly differ between septic and non-septic sheep.

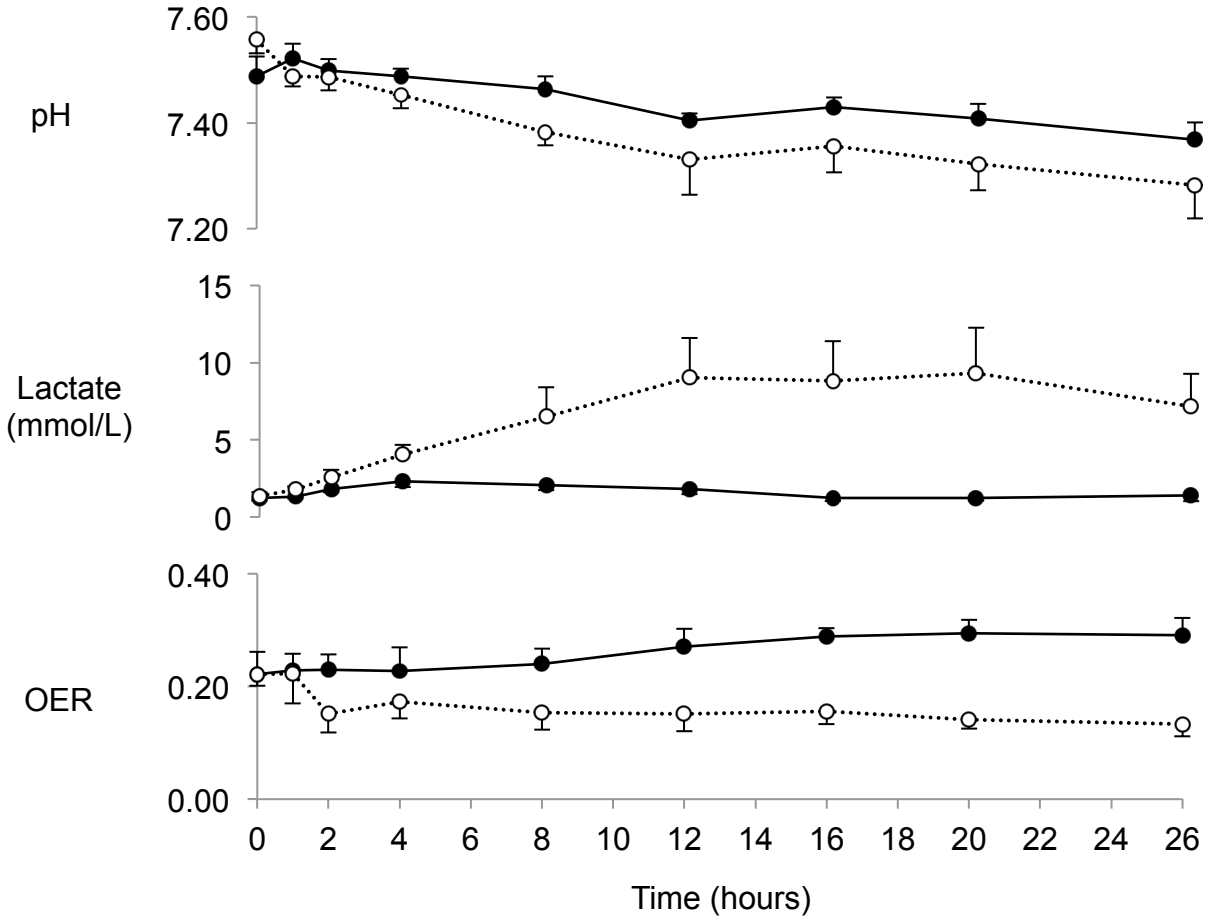

Figure S1.8: pH, lactate and oxygen extraction ratio (OER) in non-septic (●, n=5) and septic (○, n=6) sheep managed with intensive care support for 26 hours.

pH: Group x Time p = 0.29, Group p = 0.33, Time p < 0.01

Lactate: Group x Time p = 0.04

OER: Group x Time p = 0.03

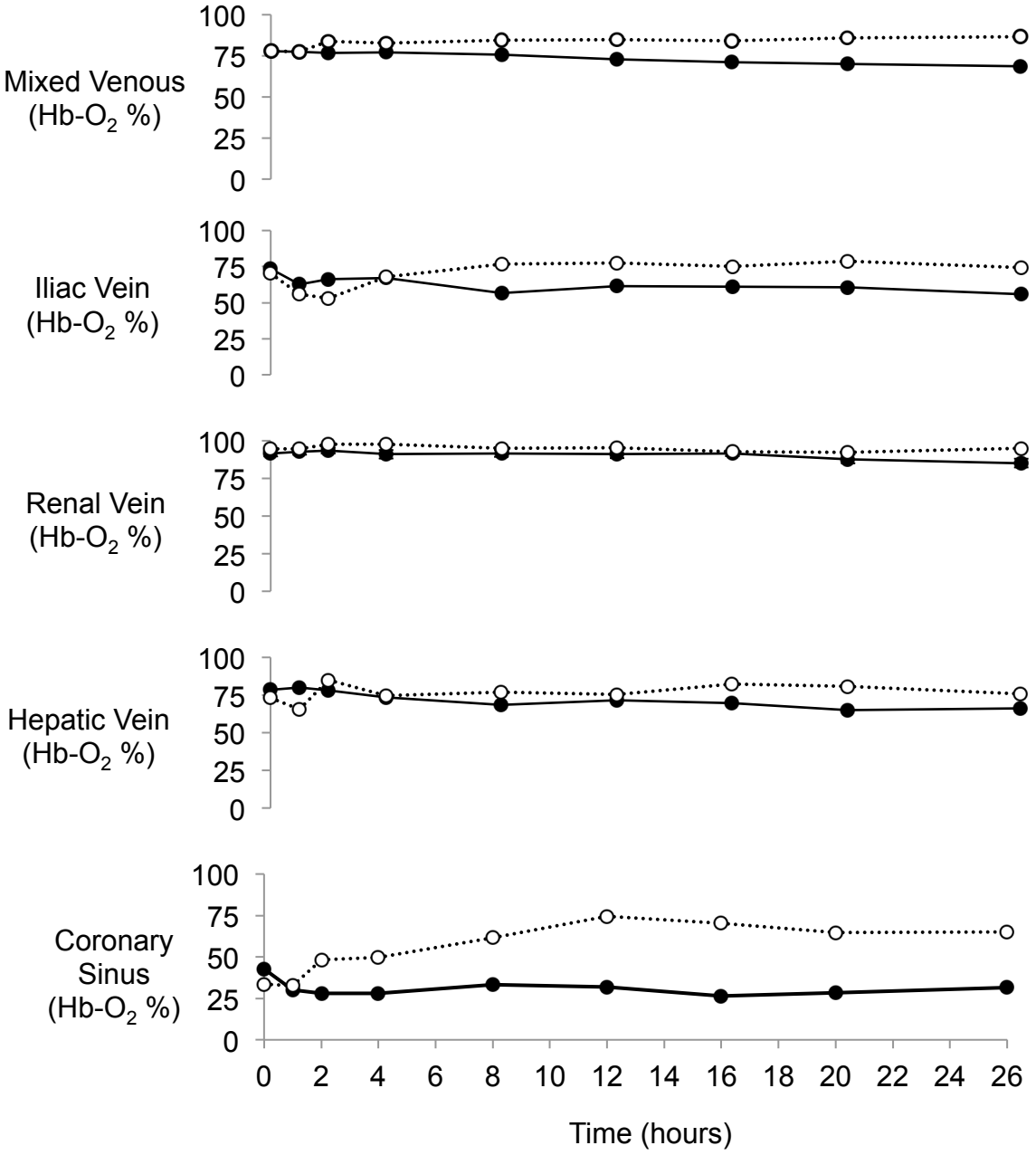

Figure S1.9: Venous haemoglobin-O_2 saturation (Hb-O_2 %) from pulmonary artery (mixed venous), coronary sinus, iliac, renal, and hepatic veins in non-septic (●, n=5) and septic (○, n=6) sheep managed with intensive care support for 26 hours.

Mixed Venous: Group x Time p = 0.03

Iliac Vein: Group x Time p = 0.32, Group p = 0.29, Time p = 0.26

Renal Vein: Group x Time p = 0.16, Group p = 0.09, Time p = 0.39

Hepatic Vein: Group x Time p = 0.17, Group p = 0.13, Time p = 0.36

Coronary Sinus: Group x Time p = 0.05

Endocrine Parameters (*Table S1.5*)

Plasma free T3 and free T4 concentrations measured at baseline were within normal ranges previously measured in sheep using the same assay techniques (6). Plasma free T3 declined over time and was lower in the septic group of sheep, but there was no difference between groups over 26 hours. The lack of a statistically significant difference between groups over time likely reflects small sample size and baseline variability. Plasma free T4 levels decreased and rT3 increased, with changes being significantly greater in septic sheep.

Normal plasma cortisol levels in sheep range between 42 – 82 nmol/L (5). At baseline, both septic and non-septic sheep had elevated plasma cortisol levels. Cortisol levels declined to normal levels by 12 hours in non-septic sheep but remained elevated in septic sheep.

Table S1.5: Plasma thyroid hormones and cortisol in non-septic (n = 5) and septic sheep (n = 6).

* $p < 0.05$ for differences of adjusted means in non septic vs. septic sheep.

		0 hrs	**2 hrs**	**6 hrs**	**12 hrs**	**26 hrs**	Group x Time	Time	Group
Free-T3 (pmol/L)	Non septic	5.6 ± 0.5	5.7 ± 0.5	5.3 ± 0.7	4.8 ± 0.4	4.4 ± 0.3	p = 0.12	p < 0.01	p < 0.01
	Septic	5.1 ± 0.3	5.1 ± 0.3	5.0 ± 0.4	4.0 ± 0.4	3.6 ± 0.2			
Free-T4 (pmol/L)	Non septic	10.8 ± 1.6	12.0 ± 1.9	11.2 ± 2.7	9.2 ± 2.4	7.0 ± 1.2	p = 0.02		
	Septic	11.3 ± 1.5	11.0 ± 1.7	10.7 ± 1.9	6.5 ± 2.1	3.2 ± 2.2 *			
Reverse-T3 (nmol/L)	Non septic	2.3 ± 0.2	2.7 ± 0.4	3.1 ± 0.3	4.4 ± 0.7	3.7 ± 2.1	p = 0.03		
	Septic	2.2 ± 0.4	2.7 ± 0.4	4.0 ± 1.1	7.6 ± 1.2 *	4.9 ± 1.1			
Cortisol (nmol/L)	Non septic	241 ± 45	225 ± 85	132 ± 81	37 ± 27	144 ± 169	p = 0.08	p < 0.01	p = 0.20
	Septic	247 ± 64	289 ± 55	282 ± 105	168 ± 90	124 ± 79			

F) REFERENCES

1. Adams D, McKinley M, Colditz I, et al. The Sheep. In: Australian & New Zealand Council for the Care of Animals in Research and Teaching. Fact Sheet A9 ed: University of Adelaide; 2009.

2. Dehring DJ, Fader RC, Traber LD, et al. Cardiopulmonary changes occurring with pulmonary intravascular clearance of live bacteria in sheep. Circ Shock 1989;29(3):245-256.

3. Bocking AD, McMillen IC, Harding R, et al. Effect of reduced uterine blood flow on fetal and maternal cortisol. J Dev Physiol 1986;8(4):237-245.

4. Rahman AN, Snibson KJ, Lee CS, et al. Effects of implantation and early pregnancy on the expression of cytokines and vascular surface molecules in the sheep endometrium. J Reprod Immunol 2004;64(1-2):45-58.

5. Jackson PGG, Cockcroft PD. Clinical examination of farm animals. In. Oxford: Blackwell Science; 2002. p. 301-305.

6. Dias IR, Viegas CA, Silva AM, et al. Haematological and biochemical parameters in Churra-da-Terra-Quente ewes from the northeast of Portugal. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 2010;62(2):265-272.

7. Wilhelmi MH, Tiede A, Teebken OE, et al. Ovine blood: establishment of a list of reference values relevant for blood coagulation in sheep. ASAIO J 2012;58(1):79-82.