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Research article

EFFECTS OF A COMBINATION OF PRONE POSITIONING AND HIGH-FREQUENCY OSCILLATORY VENTILATION ON BLOOD GAS EXCHANGE IN AN EXPERIMENTAL PIG MODEL OF ACUTE RESPIRATORY DISTRESS SYNDROME

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This animal study was conducted in order to verify the effects of combining high-frequency oscillatory ventilation and prone positioning and the order of application of both methods on blood gas exchange in an experimental model of *acute respiratory distress syndrome.*

Forty domestic pigs were used for the study. Saline solution washout was produced by bilateral lung lavage. The lavage process was repeated until adequate impairment of gas exchange (defined as PaO2 < 100 mmHg) 60 min following the last lavage was achieved. Subsequently, lung injury was established and each model was randomized to one of five groups, with differences in the type of mechanical ventilation used (conventional mechanical ventilation in accordance with the principles of protective lung ventilation or high-frequency oscillatory ventilation) and also in the positioning of the experimental model (supine position or mode changing prone and supine positions in a ratio 18:6 hours).

The best oxygenation was achieved in the group prone position + high-frequency oscillatory ventilation. The most favorable combination in terms of carbon dioxide elimination is the high-frequency oscillatory ventilation + prone position. The best results in terms of oxygenation index value were obtained in the combination of a prone position with the high-frequency oscillatory ventilation and in the prone position.

In conclusion, by using combinations of prone positioning and high-frequency oscillatory ventilation, one can achieve better blood gas parameters during *acute respiratory distress syndrome.*

Key words: acute respiratory distress syndrome; high frequency oscillatory ventilation; prone position, experimental model; gas exchange

INTRODUCTION

Acute respiratory failure (ARF) causes significant morbidity and mortality in children, often leading to acute respiratory distress syndrome (ARDS) and other organ system

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failures [1]. Incidences of ARF and ARDS in developed countries are 0.7%–4.2% of the total PICU admissions and the mortality varies by about 20%–30% [2].

Gas exchange is a process through which a given amount of oxygen (O_2) is exchanged with a comparable amount of carbon dioxide (CO_2). For an efficient mechanism, there must be an optimum matching between ventilation and perfusion. In normal conditions the amount of alveolar ventilation nearly equals the cardiac output value producing a global ventilation/perfusion (VA/Q) ratio close to unity [3]. Ventilator settings are often adjusted to achieve predestined arterial blood gas tensions. This approach is adequate with respect to oxygenation and carbon dioxide elimination [4].

The prone position (PP) has been demonstrated to cause an increase in both end-expiratory lung volume and alveolar recruitment. Changes in patient positioning can have a serious effect on oxygenation and ventilation in severe ARDS [5].

High-frequency oscillatory ventilation (HFOV) is one form of ventilation aimed at treating poor oxygenation associated with ARDS. This ventilation treatment strategy is used to recruit alveoli while reducing sheer forces within the lung, which can exacerbate ARDS [6]. Patient's lungs are held inflated to maintain oxygenation. Carbon dioxide is cleared by small volumes of gas moved in and out of the respiratory system at 3 to 15 Hz. This action is thought to minimize the repeated process of opening and collapsing of lung that causes lung damage during conventional mechanical ventilation [7].

The aim of this our study was to verify the effect of combining HFOV and prone positioning and order of application of both methods on blood gas exchange in an experimental model of ARDS.

MATERIAL AND METHODS

The study protocol was approved by the Institutional Review Board for the care of animal subjects (University of Veterinary and Pharmaceutical Sciences Brno). The care and handling was in accord with National Institutes of Health guidelines for ethical animal research.

Forty anesthetized domestic pigs (mean weight, 38 ± 5kg SD) were used for the study. The animals were premedicated with intramuscular tiletamin-zolazepam (Zoletil 50®, Virbac) and xylazin (Rompun®, Bayer). Anesthesia was induced with propofol (1% Propofol, Fresenius) via an ear vein. The trachea was isolated and cannulated using a 9-mm inner-diameter cuffed endotracheal tube. After the airway was secured, an additional 2 mg/kg bolus of propofol along with 100 ug fentanyl (Fentanyl, BBraun) was administered. Anesthesia was maintained by continuous infusion of propofol, midazolam (Midazolam, BBraun), and fentanyl throughout the experiment. The animals were put in a supine position and administered mechanical ventilation (Siemens Servo Ventilator 300; Siemens-Elema AB; Solna, Sweden) in the volume-controlled mode (CMV) with a PEEP of 5 cm H_2O, an inspiratory/expiratory ratio of 1:2, and a fraction of inspired oxygen of 1.0. A tidal volume of 10 mL/kg and a respiratory rate

of 14 to 18 breaths/min were applied to maintain a $PaCO_2$ value within the range of 35 to 45 kPa.

Initially, a continuous infusion of balanced solution at a rate of 10 mL/kg/h was administered and increased up to 15 mL/h as indicated by cardiac filling pressures.

Central venous and pulmonary artery pressures were measured using a 7.5F flow directed thermodilution fiber optic pulmonary artery catheter (Arrow International, Inc.; Cleveland, OH). The right carotid artery was cannulated with a 20-gauge catheter (Arrow International, Inc.; Cleveland, OH) for blood sampling and arterial pressure monitoring. Continuous ECG monitoring was performed. For hemodynamic monitoring, a monitor and corresponding software (Datex-Ohmeda, Finland) were used. Cardiac output was measured using the pulmonary artery. Arterial and mixed venous blood gases were analyzed (Gastat 600, Techno Medica Co., Ltd., Japan). All catheters were intermittently flushed with normal saline containing a low dose of heparin (10 U/ml of infusion fluid) to avoid clotting within the catheters.

Experimental protocol

After the surgical procedure, a stabilization period of at least 30 minutes followed and then baseline values were obtained. Saline solution washout (SW) was produced by bilateral lung lavage. The endotracheal tube was disconnected from the ventilator, and warmed saline solution was instilled from a height of 50 cm until a meniscus was seen in the tube. The fluid was retrieved via gravity drainage after 45s of apnea followed by endotracheal suctioning. Instilled and retrieved volumes were measured. Between the lavages, the pigs received manual ventilation with a fraction of inspired oxygen of 1 using a self-inflating bag. The lavage process was repeated until adequate impairment of gas exchange (defined as $PaO_2 < 100$mmHg) 60min following the last lavage was achieved. Subsequently, lung injury was established and each model was randomized to one of the five groups (eight models in a group), with differences in the type of used mechanical ventilation (conventional mechanical ventilation in accordance with the principles of protective lung ventilation or HFOV), and also in the position of the experimental model (supine position or mode changing prone and supine positions in the ratio 18:6 hours). Ventilator settings (CMV) after randomization were as follows: tidal volume of 6 mL/kg, respiratory rate of 30 breaths/min, PEEP of 8 cm H_2O, an inspiratory/expiratory ratio of 1:2. The fraction of inspired oxygen of 1.0 HFOV (SensorMedics 3100B) was initiated at the following settings: fraction of inspired oxygen 1.0; oscillation frequency, 5 Hz; percent inspiratory time, 33%; and bias flow, 20 L/min. Mean airway pressure was set at 5 cm H_2O greater than mean airway pressure measured during CMV.

Group 1 (control) was throughout the experiment ventilated conventionally in the supine position;

Group 2 (PP) was ventilated first conventionally in mode rotation of prone (18 hours) and then supine position (6 hours);

Group 3 (HFOV) was throughout the experiment ventilated by HFOV in the supine position;

Group 4 (HFOV + PP) was ventilated first in the supine position by HFOV, after 6 hours was ranked mode alternating prone and supine positions;

Group 5 (PP + HFOV) was ventilated conventionally in mode changing prone and supine position. After 6 hours the conventional ventilation was converted to HFOV (Figure 1).

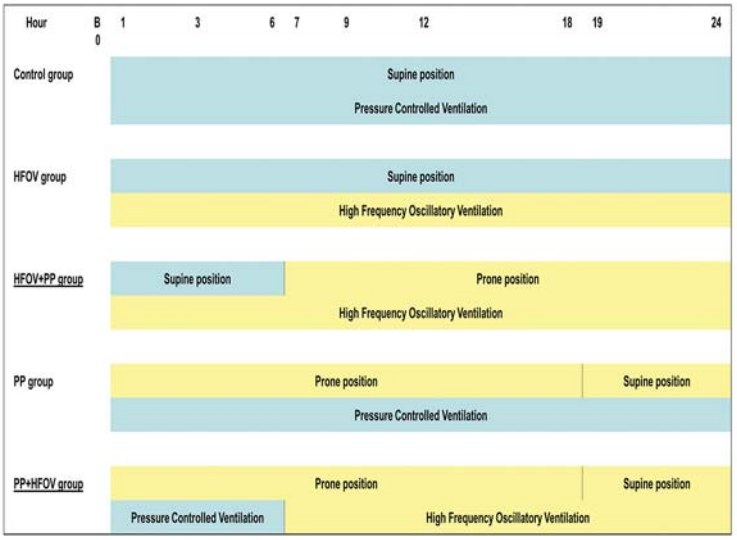

Figure 1. Graphical representation of ventilation techniques and ventilation positions in five groups during the 24 hour experimental model of ARDS
PCV - Pressure Control Ventilation; HFOV - High Frequency Oscillatory Ventilation; PP – prone position

Setting mode of ventilation was monitored in all animals (CMV: inhaled oxygen fraction - FiO_2, respiratory rate - RR, tidal volume - VT, peak airway pressure - PIP, end-expiratory pressure - PEEP, mean airway pressure - Paw; HFOV: inhaled oxygen fraction - FiO_2, frequency of oscillation - f, amplitude - ΔP).

Systemic arterial pressure, central venous pressure (CVP), pulmonary artery pressure (PAP), pulmonary capillary wedge pressure (PAWP) were measured invasively. The monitored parameters [PaO_2, $PaCO_2$ and oxygenation index (OI = Paw x 100 x FiO_2 / PaO_2)] were recorded after instrumentation (baseline), after induction of ARDS (time 0 h) and always immediately before and 60 minutes after changing position or ventilation mode - time 1, 3, 6, 7, 9, 12, 18, 19, 24 hours.

After finishing the protocol, all the animals were sacrificed under deep anesthesia with a bolus injection of thiopental followed by 40 ml of potassium chloride i.v.

Statistical analysis

Data were analyzed using the ANOVA test. Scale of values in each of the groups is shown in graph in the form of mean value and its standard error (SE). To comprehensively elucidate the differences in values and dynamics of evaluated parameters in between the groups we divided our data to 4 phases based on the time of performed interventions: Baseline until Hour 0; Hour 0 until Hour 6; Hour 6 until Hour 18; Hour 18 until Hour 24.

Scatter analysis was performed for each of these phases. Significance values for the differences in main effects (between groups in the corresponding interval in total, for all groups in time) and their interactions (difference in dynamics) were obtained.

To clarify the significant point of difference in values, with respect to correction for repeated testing, we consequently performed the post-hoc test (Fisher LSD test). Table shows test results for 4 examined group pairs together with basic summary statistics (count, mean, SE). The pairs of the examined groups were as follows:

Pair A: HFOV x PP

Pair B: HFOV x HFOV+PP

Pair C: PP x PP+HFOV

Pair D: HFOV+PP x PP+HFOV

Data not fulfilling normality requirements were transformed and the analysis was performed with those transformed data. Graph shows data in the transformed scale and it also includes the scale in back-transformation for better orientation in the original data (Y axis on the right side). Transformation was performed in OI values (logarithmic transformation $x' = \log10(x)$, values of the transformed variable are 0=1, 1=10, 2=100, 3=1000…), arterial and venous blood saturation and lung shunt values in percent (ArcSin transformation $x' = \arcsin(\sqrt{x})$, values of the transformed variable are 0°...90°, which is corresponding to 0%...100%).

RESULTS

PaO_2 values are shown in Table 1, trends of values are shown in Figure 2. The best oxygenation was achieved in group PP+HFOV, which is comparable with group PP. Prone positioning in animals ventilated using HFOV (group HFOV+PP) had only a moderate and temporary effect on oxygenation improvement compared with group HFOV. Groups PP and HFOV are comparable in terms of oxygenation.

$PaCO_2$ values in all groups are shown in Table 2, trends of values are shown in Figure 3. The most favorable combination in terms of CO_2 elimination was HFOV+PP. Reversed usage of both methods (PP+HFOV) is less effective for CO_2 elimination. Similarly, as with the effect on oxygenation, the addition of prone positioning in animals ventilated using HFOV (HFOV+PP) had only a moderate effect on ventilation improvement compared with group HFOV. Groups PP and HFOV are equally effective in CO_2 elimination.

Table 1. ANOVA models results of partial pressure of oxygen in arterial blood in each experiment phase

PaO_2	Significance levels		
Phase	**Group**	**Time**	**Group*Time**
1. B -> 0h	0.228	<001	0.144
2. 0h -> 6h	0.007	<001	0.001
3. 6h -> 18h	0.023	<001	<0.001
4. 18h -> 24h	0.577	0.320	0.696

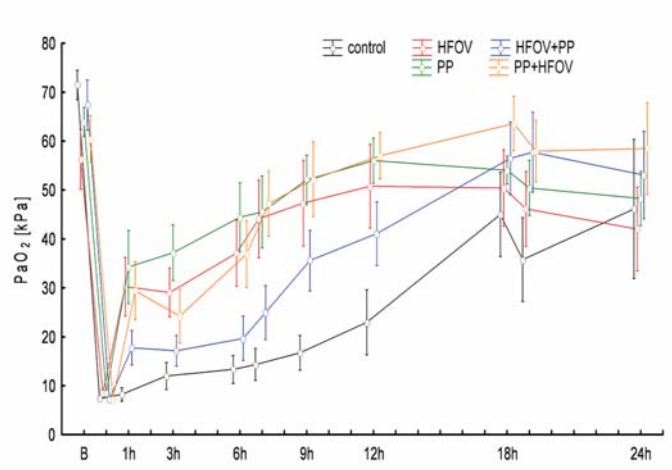

Figure 2. The course of PaO_2 (kPa) in each group
HFOV – High Frequency Oscillatory Ventilation; PP – prone position

Table 2. Post-hoc comparison of partial pressure of oxygen in arterial blood at each time point

	PaO_2			
Time	Pair A	Pair B	Pair C	Pair D
B	0.293	0.216	0.528	0.396
0h	0.944	0.851	0.909	0.981
1h	0.549	0.254	0.780	0.081
3h	0.648	0.160	0.229	0.326
6h	0.729	**0.048**	0.742	**0.018**
7h	0.791	**0.031**	0.788	**0.013**
9h	0.689	0.181	0.984	**0.046**
12h	0.647	0.273	0.963	0.079
18h	0.717	0.678	0.457	0.496
19h	0.772	0.431	0.524	0.959
24h	0.786	0.447	0.250	0.562

Abbreviations: B – values after instrumentation of the model - baseline; Pair A: HFOV vs. PP; Pair B: HFOV vs. HFOV+PP; Pair C: PP vs. PP+HFOV; Pair D: HFOV+PP vs. PP+HFOV

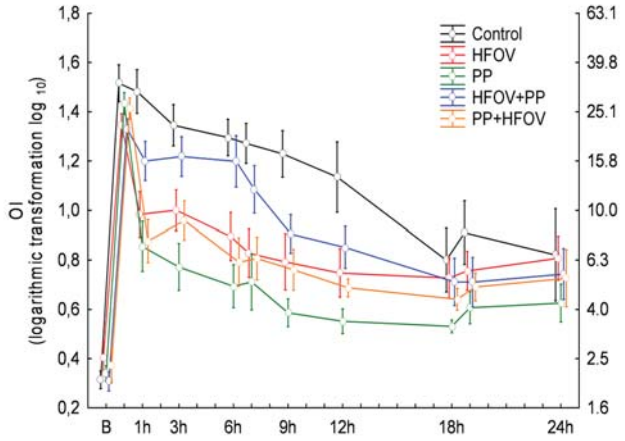

Figure 3. The course of OI (log_{10} OI) in each group
HFOV – High Frequency Oscillatory Ventilation; PP – prone position

Table 3. ANOVA models results of oxygenation index in each experiment phase

Log(OI)	Significance levels		
Phase	**Group**	**Time**	**Group*Time**
1. B -> 0h	0.537	<0.001	0.067
2. 0h -> 6h	<0.001	<0.001	<0.001
3. 6h -> 18h	0.002	<0.001	<0.001
4. 18h -> 24h	0.364	0.163	0.811

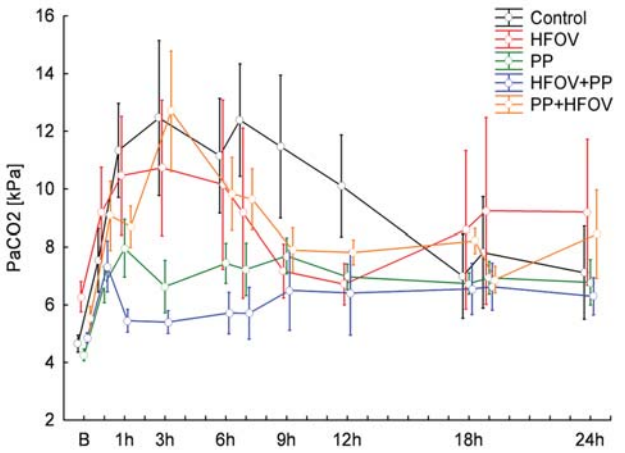

Figure 4. The course of $PaCO_2$ (kPa) in each group
HFOV – High Frequency Oscillatory Ventilation; PP – prone position

Oxygenation Index (OI) values are shown in Table 3 and Figure 4. Data were logarithmically transformed because in order to allow parametric evaluation in the factorial design. That means the lower the OI value (OI = 100 x Paw x FiO_2/PaO_2),

the better signs of oxygenation. Best results in terms of OI value were obtained, as in terms of oxygenation, in the prone position and in the combination of prone positioning with HFOV (PP + HFOV).

Table 4. Post-hoc comparison of oxygenation index at each time point

	Log(OI)			
Time	**Pair A**	**Pair B**	**Pair C**	**Pair D**
B	0.293	0.216	0.528	0.396
0h	0.944	0.851	0.909	0.981
1h	0.549	0.254	0.780	0.081
3h	0.648	0.160	0.229	0.326
6h	0.729	**0.048**	0.742	**0.018**
7h	0.791	**0.031**	0.788	**0.013**
9h	0.689	0.181	0.984	**0.046**
12h	0.647	0.273	0.963	0.079
18h	0.717	0.678	0.457	0.496
19h	0.772	0.431	0.524	0.959
24h	0.786	0.447	0.250	0.562

Abbreviations: B – values after instrumentation of the model - baseline; Pair A: HFOV vs. PP; Pair B: HFOV vs. HFOV+PP; Pair C: PP vs. PP+HFOV; Pair D: HFOV+PP vs. PP+HFOV

Table 5. ANOVA models results of partial pressure of carbon dioxide in arterial blood in each experiment phase

$PaCO_2$	**Significance levels**		
Phase	**Group**	**Time**	**Group*Time**
1. B -> 0h	0.095	<0.001	0.951
2. 0h -> 6h	0.023	0.454	0.221
3. 6h -> 18h	0.138	0.809	0.045
4. 18h -> 24h	0.526	0.702	0.655

Table 6. Post-hoc comparison of partial pressure of carbon dioxide in arterial blood at each time point

	$PaCO_2$			
Time	**Pair A**	**Pair B**	**Pair C**	**Pair D**
B	0.477	0.769	0.302	0.587
0h	0.423	0.778	0.067	0.220
1h	0.602	0.055	0.708	**0.045**
3h	0.438	0.204	**0.000**	**0.000**
6h	0.612	0.606	0.220	**0.016**
7h	0.727	0.608	0.145	**0.015**
9h	0.670	0.803	0.999	0.429
12h	0.649	0.834	0.656	0.463
18h	0.996	0.995	0.246	0.247
19h	0.818	0.951	0.908	0.754
24h	0.900	0.798	0.196	0.149

Abbreviations: B – values after instrumentation of the model - baseline; Pair A: HFOV vs. PP; Pair B: HFOV vs. HFOV+PP; Pair C: PP vs. PP+HFOV; Pair D: HFOV+PP vs. PP+HFOV

DISCUSSION

Animal models provide a bridge between patients and laboratory. A hypotheses generated in human studies can be tested directly on animal models, and the results of studies can be tested in animal models to assess their relevance in intact living systems. Ideally, animal models of ALI should reproduce the mechanisms of ALI in humans, including the physiological and pathological changes that occur [8].

The saline lavage model was developed by Lachmann et al. in 1979 based on the observation that ARDS is associated with depletion of the surfactant from the air spaces and reduced concentrations of surfactant-associated proteins in the bronchoalveolar fluid [9]. The major advantage is that the saline lavage model provides an ideal manner to test the effects of ventilation strategies on the development of tissue injury because the tissue injury results more from the ventilation strategy than from the saline lavage [8].

The aim of our study was to assess the effect of combining prone positioning and high-frequency oscillatory ventilation on oxygenation and elimination of carbon dioxide. Our main results were as follows: We can reach better oxygenation parameters of the organism with ARDS, as predicted from values of PaO_2 and OI, when using conventional ventilation in the prone position (PP) or mainly when using prone positioning and high-frequency oscillatory ventilation (PP+HFOV). Changing body position from supine to prone alters the ventral-dorsal pleural pressure gradient and ultimately the regional trans-pulmonary pressure. These changes occur by altering gravitational forces and by reducing the compressive effects of the heart, mediastinal structures and abdominal wall.

For the elimination of CO_2 it is best to use HFOV at first. Figure 3 shows values and trends from comparison of groups on HFOV ventilation and the changes between prone and supine positioning. We saw excellent CO_2 elimination even in the first hours of our experiment in the group HFOV + PP, where we can see statistically significant differences in $PaCO_2$ values in the 1st, 3rd and 6th hour. Most significant for CO_2 elimination and for successful ventilation is HFOV ventilation, prone positioning is not as much contributing in a successful CO_2 elimination. We have not found any statistically significant differences in $PaCO_2$ values from the 9th hour until 24th hour of our experiment. HFOV improves efficiency of alveolar ventilation by a decreased physiological dead space.

Since therapeutic alternatives are lacking and the underlying concepts sound reasonable, multimodal therapeutic approaches are commonly used for salvage therapy in patients with ARDS. Papazian et al. found that the prone position combined with HFOV and pressure-controlled ventilation (PCV) is superior to HFOV and supine positioning in terms of oxygenation, but failed to demonstrate additive effects. However, the inflammatory mediators were elevated during HFOV-prone but not during HFOV-supine [10]. This calls for long term experiments with large animals

comparing conventional lung protective ventilation and HFOV with and without prone positioning looking not only at gas exchange and respiratory mechanics but also at histology and inflammatory mediators.

Another study in 43 patients with ARDS compared three groups of patients: a) conventional lung-protective mechanical ventilation in the prone position (12 hrs) followed by a 12-hr period of conventional lung-protective mechanical ventilation in the supine position (CV prone – CV supine); b) conventional lung-protective mechanical ventilation in the supine position (12 hrs) followed by HFOV in the supine position (12 hrs) (CV supine – HFOV supine); and c) conventional lung protective mechanical ventilation in the prone position (12 hrs) followed by HFOV in the supine position (CVprone-HFOVsupine group). They found that the sequence prone positioning followed by HFOV maintained the improvement in oxygenation related to prone positioning, whereas there was no persistent improvement when the prone position was followed by a 12-hr period of supine positioning with conventional ventilation [11]. According to the study by Ferguson et al., among patients with moderate-to-severe ARDS, early application of HFOV was associated with higher mortality than in control ventilation strategy targeting lung recruitment with the use of low tidal volumes and high positive end-expiratory pressure [12]. The higher mortality in the HFOV group was probably because of elevated mean airway pressures.

The importance of prone positioning may also consist in reducing mortality. In patients with severe ARDS, early application of prolonged prone-positioning leads to a significantly decreased 28^{th}-day and 90^{th}-day mortality [13]. In an animal experimental study Brederlau et al. evaluated the effects of prone positioning on gas-exchange, hemodynamics and respiratory parameters in HFOV-ventilated pigs with severe lavage induced acute lung injury [14]. The authors came to similar conclusions as in the present work. They found that HFOV and prone positioning improves oxygenation at a lower P mean than HFOV and supine positioning. This improvement is achieved with lower mean airway pressure, which also correlates with our findings - oxygenation expressed OI. In contrast to the cited works, which found no differences in the elimination of CO_2 in both groups, we have found a good influence of HFOV on ventilation, which is slightly potentiated by a subsequent use of prone positioning (HFOV + PP). Inverted use of both methods, a first PP and then HFOV, resulted in a small effect on CO_2elimination.

The major limitation of this study is that it was performed on a piglet model. The results can not be directly applied to other models or to humans. It is possible that the use of a different model might yield different results. In patients (except newborns) not surfactant deficiency, but alveolar flooding, is the predominant mechanism in ARDS-development. In addition, the high inspiratory oxygen fraction used in this study may have promoted the occurrence of reabsorption atelectasis especially at low levels of PEEP (8cm H_2O) [15]. Titration of PEEP remains a controversial issue, thus optimal PEEP might be different in the prone position.

CONCLUSIONS

In this saline lavage induced porcine model of ARDS, we showed in a clinically relevant scenario, that the combination of HFOV and prone positioning improved oxygenation and CO_2 elimination. Having in view a long history of failed multimodal treatment approaches in ARDS research, we have concluded from our results that a combination of HFOV and prone positioning seems promising and should be further investigated systematically and compared to conventional lung protective ventilation. Long term trials in large animals and acquisition of histological and immunological data seem clearly justified.

Acknowledgement

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EFEKTI KOMBINACIJE LEŽEĆEG POLOŽAJA I OSCILATORNE PLUĆNE VENTILACIJE VISOKE FREKVENCIJE NA RAZMENU GASOVA U KRVI NA MODELU SINDROMA AKUTNOG RESPIRATORNOG ŠOKA KOD SVINJA

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Ispitivanje je sprovedeno sa ciljem utvrđivanja efekata kombinacije oscilatorne ventilacije visoke frekvencije i ležećeg položaja, na razmenu gasova u krvi u eksperimentalnom modelu sindroma akutnog respiratornog šoka, kod 40 svinja. Urađena je bilateralna lavaža pluća fiziološkim rastvorom. Postupak lavaže je ponovljan dok nije postignut adekvatan poremećaj razmene gasova (definisano kao PaO2<100 mmHg) 60 minuta posle poslednje lavaže. Posle toga, uočena je ozleda pluća i svaki model je nasumično raspoređen u jednu od pet grupa sa različitim tipom mehaničke ventilacije kao i prema poziciji eksperimentalnog modela (leđni položaj ili izmena stomačnog i leđnog položaja). Najbolja oksigenacija je postignuta u grupi sa stomačnim položajem + oscilatorna ventilacija visoke frekvencije. Najbolja kombinacija u odnosu na eliminaciju ugljen dioksida je bila oscilatorna ventilacija visoke frekvencije + stomačni položaj. Najbolji rezultati u odnosu na indeks oksigenacije dobijeni su primenom kombinacije stomačnog položaja + oscilatorna ventilacija visoke frekvencije kao i samo stomačni položaj. Može da se zaključi da se primenom kombinacije stomačnog položaja i oscilatorne ventilacije visoke frekvencije dobijaju bolje vrednosti gasova u krvi tokom sindroma akutnog respiratornog šoka.