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(57) Abrégé/Abstract:
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A THERAPEUTIC COMBINATION COMPRISING A PULMONARY SURFACTANT AND A STEROID FOR THE TREATMENT OF EVOLVING BPD

TECHNICAL FIELD

The invention relates to compositions for the treatment of diseases of prematurity and methods thereof. In particular the invention relates to the use of a pulmonary surfactant in combination with a steroid for treating evolving broncho-pulmonary dysplasia in preterm neonates.

BACKGROUND OF THE INVENTION

The human lung is composed of a large number of small air sacs, called alveoli, in which gases are exchanged between the blood and the air spaces of the lungs. In healthy individuals, this exchange is mediated by the presence of a protein-containing surfactant complex that prevents the lungs from collapsing at the end of expiration.

The lung surfactant complex is composed primarily of lipid and contains minor amounts of various proteins. An absence of adequate levels of this complex results in malfunction of the lungs. This syndrome is called Respiratory Distress Syndrome (RDS) and it commonly affects preterm neonates.

The mainstay of the treatment of RDS is the replacement therapy with exogenous pulmonary surfactant preparations extracted from animal lungs, known as modified natural surfactants. For instance, modified natural surfactants used in the clinical practice are poractant alfa derived from porcine lung, and sold under the trademark of Curosurf®, beractant (Surfacten® or Survanta®) bovactant (Alveofact®), both derived from bovine lung, and calfactant derived from calf lung (Infasurf®).

Exogenous pulmonary surfactants are currently administered by endotracheal instillation as suspension in a saline aqueous solution to intubated pre-term infants kept under mechanical ventilation with oxygen.

Although said therapy has greatly increased postnatal survival, children that survive RDS have a high risk of developing broncho-pulmonary dysplasia (BPD), a common and

serious complication of prematurity, associated with significant mortality, morbidity and healthcare resource utilization. Despite advances in both prenatal and neonatal care the incidence of the condition continues to rise. The management of BPD and its related problems remains a major challenge for neonatologists and paediatricians. Multiple interventions have been proposed to prevent and treat BPD but many are still not evidence based. Current treatments appear to have reduced the severity of BPD but have had little effect on its incidence. BPD is an evolving process of lung injury and its pathophysiology varies at different stages of the disease. Its management therefore is unlikely to be in the form of a single intervention but rather a combined approach with different strategies used to target different factors and/or stages of the disease.

For this reason, it is useful to categorize intervention for BPD at three subsequent stages when designing an overall management plan. These are: i) prevention of BDP; ii) treatment of evolving BPD; and iii) treatment of established BPD (see Bowen P et al Pediatrics and Child Health 2013, 24:1, 27-31).

The prevention of BPD in neonates affected by RDS has been managed by systemic administration of a corticosteroid, antenatal or within few hours postnatal. However, the effectiveness of postnatal corticosteroid administration is offset by possible adverse systemic effects, e.g., hypertension, hyperglycemia, gastrointestinal complications, and neurodevelopmental disability.

As an alternative to systemic administration, delivery of corticosteroid by inhalation or intracheal instillation has been proposed for the prophylaxis of BDP.

For example, US 2010-0317636 discloses a method for the prophylaxis of BPD in an infant suffering from respiratory distress syndrome by administering to the infant a combination of a corticosteroid having a high local to systemic anti-inflammatory activity and a lung surfactant.

Yeh et al (Pediatrics 2008, 121(5), e1310-e1318) proposed the intratracheal instillation of budesonide using the pulmonary surfactant Survanta® as a carrier. While Dani et al (Pediatr Pulmonol 2009, 44, 1159-1167) have proposed the intratracheal

instillation of beclometasone dipropionate in combination with Cursourf®.

However, through these approaches as well, a large population of preterm neonates would be exposed to corticosteroids, many without benefit if otherwise they would not develop BPD (see Bancalari E Am J Respir Crit Care Med 2016, 193:1, 12).

On the other hand, serious concerns were raised on the efficacy of corticosteroids in established BDP, as said disease is hallmarked by strong and persistent airway inflammation, fibrosis, and smooth muscle hypertrophy.

Postnatal corticosteroids could find their place in therapy in the treatment of evolving BPD as in this way they will be administered to patients in need thereof.

However, due to the observed side effects or to the lack of clear sign of efficacy, the systemic postnatal administration of dexamethasone and hydrocortisone is not currently recommended routinely.

In view of the above considerations, there is still a need to develop a more compliant corticosteroid-based medicament for the treatment of evolving BPD in premature neonates.

Furthermore, it would be advantageous to provide a medicament that may be administered locally either by inhalation or intra-tracheal instillation.

Finally, it would be of particular advantage to provide a medicament able of promoting the lung development.

SUMMARY OF THE INVENTION

The present invention is directed to a pulmonary surfactant in combination with budesonide at a dose comprised from 0.1 to 1.5 mg/kg for use for the treatment of evolving bronchopulmonary dysplasia (BPD) in preterm neonates.

Preferably, the combination of the invention increases the mRNA expression of some protein indicators of lung maturation, more preferably the mRNA expression of the surfactant proteins SP-A, SP-B and SP-C.

Advantageously, said combination is administered from the 2nd to the 28th day of life, preferably from the 5th to the 15th day of life, more preferably from the 7th to the 10th day of life.

The invention is also directed to the use of a pulmonary surfactant in combination with budesonide at a dose comprised from 0.1 to 1.5 mg/kg in the manufacture of a medicament for the treatment of bronchopulmonary dysplasia (BPD) in preterm neonates. Advantageously, said combination is administered from the 2nd to the 28th day of life, preferably from the 5th to the 15th day of life, more preferably from the 7th to the 10th day of life.

Preferably the dose of budesonide may be comprised between 0.2 and 1.0 mg/kg.

The medicament of the invention could be administered simultaneously, sequentially or separately, preferably for simultaneous administration as fixed combination.

In a particular embodiment, said medicament is in form of pharmaceutical composition for inhalation or intratracheal administration comprising said fixed combination.

In a further embodiment, the invention is directed to a method for the treatment of evolving bronchopulmonary dysplasia comprising the administration to a preterm infant in need of such treatment pulmonary surfactant in combination with budesonide at a dose comprised from 0.1 to 1.5 mg/kg, wherein said combination is administered from the 2nd to the 28th day of life, preferably from the 5th to the 15th day of life, more preferably from the 7th to the 10th day of life.

DEFINITIONS

The term "bronchopulmonary dysplasia (BPD)" refers to a chronic pulmonary disorder, also known as chronic lung disease (CLD), which is the consequence of unresolved or abnormally repaired lung damage.

BPD typically occurs in very low birth weight (VLBW) infants who sustain lung damage as a result of oxygen toxicity and barotrauma from mechanical ventilation early in life. The definition and classification of BPD have changed since its original description by Northway et al. in 1967. The National Institute of Child Health and Human Development (NICHD) defined BPD in a consensus statement in 2001. This definition uses supplemental oxygen requirement for 28 days and then identifies 3 grades of severity, dependent on the

respiratory support required at either 36 weeks postmenstrual age (PMA) or at discharge for those born at <32 weeks gestation or at 56 days of life or discharge for those born at >32 weeks gestation.

According to more recent definitions, BPD may be primarily considered an arrest of lung development (see Jobe A et al Ped Res 1999, 46, 641).

In 2001 Jobe A et al (*Am J Respir Crit Care Med*; 163(7) 1723-1729) proposed a new definition including specific criteria for 'mild,' 'moderate' and 'severe' BPD.

Mild BDP is defined as the disease requiring supplemental oxygen for ≥ 28 days and on room air at 36 weeks PMA or at discharge (for infants <32 weeks at birth) or at 56 days or at discharge (for infants ≥ 32 weeks at birth).

Moderate BDP is defined as the disease requiring supplemental oxygen for ≥ 28 days and a need for supplemental oxygen <30% at 36 weeks PMA/discharge (for <32 weeks) or at 56 days/discharge (for infants ≥ 32 weeks).

Severe BPD is defined as the disease requiring supplemental oxygen for ≥ 28 days and a need for ≥ 30% oxygen or on nasal CPAP or mechanical ventilation at 36 weeks PMA/discharge (<32 weeks) or at 56 days/discharge (≥ 32 weeks).

The term "evolving BPD", sometimes known as early BPD, refers to the initial phase of the chronic process leading to established BDP and indicates the disease characterised by oxygen and/or ventilator-dependency from 7th to 14th day of life (Walsh MC et al Pediatrics 2006, 117, S52-S56).

The term "modified natural surfactant" refers to a lipid extract of minced mammalian lung. Due to the lipid extraction process used in the manufacture process, the hydrophilic proteins SP-A and SP-D are lost. These preparations have variable amounts of two hydrophobic, surfactant-associated proteins SP-B and SP-C and, depending on the method of extraction, may contain non-surfactant lipids, proteins or other components.

The term "poractant alfa" refers to a modified natural surfactant extracted from porcine lungs substantially consisting of polar lipids, mainly phospholipids and the proteins, SP-B and SP-C. Poractant alfa is available under the trademark Curosurf®.

The term "artificial" pulmonary surfactants refers to simply mixtures of synthetic compounds, primarily phospholipids and other lipids that are formulated to mimic the lipid composition and behavior of natural pulmonary surfactant. They are devoid of pulmonary surfactant proteins.

The term "reconstituted" pulmonary surfactants" refers to artificial pulmonary surfactants to which have been added pulmonary surfactant proteins/peptides isolated from animals or proteins/peptides manufactured through recombinant technology such as those described in WO 95/32992 or synthetic pulmonary surfactant protein analogues such as those described in WO 89/06657, WO 92/22315 and WO 00/47623.

The term "non-invasive ventilation (NIV) procedure" defines a ventilation modality that supports breathing without the need for intubation such as nasal Continuous Positive Airway Pressure (nasal CPAP). Other non-invasive ventilation procedures are nasal intermittent positive- pressure ventilation (NIPPV), High Flow Nasal Cannula (HFNC), and bi-level positive airway pressure (BiPAP).

The term "respiratory support" includes any intervention that treats respiratory illness including, for example, the administration of supplemental oxygen, mechanical ventilation, and nasal CPAP.

The term "treatment" refers to the use for curing, symptom-alleviating, symptom-reducing of the disease or condition, e.g., BPD in the patient.

The term "prevention" refers to the use for progression-slowing and/or onset delaying of the disease or condition, e.g., BPD, in the patient.

The term "pre-term neonates", or preterm infants, includes extremely low birth weight (ELBW), very-low-birth -weight (VLBW), and low-birth weight (LBW) neonates of 24-35 weeks gestational age.

The term "fixed combination" means a combination wherein the active substances are in a fixed quantitative ratio.

"Pharmaceutical acceptable" is a term used herein that refers to a medium that does not produce an allergic or similar untoward reaction when administered to an infant.

"Surfactant activity" for a surfactant preparation is defined as the ability to lower the surface tension.

The *in vitro* efficacy of exogenous surfactant preparations is commonly tested by measuring their capability of lowering the surface tension using suitable apparatus such as Wilhelmy Balance, Pulsating Bubble Surfactometer, Captive Bubble Surfactometer and Capillary Surfactometer.

The *in vivo* efficacy of exogenous surfactant preparations is tested by measuring lung mechanics in pre-term animal models according to known methods.

In the context of the present description, the term "synergistic" means that the activity of the pulmonary surfactant plus that of budesonide is more than would be expected by that of the surfactant or the budesonide alone.

With the term "biosimilar of poractant alfa, it is meant a modified natural pulmonary surfactant which is therapeutically equivalent to poractant alfa, but having a similarity in the composition of at least 80% with respect to it and a viscosity of less than 15 mPas (cP) at room temperature when it is suspended in an aqueous solution at a concentration of 80/mg/ml. The viscosity can be determined according to known methods.

FIGURES

Figure 1 - Scheme of the protocol for this complex series of interventions.

Figure 2 - Oxygenation

Figure 3 - Static Lung gas volume at 40 cmH_2O measured from the Pressure-Volume curves.

Figure 4 - Lung weight

Figure 5 - Lung Gas Volume Relative to Lung Weight

Figure 6 - Lung Dry to Wet Weight Ratios

Figure 7 - mRNA Indicators of Lung Maturation SP-A, SP-B and SP-C

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based in part on the unexpected finding that budesonide at

a dose comprised between 0.1 mg/kg and 1.5 mg/kg could be combined with a pulmonary surfactant such as poractant alfa to treat evolving bronchopulmonary dysplasia (BPD) without altering the surface activity of the surfactant.

The advantages of combining a pulmonary surfactant with the claimed dose of budesonide will be apparent from the following findings.

It has indeed surprisingly been found, in a study in preterm lambs with RDS subjected to nasal CPAP ventilation, that a pulmonary surfactant such as poractant alfa in combination with budesonide significantly increases the mRNA expression of some protein indicators of lung maturation, while unexpectedly the pulmonary surfactant alone caused a decrease in such an mRNA expression.

The addition of budesonide also increases significantly the lung gas volume as well as decreases the lung weight relative to pulmonary surfactant alone.

The decrease of lung weight is in turned linked to a loss of water that indicate loss of mesenchymal cells and a maturation response.

Furthermore, the addition of budesonide to the surfactant decreases the airways wall thickness as well as the collagen deposition, both indexes of lack of lung maturation.

Since budesonide is a highly lipophilic corticosteroid, this might favor its mucosal absorption and uptake across phospholipid cell membranes with a negligible systemic absorption, making the combination safe for therapeutic use in preterm neonates.

On the other hand, the pulmonary surfactant may favor the spreading of the corticosteroid by Marangoni effect, favoring its distribution and hence the reaching of all the interested pulmonary area.

Any pulmonary surfactant currently in use, or hereafter developed for use in respiratory distress system and other pulmonary conditions could be suitable for use in the present invention. These include modified natural, artificial and reconstituted pulmonary surfactants.

Current modified natural pulmonary surfactants include, but are not limited to, bovine lipid pulmonary surfactant (BLES™, BLES Biochemicals, Inc. London, Ont),

calfactant (Infasurf™, Forest Pharmaceuticals, St. Louis, Mo.), bovactant (Alveofact™, Thomae, Germany), bovine pulmonary surfactant (Pulmonary surfactant TA™, Tokyo Tanabe, Japan), poractant alfa (Curosurf™, Chiesi Farmaceutici SpA, Parma, Italy), and beractant (Survanta™, Abbott Laboratories, Inc., Abbott Park, 111).

Examples of reconstituted surfactants include, but are not limited to, the compositions disclosed in EP 2152288, WO 2008/011559, WO 2013,/120058, the products lucinactant (Surfaxin™, Windtree-Discovery Laboratories Inc., Warrington, Pa.) and the product having the composition disclosed in Table 2 of Example 2 of WO 2010/139442, i.e.

1.5% of SP-C33(leu) acetate;

0.2% of Mini-B(leu) acetate; and

DPPC:POPG in a 50:50 weight ratio.

The pulmonary surfactant selected for use in the medicament of the invention can be the same as, or different from, the pulmonary surfactant utilized for RDS. In a preferred embodiment, the same pulmonary surfactant is used.

In a preferred embodiment, the pulmonary surfactant is a modified natural pulmonary surfactant.

More preferably the pulmonary surfactant is poractant alfa (Curosurf™) or a biosimilar thereof as above defined, as it is endowed with very low viscosity, and hence it can be administered at high concentrations using a small volume of aqueous carrier.

In another embodiment, the pulmonary surfactant is a reconstituted surfactant having the composition disclosed in Table 2 of Example 2 of WO 2010/139442.

The dose of the pulmonary surfactant to be administered will vary with the weight and gestational age of the preterm neonate, as well as with the severity of the neonate condition. Those of skill in the relevant art will be readily able to determine these factors and to adjust the dosage accordingly.

Advantageously, the dose of the pulmonary surfactant could be of 100-200 mg/kg.

In a preferred embodiment of the invention, poractant alfa at a dose comprised

between 100 and 200 mg/kg could be used.

In a preferred embodiment, the dose could be of 100 mg/kg, while in another preferred embodiment, the dose could be of 200 mg/kg.

Advantageously, the dose of budesonide is comprised between 0.1 and 1.5 mg/kg, more advantageously between 0.2 and 1.0 mg/kg, even more advantageously between 0.25 and 1.0 mg/kg.

In certain embodiments, when an effect on the lung maturation is primarily pursued, the dose of budesonide might be comprised between 0.1 and 0.5 mg/kg, while in other embodiments the dose of budesonide might be comprised between 0.5 and 1.0 mg/kg.

Preferably, the combination of the invention is administered to pre-term neonates kept under non-invasive ventilation procedures, more preferably kept under nasal CPAP, even more preferably with a nasal device, at a pressure of from 1 to 12 cm water.

The combination of pulmonary surfactant and budesonide at the claimed doses may be administered sequentially, separately or together. Advantageously, when the two active substances are administered together, they are administered as a fixed combination.

Therefore, the present invention also concerns the use of the combination of the invention as a fixed combination in the manufacture of a medicament for treating evolving BPD.

The medicament may be in form of pharmaceutical composition.

Said formulations may be administered in the form of a solution, dispersion, suspension or dry powder. Preferably, said compositions comprise the claimed combination suspended in a suitable physiologically tolerable solvent.

More preferably, the formulation comprises an aqueous solution, preferably sterile, which may also comprise pH buffering agents and other pharmaceutically acceptable excipients such as polysorbate 20, polysorbate 80 or sorbitan monolaurate as wetting agents and sodium chloride as isotonicity agent.

The formulations may be distributed in unit-dose or multi-dose containers, for example sealed ampoules and vials, or may be stored in a frozen or freeze-dried

(lyophilized) condition requiring only the addition of sterile liquid carrier immediately prior to use.

Preferably, the formulation is supplied as sterile suspension in a buffered physiological saline (0.9% w/v sodium chloride) aqueous solution in single-use vials.

The administration of the claimed formulation may be carried out according to known methods, e.g. by endotracheal instillation, by spray administration, or nebulisation by jet ultrasonic, or mesh-vibrating nebulisers commonly available on the market.

When the formulation is administered by endotracheal instillation, depending on the severity of the respiratory distress syndrome, different methods can be appropriate. For example the claimed formulation may be administered through the endotracheal tube to pre-terns neonates kept under mechanical ventilation.

Alternatively, the formulation may be administered by the use of a thin catheter placed in the trachea and the neonate respiration supported through specially designed nasal devices such as masks, prongs or tubes according to methodology known as nasal Continuous Positive Airway Pressure (nCPAP), according to the procedure described in. WO 2008/148469.

The latter approach would be only possible with an exogenous surfactant such as poractant alfa having a low viscosity, as a high viscosity would make the passage of the surfactant through the thin catheter more difficult.

The volume of the aqueous solution in which the two combined active substances are suspended will depend on the desired concentration.

Advantageously, the volume of the formulation should be not more than 5.0 ml, preferably comprising between 4.5 and 2.0 ml, more preferably between 3.5 and 2.5 ml.

In other embodiments, when the pulmonary surfactant and budesonide are administered separately, the individual active substances could be formulated separately. In this case, the two individual active substances do not unconditionally have to be taken at the same time.

In the case of such a separate administration, the formulation of the two individual

active substances can be packed at the same time in a suitable container mean. Such separate packaging of the components in a suitable container mean is also described as a kit.

Therefore, the invention is also directed to a kit for the treatment of evolving broncho-pulmonary dysplasia, said kit comprising: a) a pulmonary surfactant at a dose comprised between 100 and 200 mg/kg and a pharmaceutically acceptable carrier or diluent in a first unit dosage form; b) budesonide at a dose comprised from 0.1 to 1.5 mg/kg and a pharmaceutically acceptable carrier or diluent in a second unit dosage form; and c) container means for containing said first and second dosage forms.

The combination of the invention which could be administered to the preterm neonate after birth according to conditions which shall be established by the skilled person in the art, is suitable to treat any form of evolving bronchopulmonary dysplasia.

The frequency of administration will vary with the size and gestational age of the preterm neonate, as well as with the severity of the neonate condition and the route of administration. Those of skill in the relevant art will be readily able to determine it.

For instance, the medicament of the invention could be administered once or twice a day.

Advantageously, the combination of the invention is administered from the 2nd to the 28th day of life, preferably from the 5th to the 15th day of life, more preferably from the 7th to the 10th day of life.

Within the above interval time, the treatment could be continued for a period of time deemed by a physician or other medical practitioner as appropriate to achieve the therapeutic effect.

The preterm neonate requiring the medicament of the invention may or may not exhibit respiratory distress syndrome (RDS). In one embodiment, the administration of the medicament of the invention is initiated in a neonate exhibiting RDS, following treatment of such syndrome with pulmonary surfactant or by another means (e.g., ventilation) or a combination thereof.

In certain embodiments, neonates to be treated with the medicament of the present invention require respiratory support but do not necessarily exhibit respiratory distress syndrome. These infants either have not been diagnosed with RDS or have not been treated with pulmonary surfactants for RDS.

All pre-term neonates could be eligible for the administration of the medicament of the invention including extremely low birth weight (ELBW), very-low-birth -weight (VLBW), and low-birth weight (LBW) neonates of 24-35 weeks gestational age. Preferably, the medicament is administered to VLBW neonates with severe RDS who will have a higher incidence of BPD.

In general terms, since management of evolving BPD is unlikely to be in the form of a single intervention but rather a combined approach, the physician shall evaluate whether preterm neonates also require concomitant respiratory support and/or other suitable drugs such as vitamin A and antibiotics.

The following examples illustrate the invention in greater detail.

EXAMPLES

EXAMPLE 1 - IN VITRO EVALUATION OF THE SURFACE ACTIVITY OF PORACTANT ALFA IN THE PRESENCE OF BUDESONIDE BY CAPILLARY SURFACTOMETER

The surface activity of poractant alfa (in the presence of budesonide, 2 ml, 1.0 mg) is evaluated in comparison to poractant alfa alone by a capillary surfactometer commercially available from Calmia Medical, Inc., USA.

Two samples are prepared: one from a vial of poractant alfa (1.5 ml, 80 mg/ml) by diluting with saline to a concentration 1 mg/ml in phospholipids, and the other from a vial of poractant alfa (1.5 ml, 80 mg/ml) mixed with a vial of budesonide (2 ml, 1.0 mg) and diluted with saline to the same concentration (1 mg/ml phospholipids). A 0.5 ml sample of both solutions is then assessed in the Capillary Surfactometer.

The principle of the capillary surfactometer is to simulate terminal human airways. The sample is introduced into the narrow section of a glass capillary, where the inner

diameter is 0.25 mm, similar to that of a terminal human airway. At one end the capillary is connected to a bellows and a pressure transducer. When the bellows is slowly compressed, pressure is raised and recorded. The increasing pressure causes the sample to be extruded from the narrow section of the capillary. As air gets through, pressure is abruptly lowered. If the sample contains well-functioning pulmonary surfactant the sample liquid will not return to the narrow section. The steady airflow obtained by the continuous compression of the bellows will meet no resistance and the pressure recorded will be zero. If on the other hand the sample does not contain a well-functioning pulmonary surfactant, the sample liquid will return repeatedly.

The behavior of poractant alfa in the presence of budesonide turns out to be statistically indistinguishable from that of poractant alfa alone, indicating that budesonide at said dose does not affect the surface activity of the surfactant.

EXAMPLE 2 - IN VIVO EVALUATION OF THE ACTIVITY OF PORACTANT ALFA IN THE PRESENCE OF BUDESONIDE IN A LAMB MODEL OF BPD

An experiment for the study of neonatal resuscitation and lung injury with an assessment of a surfactant with budesonide treatment to decrease lung injury was performed. The experiment was aimed at checking whether a stretch injury to the fetal lung would modulate a second ventilation mediated injury 24 hours after intrauterine recovery.

This was a study to test for preconditioning or tolerance response potentials of the fetal lung. The treatment with surfactant with or without budesonide after the initial stretch injury was to test if the steroid had anti-inflammatory effects that would protect the fetal lung. The groups of animals included CPAP exposure and no initial exposure for comparison with the stretch injury. The groups and their characteristics and treatments are given in Table 1. The legend to Table 1 gives details about the interventions. Figure 1 is a schematic of the protocol for this complex series of interventions.

Table 1

Groups	Animal Number	Gestational Age	Fetal Lung injury Intervention	Treatment after Intervention	Ventilation Test at 24 hrs.	Birth Weight (kg)
CPAP +Surf + Vent	5	125.8	CPAP only	Surf + Saline	Yes	2.9
CPAP + Surf + Bud 0.25 mg.kg + Vent	6	125.8	CPAP only	Surf + Bud – 0.25	Yes	2.6
V_t15 + Surf + Vent	7	126	Yes	Surf + Saline	Yes	3.1
V_t15 + Surf + Bud 0.25 mg/kg+ Vent	8	126.1	Yes	Surf + Bud – 0.25	Yes	3.0
V_t15 +Surf + Bud 1.0 mg/kg + Vent	7	126.6	Yes	Surf + Bud – 1mg	Yes	2.7
Nothing + Vent	6	126.3	No	Nothing	Yes	2.9
Nothing + Nothing	5	125.4	No	Nothing	No	2.8

Legend to Table

- CPAP: Animals placed on 5 cm H_2O CPAP for 15 min as a control for the anesthesia and surgery related to the injury intervention.
- Fetal lung injury intervention: Yes = Head and chest of animal is exposed; 4.5 mm endotracheal tube is secured in the trachea. The fetus then is ventilated with 100% humidified nitrogen with R=30, IT=1 sec, PEEP=0, maximal pressure to 55 cm H_2O. The goal was to achieve an estimated V_t of 7 ml/kg at 4 min, 12 ml/kg at 8 min, and 15 ml/kg at 12 min. The total period of ventilation was 15 min.
- Pulmonary surfactant (Surf): After the CPAP or fetal lung injury intervention, animals were treated with 100 mg/kg Curosurf assuming 3Kg weight. Curosurf plus budesonide was diluted with saline to 10 ml. Surfactant was given through endotracheal tube and mixed by syringe with fetal lung fluid. Following surfactant treatment, the trachea was ligated to prevent loss of surfactant.

- Budesonide (Bud): Pulmicort Respules (Astra Zeneca, Sweden) containing 0.5 mg micronized budesonide in 1 ml was mixed with Curosurf plus saline to deliver 0.25 or 1.0 mg/kg budesonide and surfactant in a 10 ml suspension.
- Ventilation test at 24 hours: Head of animal was again exposed and a 4.5 mm endotracheal tube was placed. Fetal lung fluid was aspirated by syringe and the lamb was delivered and ventilated with a rate of 40, an inspiratory time of 0.45 sec, a PEEP of 5 cmH_2O and a maximal peak inspiratory pressure of 40 cm H_2O with 100% humidified oxygen.

The experiment was designed for 46 fetal sheep, and the final total was 44 as one ewe had no fetus and one twin was a singleton. The experimental procedures were successfully completed with all other lambs. The number of animals per group were adjusted to increase the animals in the V_t 15 injury and surfactant groups in order to increase statistical power for those groups. Specific comments about important elements of the experimental design follow.

- The ventilation injury targeted to an estimated 15 ml/kg tidal volume at 15 min only achieved a volume of 11-13 ml/kg despite use of the maximal pressure of 55 cm H_2O, indicating that the fetal lungs were immature and surfactant deficient.
- The V_t injury and CPAP groups (assuming 3 kg birth weight) were administered with 100 mg/kg Curosurf or Curosurf plus budesonide diluted to 10 ml with saline. The trachea was ligated following treatment to assure that the treatments stayed in the lung for 24 hrs in utero prior to assessment of lung lunction.
- Budesonide was used as 0.5 mg/ml Pulmicort Respules so as to have a standardized sterile product for exposure of the fetal lung.
- At delivery 24 hrs after the initial intervention and treatment, an endotracheal tube was placed and any freely flowing fetal lung fluid was withdrawn with a syringe. Large amounts of fluid were recovered from CPAP exposed lungs. There was no fetal lung fluid and only small amounts of thick secretions were aspirated from the V_t injured lungs.
- The post-delivery ventilation period of 30 min was successful in all ventilated

lambs. Some lungs had air collections within lung tissue and pleural blebs, but most of the pressure volume curves were successful.

Results

There were no important differences in gestational ages or birth weights between groups (Table 1). These experiments are complex to analyse because there are 7 groups. The Nothing + Nothing animals will be used only for tissue collection for the baseline measurements.

Oxygenation

The results are reported in Figure 2.

Oxygenation measured as arterial PO_2 while the animals were ventilated with 100% oxygen, was very low for the uninjured and not surfactant treated ventilated twins only exposed to anaesthesia 24 hours before the ventilation - mean value 54 mmHg. This result verifies that the lambs had immature lungs prior to any intervention. Despite surfactant with or without budesonide, the injury ablated an oxygenation response. The 15 min CPAP + surfactant treatment resulted in a striking increase in mean PO_2 to 380 mmHg and the addition of budesonide resulted in a mean PO_2 of 417 mmHg, indicating that said addition slightly increases oxygenation.

In the non-ventilated groups, even though rather low, a slight improvement trend in presence of increasing concentration of budesonide can be appreciated from the comparison of groups median values.

Static Lung gas volume at 40 cmH_2O measured from the Pressure-Volume curves.

The results are reported in Figure 3.

The maximal lung gas volumes expressed relative to body weight measured following oxygen adsorption for inflation measurements on a lung without gas lung were low in the ventilated twin lungs. The CPAP + surfactant group had a large increase in lung gas volume/kg body weight, and budesonide significantly increased the volume relative to CPAP + surfactant. As for oxygenation, there were no large effects on lung gas volumes

for animals in the V_t 15 injured groups.

Lung Weight

The results are reported in Figure 4.

Large changes in lung weight across the groups at necropsy were surprisingly observed. Lung weights for the ventilated twin, the Vt 15 injury + surfactant and CPAP + surfactant were similar. However, in each of the budesonide groups, lung weights were lower with the largest effect noted for the 1.0 mg/kg budesonide group. Much change in lung weight with fetal exposure to maternal steroids was never measured in other experiments, so the observed very large changes in lung weights are rather unexpected.

Lung Gas Volume Relative to Lung Weight

The results are reported in Figure 5.

Of note, while lung weight decreased for the CPAP + surfactant + budesonide group relative to the CPAP + surfactant group, the lung gas volume increased. Therefore, the lung volume to lung weight ratios is shown. This ratio emphasizes the combined effects of lighter lungs that hold more gas. CPAP + Budesonide lungs held more gas than the CPAP lungs. This ratio also demonstrates that the V_t 15 + surfactant lung held less gas than those exposed to lung budesonide.

Lung Dry to Wet Weight Ratios

The results are reported in Figure 6.

These large changes in lung weight associated with budesonide must be primarily loss of lung water over 24 hours. That loss of water could indicate loss of mesenchymal cells and a maturation response.

mRNA Indicators of Lung Maturation

mRNA was analysed for surfactant proteins (SP) A, B, C, and D.

It is well known that the expression of the surfactant proteins are a sign of lung maturation.

The results for SP-A, SP-B, and SP-C, are reported in Figure 7.

There were consistent decreases in mRNA for the CPAP + surfactant group relative

to the unmanipulated controls and ventilated twins. Said suppression of surfactant protein mRNA is rather unexpected. Of interest, the combination of CPAP + surfactant with budesonide increased the surfactant proteins significantly.

EXAMPLE 3 - Formulation in form of aqueous suspension according to the invention.

Ingredients	Quantity For pharmaceutical unit
Poractant alfa	160 mg
micronised budesonide	0.5 mg
Polysorbate (Tween) 20	2.0 mg
Sorbitan monolaurate	0.4 mg
Sodium chloride	18 mg
Water for injection q. s. for	2.0 ml

EXAMPLE 4 - Airway thickness determination

It is known that the large and small airway appearance and thickness is altered by both CPAP and mechanical ventilation.

In particular, in the lack of proper lung maturation, an increase of the wall thickness as well as an increase of collagen deposition is observed.

Therefore, the thickness of large airway/bronchioles and the collagen deposition was measured in lambs exposed to hyperoxia according to the method reported in Wang H et al Am J Physiol. Lung Cell Mol Physiol 2014, 307, L295-L301.

The thickness was measured on blinded sections with 3 measurements per slide image, and 5 slide images per animal, and 4 to 6 animals per group.

The results are reported in Table 2.

Animals receiving mechanical ventilation had thickened small and large airways. Furthermore, quantification of collagen within the airways demonstrated increased collagen staining in animals receiving mechanical ventilation.

On the contrary, a decrease was observed for the CPAP + surfactant + budesonide groups, particularly significant for the CPAP + surfactant + budesonide 0.25 mg/kg group.

Table 2

Groups	**Large Airway Thickness**	**Large Airway Collagen**	**Bronchiole Thickness**	**Bronchiole Collagen**
	Microns	% staining	Microns	% staining
CPAP +Surf + Vent	65.4±8.9*	21.4±5.7*	16.3±4.1	51.3±13.5*
CPAP + Surf + Bud 0.25 mg.kg + Vent	35.1±7.1	7.4±1.6	14.1±1.9	22.9±10.6
V_t15 + Surf + Vent	70.1±7.9*	29.4±5.6*	21.3±5.5*	84.8±19.1*
V_t15 + Surf + Bud 0.25 mg/kg+ Vent	30.1±7.6	8.3±1.7	13.2±2.7	14.6±1.6
V_t15 +Surf + Bud 1.0 mg/kg + Vent	35.5±4.1	18.2±1.5*	15.0±0.4*	40.0±8.1*
Unventilated Controls	28.7±11.4	3.6±1.1	11.3±1.2	13.0±3.3

CLAIMS

1. A combination of a pulmonary surfactant with budesonide at a dose comprised from 0.1 to 1.5 mg/kg for use for the treatment of evolving bronchopulmonary dysplasia (BPD) in a preterm neonate.

2. The combination for use according to claim 1, which is administered from the 2nd to the 28th day of life of the preterm neonate.

3. The combination for use according to claim 2, which is administered from the 5th to the 15th day of life.

4. The combination for use according to claim 3, which is administered from the 7th to the 10th day of life.

5. The combination for use according to any one of the preceding claims, wherein the preterm neonate is kept under a non-invasive ventilation procedure.

6. The combination for use according to claim 5, wherein the non-invasive ventilation procedure is nasal CPAP.

7. The combination for use according to any one of the preceding claims, wherein the dose of budesonide is comprised between 0.2 and 1.0 mg/kg.

8. The combination for use according to any one of claims 1 to 6, wherein the dose of budesonide is comprised between 0.1 and 0.5 mg/Kg.

9. The combination for use according to any one of claims 1 to 6, wherein the dose of budesonide is comprised between 0.5 and 1.0 mg/kg.

10. The combination for use according to any one of the preceding claims wherein the pulmonary surfactant and budesonide are administered simultaneously, sequentially or separately.

11. The combination for use according to any one of the preceding claims, wherein the dose of the pulmonary surfactant is comprised between 100 and 200 mg/kg.

12. The combination for use according to any one of the preceding claims, wherein the pulmonary surfactant is poractant alfa or a biosimilar thereof.

13. The combination for use according to any one of claims 1 to 12 to be administered as a pharmaceutical formulation by inhalation or intratracheal route.

14. The combination for use according to claim 13, wherein the pharmaceutical formulation is in form of aqueous suspension comprising a pharmaceutically acceptable carrier.

15. A kit for use for the treatment of evolving broncho-pulmonary dysplasia, said kit comprising: a) a pulmonary surfactant at a dose comprised between 100 and 200 mg/kg and a pharmaceutically acceptable carrier or diluent in a first unit dosage form; b) budesonide at a dose comprised from 0.1 to 1.5 mg/kg and a pharmaceutically acceptable carrier or diluent in a second unit dosage form; and c) container means for containing said first and second dosage forms.

Figure 1

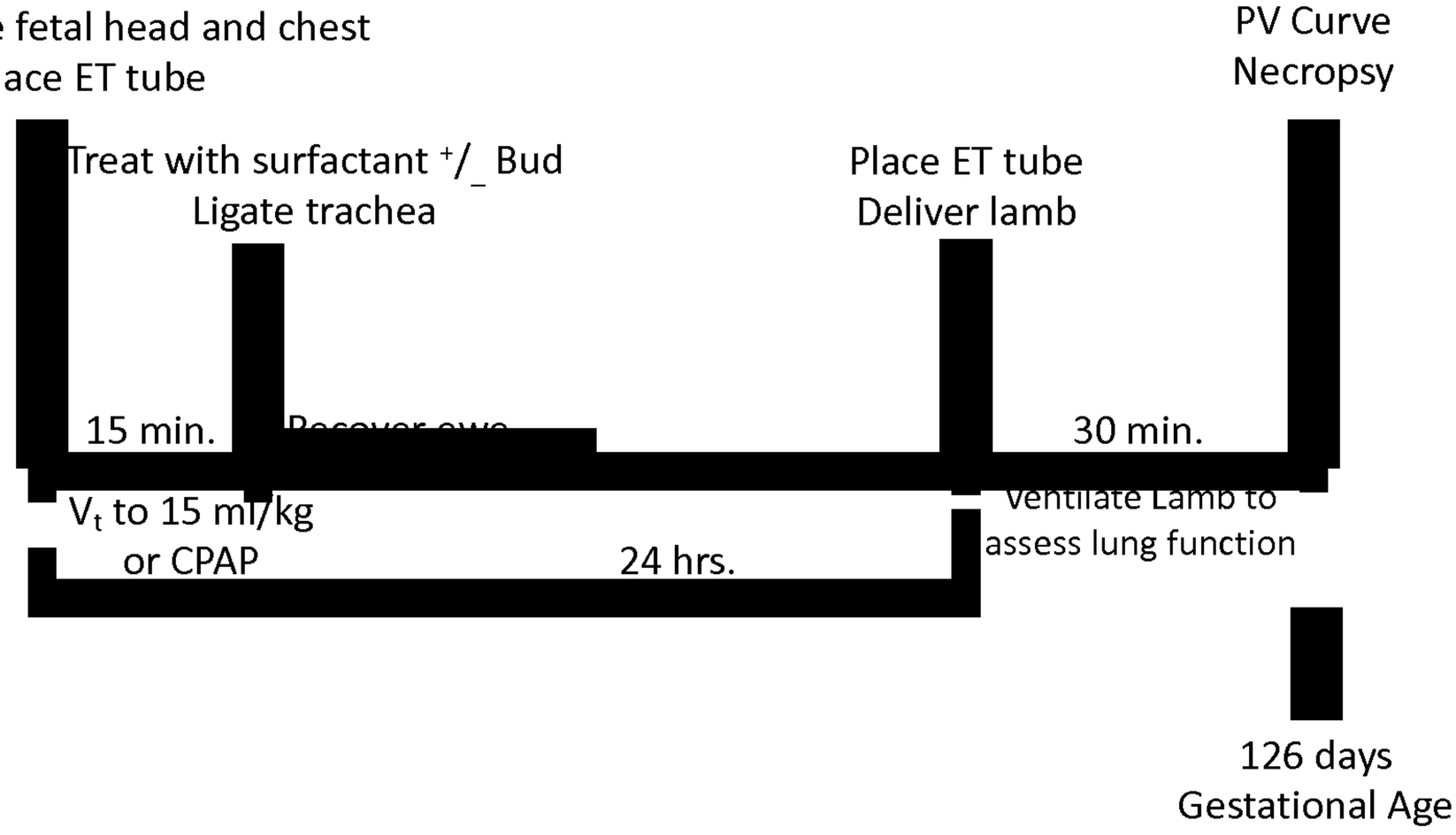

Expose fetal head and chest
Place ET tube
Treat with surfactant +/_ Bud
Ligate trachea
PV Curve
Necropsy
Place ET tube
Deliver lamb
15 min.
30 min.
V_t to 15 ml/kg
or CPAP
24 hrs.
Ventilate Lamb to
assess lung function
126 days
Gestational Age

Figure 2

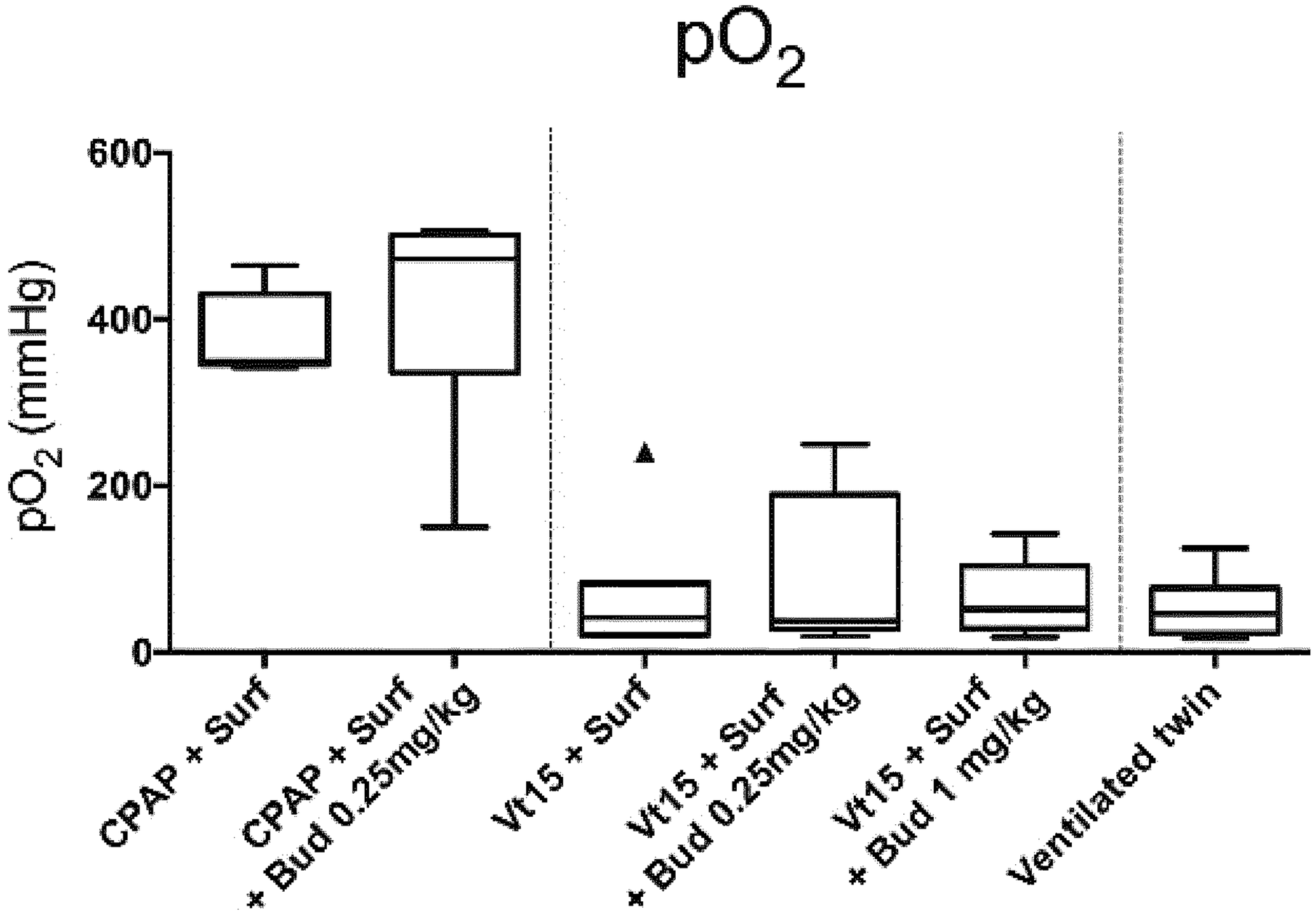

pO2
600
400
200
0
pO2 (mmHg)
CPAP + Surf
CPAP + Surf + Bud 0.25mg/kg
Vt15 + Surf
Vt15 + Surf + Bud 0.25mg/kg
Vt15 + Surf + Bud 1 mg/kg
Ventilated twin

Figure 3

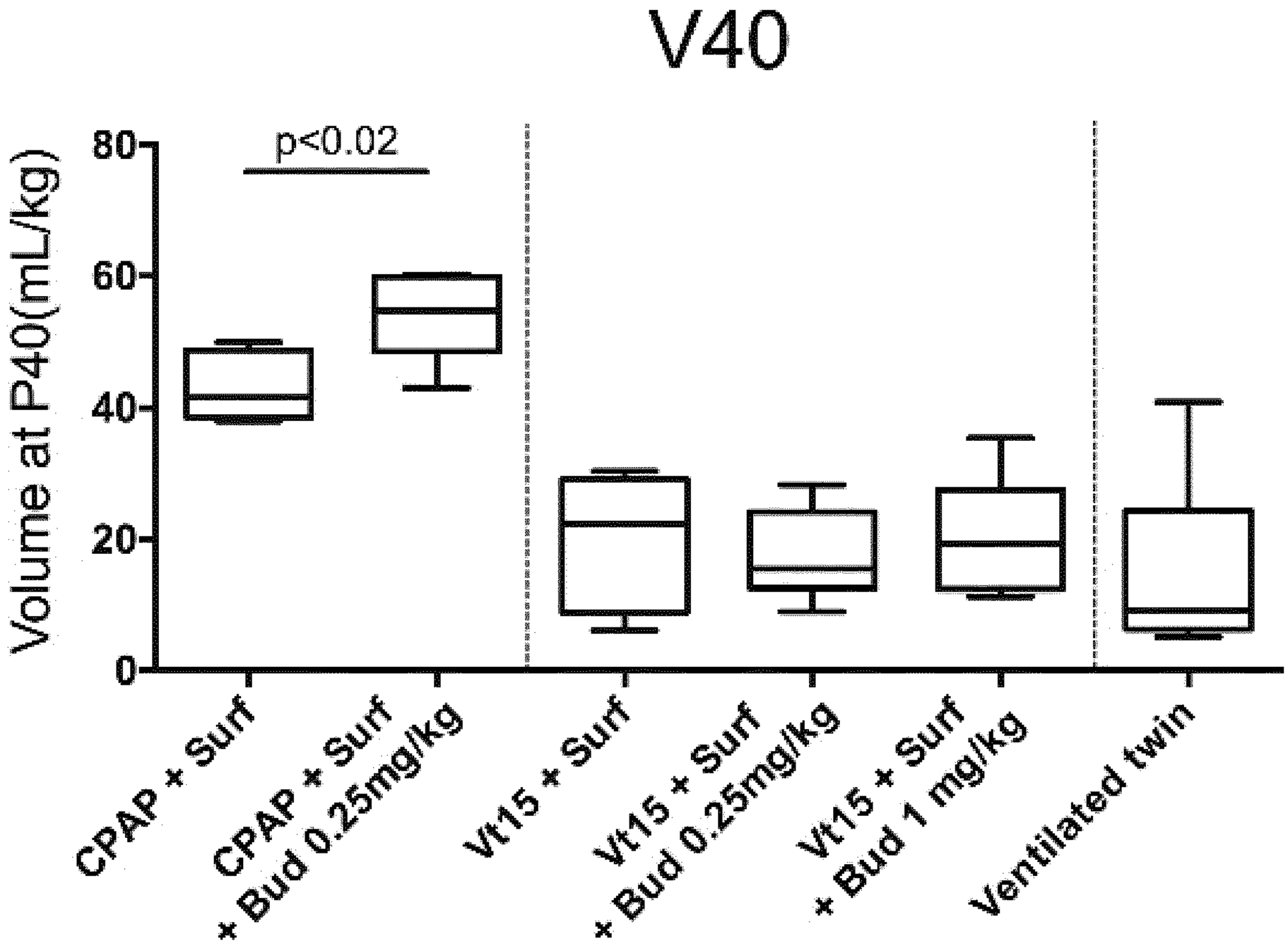

V40
p<0.02
Volume at P40(mL/kg)
80
60
40
20
0
CPAP + Surf
CPAP + Surf + Bud 0.25mg/kg
Vt15 + Surf
Vt15 + Surf + Bud 0.25mg/kg
Vt15 + Surf + Bud 1 mg/kg
Ventilated twin

Figure 4

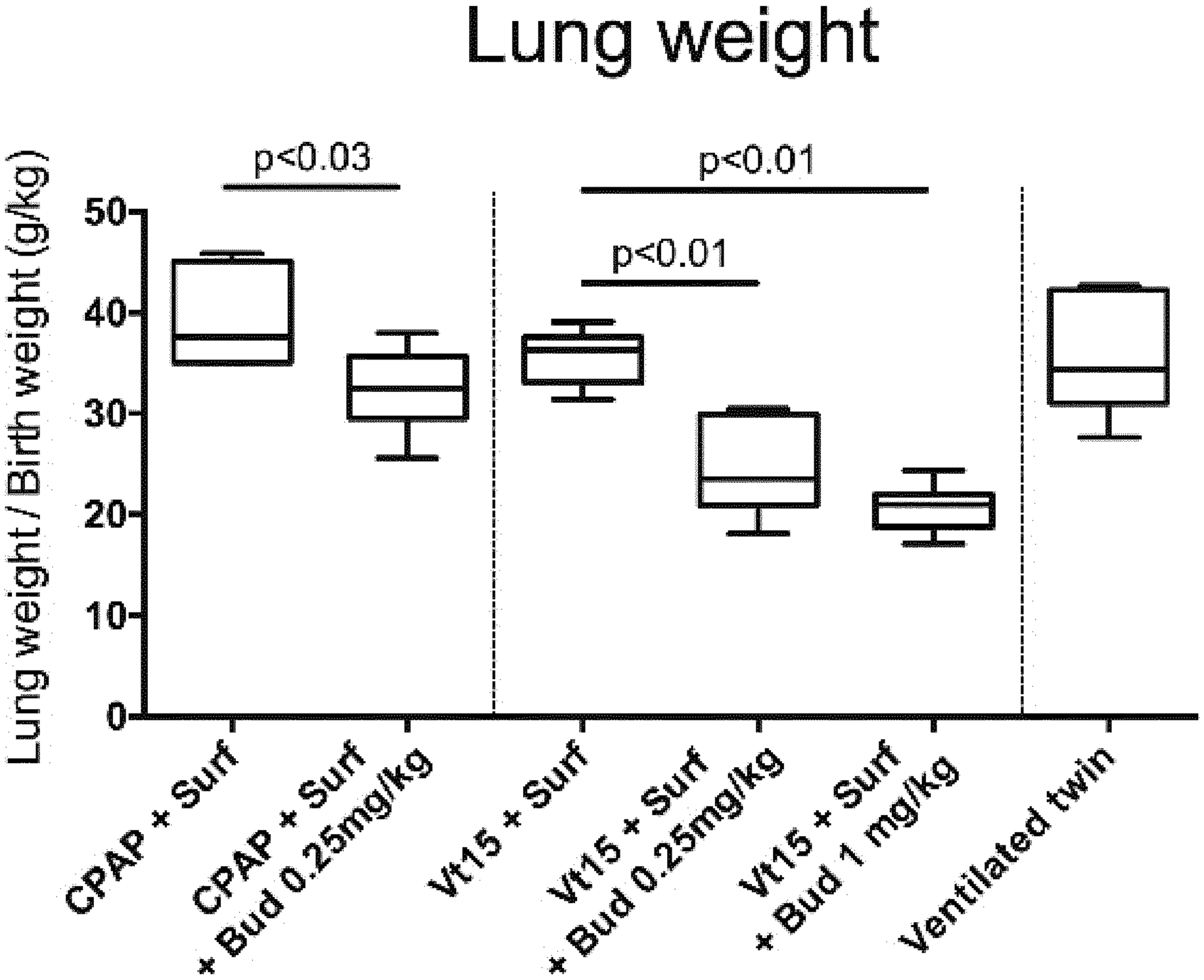

Lung weight
p<0.03
p<0.01
p<0.01
Lung weight / Birth weight (g/kg)
50
40
30
20
10
0
CPAP + Surf
CPAP + Surf + Bud 0.25mg/kg
Vt15 + Surf
Vt15 + Surf + Bud 0.25mg/kg
Vt15 + Surf + Bud 1 mg/kg
Ventilated twin

Figure 5

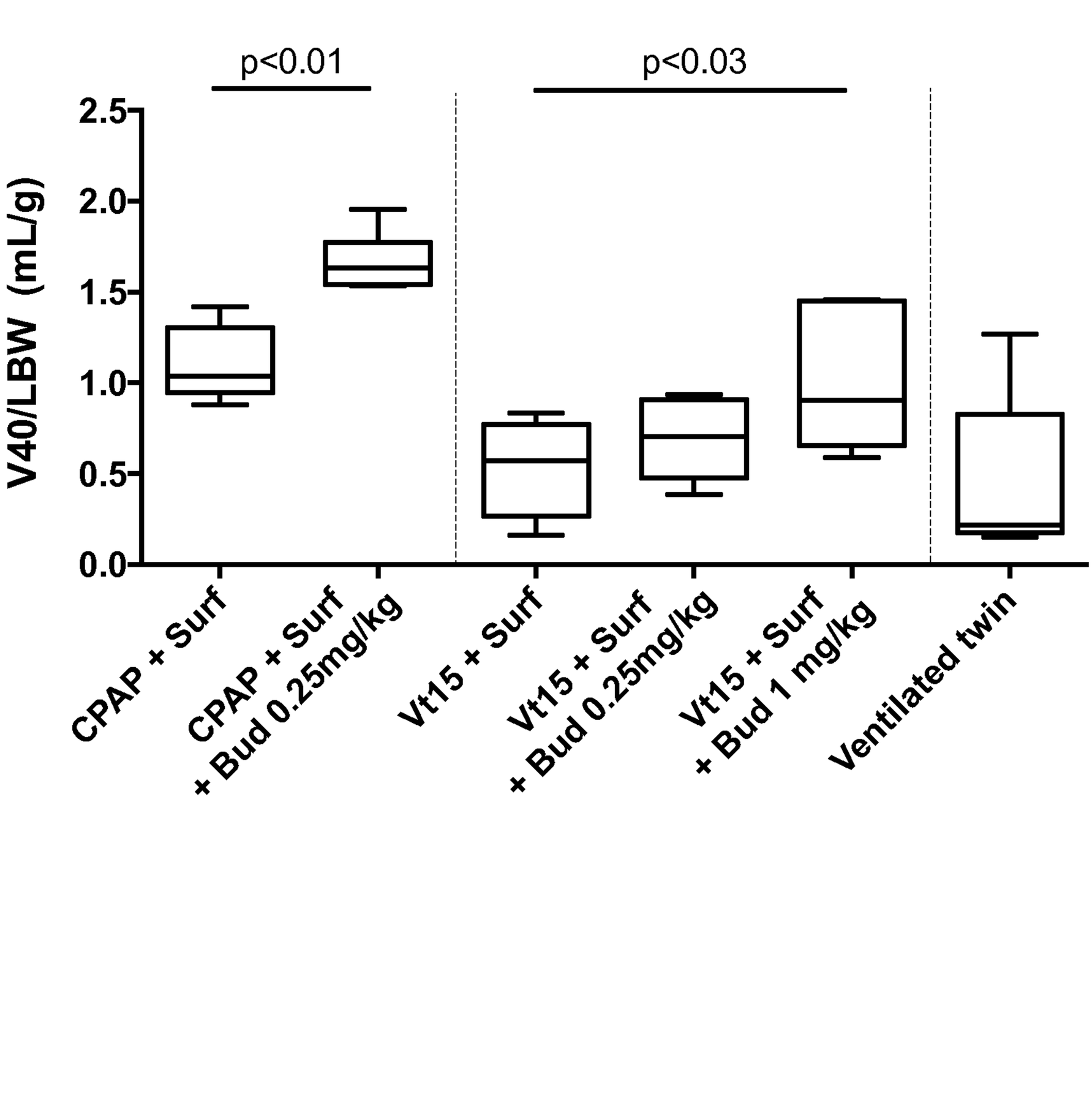
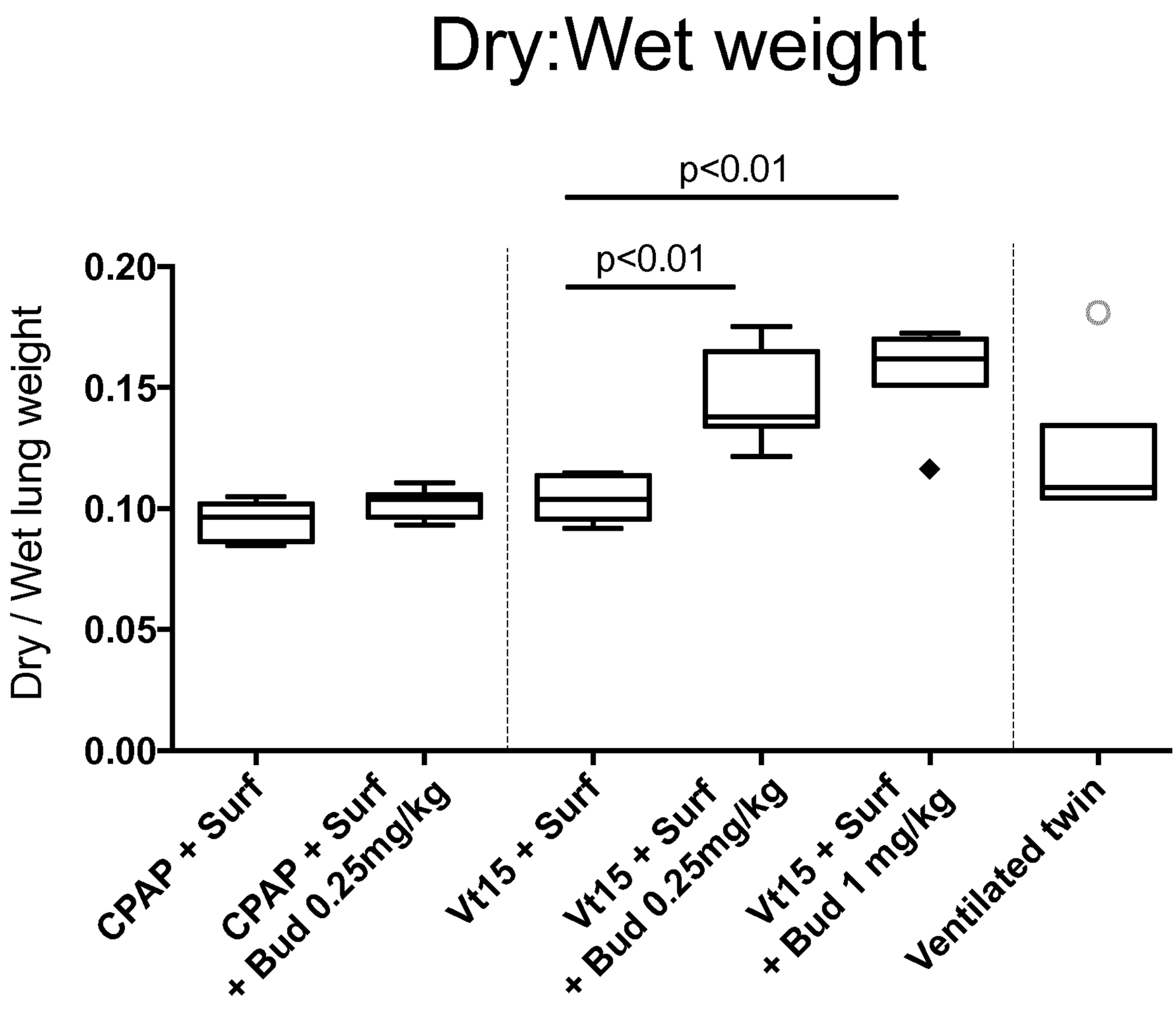

Volume / lung weight
p<0.01
p<0.03
V40/LBW (mL/g)
2.5
2.0
1.5
1.0
0.5
0.0
CPAP + Surf
CPAP + Surf + Bud 0.25mg/kg
Vt15 + Surf
Vt15 + Surf + Bud 0.25mg/kg
Vt15 + Surf + Bud 1 mg/kg
Ventilated twin

Figure 6


Dry:Wet weight
p<0.01
p<0.01
Dry / Wet lung weight
0.20
0.15
0.10
0.05
0.00
CPAP + Surf
CPAP + Surf + Bud 0.25mg/kg
Vt15 + Surf
Vt15 + Surf + Bud 0.25mg/kg
Vt15 + Surf + Bud 1 mg/kg
Ventilated twin

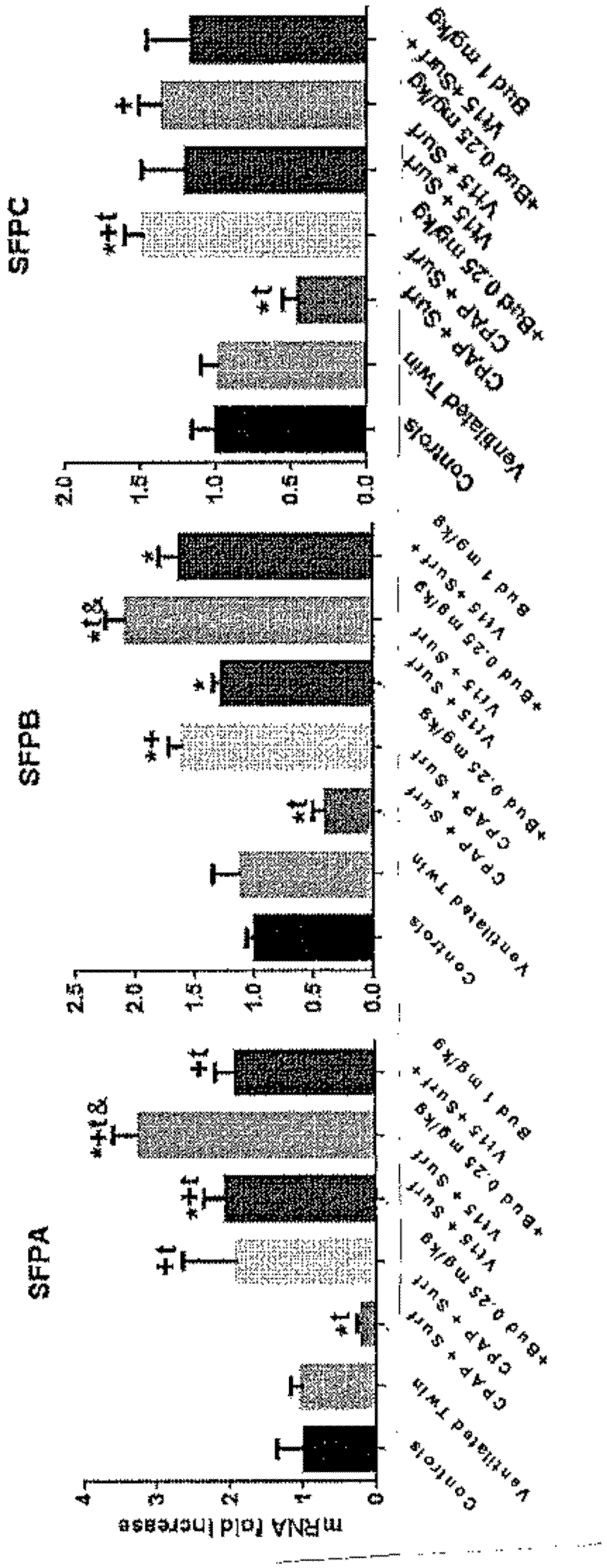

SFPC
SFPB
SFPA
mRNA fold increase
Controls
Vehicle
Budesonide 1 mg/kg
Surfactant
Budesonide 0.25 mg/kg + Surfactant
VH15 1 mg/kg
VH15 + Surfactant
Budesonide 0.25 mg/kg + VH15 + Surfactant
Ventilated Twin
CPAP
CPAP + Surfactant
Budesonide 0.25 mg/kg + CPAP + Surfactant

Figure 7