Title: "Split" ventilation in bench and porcine models of Acute Respiratory Distress Syndrome: Online Digital Supplement.

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Supplementary Methods

S1.1 "Parent" ventilator settings for benchtop testing and benchtop testing with Draeger Models of Ventilator

The "parent" Servo-I ventilator was set in a pressure control mode with the following settings; respiratory rate 20, FiO_2 0.4, positive end-expiratory pressure (PEEP) 5-10cm H_2O, pressure control above PEEP 25-30cmH_20. We performed similar benchtop testing with two other common models of ventilator with similar settings in the relevant modes of ventilation (V800, Dräger medical, Lübeck, Germany and Evita 4; Dräger medical, Lübeck, Germany). The system assembly is as described in the main manuscript and illustrated there in Figure 1. For Draeger models, an additional connection was necessary between the inflow and outflow circuitry (on the ventilator side of the one way valves) to prevent ventilator pressure differential pressure alarm activation (shown in figure E1 below).

S1.2 Animal Preparation, Treatment Groups, and mechanical ventilation settings and targets

Thirty local community-bred Landrace pigs (23 to 56kg, mean 40kg +/- 10kg) were intramuscularly premedicated with midazolam (1 mg kg^{-1}) and ketamine (10 mg kg^{-1}). A 24 gauge cannula was inserted into the ear vein and intravenous anaesthesia induced with a bolus of 2mg kg^{-1} propofol and 10mcg kg^{-1} of fentanyl. Anaesthesia was maintained with propofol (2-4mg kg^{-1} hr^{-1}), midazolam (1.25mg kg^{-1} hr^{-1}) and fentanyl (5mcg kg^{-1} hr^{-1}); paralysis was

achieved with vecuronium (bolus of 0.6mg kg^{-1}, followed by an infusion of 0.3mg kg^{-1} hr^{-1}). The anaesthesia regimen included a strong opioid (fentanyl) for analgesia. After induction of general anaesthesia, and application of lignocaine spray (1% w/v) to the larynx, direct laryngoscopy was performed, and the pigs were orally intubated (7.0mm internal diameter cuffed endotracheal tube). Prior to the administration of neuromuscular blocking agents, adequate depth of anaesthesia was confirmed by non-response to a compressive toe pinch. Following the administration of neuromuscular blocking agents, depth of anaesthesia was monitored by observing changes in heart rate and blood pressure in response to stimulating study procedures. Mechanical ventilation was commenced with the relevant ventilator, using initial settings of tidal volume (6-8ml kg^{-1} actual body weight), respiratory rate (30 breaths per minute), PEEP (5-10cmH_20), and FiO_2 of 1.0. A recruitment manoeuvre was performed in all subjects comprising an end inspiratory hold manoeuvre at 25cmH_2O for 30 seconds in all subjects prior to arterial blood gas sampling.

A 20G arterial cannula was inserted into the femoral artery under ultrasound guidance using a Seldinger technique for the purposes of continuous arterial blood pressure monitoring and blood sampling. Similarly, a triple lumen central venous catheter was inserted into the femoral vein under ultrasound guidance to facilitate infusion of medications and fluids. Intravenous compound sodium lactate was infused at a rate of 5ml kg^{-1} hr^{-1} to a maximum of 30ml kg^{-1}. Infusions of noradrenaline (0.1-0.5mcg kg^{-1} min^{-1}) and vasopressin (0-2.4 IU per hour) were used to maintain mean arterial pressure

> 65mmHg during general anaesthesia. Temperature was maintained at 37-39 degrees Celsius using electronically controlled heating pads.

Replicates were assigned to one of four main treatment groups – “single” ventilated uninjured, “single” ventilated injured, “split” ventilated uninjured and “split” ventilated injured. Block allocation of replicates was performed with a block size of 4, primarily to control for learning effects. There were 5 replicates per group. The Combi-Ventilate system necessitated ventilation of two animals simultaneously and so each pair of animals was a single replicate. In total there were 30 pigs (5 “single” ventilated uninjured, 5 “single” ventilated injured, 10 “split” ventilated uninjured, and 10 “split” ventilated injured).

Two pigs were ventilated on each study day, either two “single” ventilated or two “split” ventilated animals, to prevent confounding by resource depletion on “split” ventilation days. Pigs in the “single” ventilated groups were ventilated using a conventional ventilator (Evita 4; Dräger medical, Lübeck, Germany). Pigs in the “split” ventilated groups were ventilated using the Combi-Ventilate system with the Servo-I (Maquet) acting as the “parent” ventilator.

Animals in all groups were ventilated in a “volume control” mode of ventilation. Initial tidal volume was set at 6-8ml kg^{-1} for all pigs with a respiratory rate of 30 breaths per minute and PEEP was set at 5-10cmH_2O. For pigs ventilated with the Combi-Ventilate device, PEEP, respiratory rate and FiO_2 were set on the “parent” ventilator and tidal volume was set on the individual animal’s Combi-Ventilate module. These parameters could be adjusted to achieve a plateau pressure ≤30cmH_20, with a tidal volume of 6-8ml kg^{-1} and an arterial blood pH>7.15. If the pH target could not be achieved without breaching the

tidal volume or plateau pressure goals, we permitted intravenous administration of sodium bicarbonate 8.4% w/v up to 4ml kg^{-1} to achieve the pH goal. In “single” ventilated animals, PEEP was set according to the ARDSnet “low” PEEP protocol (based on FiO_2 to achieve oxygenation targets). In “split” ventilated animals, it was not possible to individualise PEEP and PEEP was titrated to the lowest PEEP which allowed oxygenation targets to be reached in both animals.

Animals were euthanised upon completion of the experiment, or if humane endpoints were reached, by intravenous injection of Somulose (Secobarbital Sodium 400 mg/Cinchocaine Hydrochloride 25 mg), 0.1ml kg^{-1} body weight. Humane endpoints were never reached during the experiments.

Lung Injury Protocol:

Lung injury was accomplished by endobronchial administration of acid. HCl 0.05N, pH 1.41, was prepared and instilled (8 ml kg^{-1} body weight) at the right cranial lobe bronchus, the right main bronchus and the left main bronchus, in the ratio of 1:2:3 over 3 min by means of a flexible bronchoscope (Ambu®ascope™). We instilled the acid directly after intubation and allowed 60 minutes post instillation for lung injury to become established. In uninjured animals, bronchoscopy was performed at identical timepoints, but without any instillation of acid or vehicle.

S1.3 *Respiratory Mechanics and Gas exchange*

Total respiratory system compliance was calculated as tidal volume/(plateau pressure-PEEP). For “single” ventilated animals, plateau pressure was measured during an end inspiratory hold and PEEP during an end expiratory hold. For “split” ventilated animals, plateau pressure was measured by the Combi-Ventilate spirometer during an end inspiratory hold on the “parent” ventilator. Similarly, PEEP was measured by the Combi-Ventilate spirometer system during an end expiratory hold manoeuvre on the “parent” ventilator (Combi-Ventilate measurements of PEEP and plateau pressure were validated separately).

Gas exchange was determined hourly by arterial blood sampling (ABL 5; Radiometer; Copenhagen, Denmark). SaO2 was determined by co-oximetry. PF ratios were calculated as PaO_2/FiO_2.

S1.4 *Bronchoalveolar Lavage Fluid (BALF) sampling and processing*

Bronchoalveolar lavage fluid (BALF) sampling was performed at three discrete timepoints during the experiment. The first BALF sample was obtained immediately following intubation. The second BALF sample was obtained one hour post instillation of acid or at an identical timepoint in uninjured animals. The third sample was taken after 6 hours of post injury mechanical ventilation prior to euthanasia. Samples were obtained from the right cranial lobe bronchus. 20mls of 0.9% NaCl was instilled and immediately aspirated. Samples were stored on ice and processed within 1 hour of sampling.

Samples were centrifuged at 1500rpm for 10 minutes at 4 degrees Celsius. Supernatant was centrifuged at 1200rpm for a further 8 minutes at 4 degrees Celsius. Supernatant was stored in 1ml aliquots at minus 80 degrees Celsius.

Total protein levels in BAL were quantified using the Pierce™ BCA protein assay kit from Thermo Scientific. In brief, 10μl standards and samples were added in duplicate wells to a 96-well plate. Subsequently, 200μl BCA reagent (50 parts reagent A : 1 part reagent B) was added to each well and incubated in the dark for 30 minutes at 37°C. The optical density was measured spectrophotometrically at 544nm and total protein levels were quantified using the equation of the line: y=mX+c.

ELISA kits from R&D systems were used to analyse the levels of of IL-6 (DY686) in BAL. Levels were examined in samples taken immediately before injury, 20 minutes post-injury and 6 hours later (In brief, 96-well plated were coated in 100μl capture antibody overnight. The following day, the plate was washed and blocked with 100μl reagent diluent. The plate was washed again and 100μl standards and samples were added and incubated for 2 hours. The plate was washed again and 100μl detection antibody was added to the wells and incubated for a further 2 hours. Subsequently, the plate was washed and 100μl streptavidin-HRP was added to each well and incubated for 20 minutes in the dark. The plate was washed and 100μl of substrate solution was added and incubated for 20 minutes in the dark. Finally, 50μl stop solution was added to stop the reaction and complete the ELISA. The optical density was measured spectrophotometrically at 450nm and 590nm and cytokine levels were quantified using MyCurveFit software.)

Supplementary Results

Table S1.

Maximum Discrepancy in tidal volumes achieved at varying matched compliance of two test lungs

Driving Pressure (cmH_2O)	**Compliance - Test Lungs**	**Tidal Volume Parent Ventilator**	**Tidal Volume Test Lung A**	**Tidal Volume Test Lung B**
20	80	1280	970	220
20	50	1200	870	230
20	20	750	560	240

Test	Parent Measured PEEP	Parent Measured Peak Pressure	C Lung A	C Lung B	Resistance Lung A	Resistance Lung B	PEEP Lung A	Peak Pressure Lung A	Tidal Volume Lung A	PEEP Lung B	Peak Pressure Lung B	Tidal Volume Lung B	% Decrease Tidal Volume in Partner Lung
A	9.9	39	100	100	5	5	15	20	413	13	19	399	
	9.8	39	100	80	5	5	14	20	409	13	20	403	0.97
	9.8	39	100	60	5	5	14	20	410	13	19	396	0.73
	9.7	39	100	40	5	5	14	20	410	13	23	383	0.73
	10	39	100	30	5	5	14	20	407	12	26	368	1.45
	9.9	39	80	80	5	5	14	20	408	13	18	402	
	9.8	39	80	60	5	5	14	19	405	13	21	397	0.74
	10	39	80	40	5	5	14	20	404	12	21	385	0.98
	10	39	80	30	5	5	14	20	404	12	26	365	0.98
	9.9	39	60	60	5	5	14	21	402	13	21	400	
	10	39	60	40	5	5	14	21	403	12	23	389	-0.25
	10	39	60	30	5	5	14	21	403	12	26	363	-0.25
	10	39	40	40	5	5	13	24	399	12	24	401	
	10	39	40	30	5	5	13	24	400	12	26	378	-0.25
	10	39	30	30	5	5	13	26	393	12	27	399	
	10	39	30	80	5	5	13	26	390	14	21	440	0.76
B	9.9	39	80	80	5	5	14	18	394	14	20	399	
	10	39	80	80	5	20	14	20	399	14	23	360	-1.27
	9.7	39	50	50	5	5	14	22	400	13	22	405	
	10	39	50	50	5	20	14	22	401	13	25	369	-0.25
	10	39	30	30	5	5	13	26	390	12	27	387	
	10	39	30	30	5	20	13	26	391	12	28	359	-0.26
C	10	39	30	30	5	5	13	26	388	12	28	397	
	9.4	38	30	30	5	*a*	13	26	380	-	-	-	2.06
	10	39	30	30	5	5	13	26	390	12	28	398	
	9.8	38	30	30	5	*b*	13	26	388	-	-	-	0.51
D	10	39	30	30	5	5	13	26	391	12	27	399	
	10	39	30	30	5	-	13	26	394	-	-	-	-0.77

Table S2. Demonstration of ventilatory independence of two test lungs under conditions of changing compliance or resistance in a single test lung with the V800 ventilator (Dräger medical, Lübeck, Germany) acting as the parent ventilator. Panel A displays the effect of changing one test lung's (lung B) compliance in different conditions of initially matched compliance. Panel B represents the effects of increased resistance under different conditions of matched compliance. Panel C represents the effects of a disconnect from one test lung under conditions of matched compliance where "a" represents a disconnect at the ET tube and "b" represents a disconnect distal to the Y piece. Panel D represents the effect of an occlusion at test lung B under conditions of matched compliance. C=compliance.

Test	Parent Measured PEEP	Parent Measured Peak	C Lung A	C Lung B	Resistance Lung A	Resistance Lung B	PEEP Lung A	Peak Pressure Lung A	Tidal Volume Lung A	PEEP Lung B	Peak Pressure Lung B	Tidal Volume Lung B	% Decrease Tidal Volume in Partner Lung
A	10	40	100	100	5	5	15	20	395	14	20	399	
	10	40	100	80	5	5	15	19	396	14	20	394	-0.25
	10	40	100	60	5	5	15	20	395	13	21	393	0.00
	10	40	100	40	5	5	15	20	394	13	23	376	0.25
	10	40	100	30	5	5	15	20	396	12	26	363	-0.25
	10	40	80	80	5	5	14	20	393	14	20	397	
	10	40	80	60	5	5	15	20	395	13	21	395	-0.51
	10	40	80	40	5	5	15	20	393	13	24	379	0.00
	10	40	80	30	5	5	15	20	398	13	26	357	-1.27
	10	40	60	60	5	5	14	21	395	13	21	401	
	10	40	60	40	5	5	14	21	395	13	24	384	0.00
	10	40	60	30	5	5	14	21	396	12	26	362	-0.25
	10	40	40	40	5	5	14	24	394	13	24	404	
	10	40	40	30	5	5	14	24	393	13	27	386	0.25
	10	40	30	30	5	5	13	27	386	12	27	389	
	10	40	30	80	5	5	13	27	390	14	21	429	-1.04
B	10	40	80	80	5	5	14	20	394	14	20	398	
	10	40	80	80	5	20	14	20	393	14	23	368	0.25
	10	40	50	50	5	5	14	22	394	13	22	394	
	10	40	50	50	5	20	14	22	389	13	24	359	1.27
	10	40	30	30	5	5	13	26	387	13	27	394	
	10	40	30	30	5	20	13	27	388	13	29	358	-0.26
C	10	40	30	30	5	5	13	26	386	13	27	388	
	10	39	30	30	5	*a*	13	26	389	-	-	-	-0.78
	10	40	30	30	5	5	13	27	387	13	27	394	
	10	39	30	30	5	*b*	13	26	389	-	-	-	-0.52
D	10	40	30	30	5	5	13	37	387	13	27	394	
	10	40	30	30	5	-	13	27	390	-	-	-	-0.78

Table S3. Demonstration of ventilatory independence of two test lungs under conditions of changing compliance or resistance in a single test lung with the Evita 4 ventilator (Dräger medical, Lübeck, Germany) acting as the parent ventilator. Panel A displays the effect of changing one test lung's (lung B) compliance in different conditions of initially matched compliance. Panel B represents the effects of increased resistance under different conditions of matched compliance. Panel C represents the effects of a disconnect from one test lung under conditions of matched compliance where "a" represents a disconnect at the ET tube and "b" represents a disconnect distal to the Y piece. Panel D represents the effect of an occlusion at test lung B under conditions of matched compliance. C=Compliance.

Test	Parent Measured PEEP	Parent Measured Peak Pressure	C Lung A	C Lung B	Resistance Lung A	Resistance Lung B	PEEP Lung A	Peak Pressure Lung A	Tidal Volume Lung A	PEEP Lung B	Peak Pressure Lung B	Tidal Volume Lung B	% Decrease Tidal Volume in Partner Lung
A	14	20	100	100	5	5	14	20	399	13	19	411	
	14	20	80	100	5	5	14	20	386	13	19	408	0.73
	14	21	60	100	5	5	14	21	385	13	19	411	0.00
	13	26	30	100	5	5	13	26	351	13	19	407	0.97
	13	23	40	100	5	5	13	24	372	13	19	408	0.73
	14	20	80	80	5	5	14	20	390	13	20	401	
	13	21	60	80	5	5	14	21	382	13	20	401	0.00
	13	23	40	80	5	5	12	22	390	13	20	405	-1.00
	13	26	30	80	5	5	12	25	371	13	20	401	0.00
	13	21	60	60	5	5	13	20	397	13	21	397	
	13	23	40	60	5	5	12	23	385	13	21	396	0.25
	13	26	30	60	5	5	12	25	360	13	21	397	0.00
	13	23	40	40	5	5	12	22	386	12	24	392	
	13	23	30	40	5	5	12	25	365	12	19	390	0.51
	12	26	30	30	5	5	12	25	387	12	27	388	
	13	26	80	30	5	5	13	19	424	12	27	397	-2.32
B	16	21	80	80	5	5	13	17	405	12	18	413	
	14	23	80	80	5	20	13	19	404	13	22	378	0.25
	13	22	50	50	5	5	12	21	394	12	20	390	
	13	23	50	50	5	20	12	21	392	12	23	361	0.51
	13	27	30	30	5	5	11	26	401	11	27	399	
	13	29	30	30	5	20	12	26	402	11	29	381	-0.25
C	12	27	30	30	5	5	12	26	400	11	27	404	
	1	28	30	30	5	*a*	12	27	401	-	-	-	-0.25
	13	27	30	30	5	5	12	26	401	11	27	409	
	1	28	30	30	5	*b*	12	27	389	-	-	-	2.99
D	12	27	30	30	5	5	12	26	408	11	27	406	
	13	39	30	30	5	-	11	24	337	-	-	-	17.40

Table S4. Demonstration of ventilatory independence of two test lungs under conditions of changing compliance or resistance in a single test lung with the Servo-I (Maquet, Germany) acting as the parent ventilator with original set up as shown in main text . Panel A displays the effect of changing one test lung's (lung A) compliance in different conditions of initially matched compliance. Panel B represents the effects of increased resistance under different conditions of matched compliance. Panel C represents the effects of a disconnect from one test lung under conditions of matched compliance where "a" represents a disconnect at the ET tube and "b" represents a disconnect distal to the Y piece. Panel D represents the effect of an occlusion at test lung B under conditions of matched compliance. C=compliance.

Test	Parent Measured PEEP	Parent Measured Peak Pressure	C Lung A	C Lung B	Resistance Lung A	Resistance Lung B	PEEP Lung A	Peak Pressure Lung A	Tidal Volume Lung A	PEEP Lung B	Peak Pressure Lung B	Tidal Volume Lung B	% Decrease Tidal Volume in Partner Lung
A	10	40	100	100	5	5	14	17	409	13	19	391	
	10	40	80	100	5	5	13	20	407	13	18	394	-0.77
	10	40	60	100	5	5	13	21	399	13	19	407	-4.09
	10	40	40	100	5	5	12	23	384	13	19	401	-2.56
	10	40	30	100	5	5	12	25	365	13	19	399	-2.05
	10	40	80	80	5	5	13	20	392	12	19	395	
	10	40	60	80	5	5	13	20	382	12	19	392	0.76
	10	40	40	80	5	5	12	23	367	12	19	406	-2.78
	10	40	30	80	5	5	12	25	351	12	19	396	-0.25
	10	40	60	60	5	5	13	20	387	12	20	385	
	10	40	40	60	5	5	12	23	375	12	20	385	0.00
	10	40	30	60	5	5	12	25	353	12	20	389	-1.04
	10	40	40	40	5	5	12	23	393	12	23	394	
	10	40	30	40	5	5	12	25	371	12	23	396	-0.51
	10	40	30	30	5	5	12	26	390	11	27	402	
	10	40	80	30	5	5	13	21	433	11	27	405	-0.75
B	10	40	80	80	5	5	13	20	418	13	19	395	
	10	40	80	80	5	20	13	20	406	13	22	358	2.87
	10	40	50	50	5	5	13	22	396	12	22	416	
	10	40	50	50	5	20	13	22	393	12	23	376	0.76
	10	40	30	30	5	5	12	26	391	11	27	410	
	10	40	30	30	5	20	12	26	392	12	28	374	-0.26
C	10	40	30	30	5	5	12	26	384	11	27	411	
	9	40	30	30	5	*a*	14	28	356	-	-	-	7.29
	10	40	30	30	5	5	12	27	405	11	27	409	
	10	40	30	30	5	*b*	14	28	368	-	-	-	9.14
D	10	40	30	30	5	5	12	27	410	11	27	406	
	10	40	30	30	5	-	12	27	410	-	-	-	0.00

Table S5. Demonstration of ventilatory independence of two test lungs under conditions of changing compliance or resistance in a single test lung with the Servo I (Maquet, Germany) acting as the parent ventilator and with the circuit modified to contain a connection between the inflow and outflow ports of the parent ventilator at the ventilator side of the one way flow valves. Panel A displays the effect of changing one test lung's (lung A) compliance in different conditions of initially matched compliance. Panel B represents the effects of increased resistance under different conditions of matched compliance. Panel C represents the effects of a disconnect from one test lung under conditions of matched compliance where "a" represents a disconnect at the ET tube and "b" represents a disconnect distal to the Y piece. Panel D represents the effect of an occlusion at test lung B under conditions of matched compliance. C=compliance.

Additional Supplemental Content:

Table S6. Components of Combiventilate System

Component Name	**Brand**	**Quantity**
End Expiratory Filter	Fisher and Paykel	4
Adult breathing circuit	Fisher and Paykel	2
HME Filter	Romsons HME	2
Adult patient spirometry set 2m	Intersurgical	2
One way flow valves	Intersurgical	4
T piece connectors	Intersurgical	2
Female Female adaptors	Intersurgical	4

Table S7. Sample data from validation of Combi-Ventilate plateau pressure measurements

Set Compliance Michigan Lung 1	**Set Compliance Michigan Lung 2**	**Plateau Pressure Combi-Ventilate 1**	**Intra-Pleaural Pressure Michigan Lung 1**	**% Difference Plateau vs Intra-Pleural Pressures 1**	**PEEP Lung 1**	**Tidal Volume Lung 1**	**Plateau Pressure Combi-Ventilate 2**	**Intra-Pleaural Pressure Michigan Lung 2**	**% Difference Plateau vs Intra-Pleural Pressures 2**	**PEEP Lung 2**	**Tidal Volume Lung 2**
30	30	25.5	25	1.96	12	409	26	26	0.00	12	411
50	50	20.7	20	3.38	13	407	20.9	19	9.09	12	408
80	80	18.4	18	2.17	13	400	18	17	5.56	13	397
30	80	22	22	0.00	12	396	18.9	18	4.76	13	393

Figure E1. displays the modified circuit set up for use with Drager ventilators with an additional connection between the inflow and outflow circuitry (on the ventilator side of the one-way valves) to prevent ventilator pressure differential pressure alarm activation.