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Effect of betamethasone, surfactant, and positive end-expiratory pressures on lung aeration at birth in preterm rabbits

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Crawshaw JR, Hooper SB, te Pas AB, Allison BA, Wallace MJ, Kerr LT, Lewis RA, Morley CJ, Leong AF, Kitchen MJ. Effect of betamethasone, surfactant, and positive end-expiratory pressures on lung aeration at birth in preterm rabbits. *Am J Physiol Regul Integr Comp Physiol* 121: 750–759, 2016. First published July 8, 2016; doi:10.1152/japplphysiol.01043.2015.—Antenatal glucocorticoids, exogenous surfactant, and positive end-expiratory pressure (PEEP) ventilation are commonly provided to preterm infants to enhance respiratory function after birth. It is unclear how these treatments interact to improve the transition to air-breathing at birth. We investigated the relative contribution of antenatal betamethasone, prophylactic surfactant, and PEEP (3 cmH_2O) on functional residual capacity (FRC) and dynamic lung compliance (C_{DL}) in preterm (28 day GA) rabbit kittens at birth. Kittens were delivered by cesarean section and mechanically ventilated. FRC was calculated from X-ray images, and C_{DL} was measured using plethysmography. Without betamethasone, PEEP increased FRC recruitment and C_{DL}. Surfactant did not further increase FRC, but significantly increased C_{DL}. Betamethasone abolished the benefit of PEEP on FRC, but surfactant counteracted this effect of betamethasone. These findings indicate that low PEEP levels are insufficient to establish FRC at birth following betamethasone treatment. However, surfactant reversed the effect of betamethasone and when combined, these two treatments enhanced FRC recruitment irrespective of PEEP level.

functional residual capacity; betamethasone; preterm birth; positive end-expiratory pressure; surfactant

NEW & NOTEWORTHY

Antenatal betamethasone, surfactant therapy, and positive end-expiratory pressure (PEEP) ventilation are frequently used in combination to improve lung function in preterm newborns. However, it is unclear how these factors interact to enhance functional residual capacity (FRC) and dynamic lung compliance (CDL) at birth. Contrary to expectation, betamethasone hindered FRC development and abolished the benefit of low PEEP, yet improved CDL. However, surfactant administration counteracted the adverse effect of betamethasone on FRC recruitment at birth.

VERY PRETERM INFANTS HAVE lungs that are structurally and functionally immature with a small gas exchange surface area and a large gas diffusing distance, they are commonly surfactant deficient (2, 17, 48). These infants struggle to clear their airways of liquid because of this structural immaturity, a high chest wall compliance and weak inspiratory muscles, making it difficult to develop a functional residual capacity (FRC) after birth (1, 14). As such, very preterm infants usually require interventions to facilitate the transition to pulmonary gas exchange at birth. These interventions include antenatal glucocorticoids, surfactant therapy, and respiratory support, either using continuous positive airway pressure (or CPAP) or mechanical ventilation with a positive end-expiratory pressure (PEEP). However, the relative contribution that each treatment has on facilitating lung aeration and how they interact is currently unknown. Antenatal glucocorticoids are routinely administered to women at risk of premature delivery (38) and act to induce morphological changes in lung structure (50). These include condensation of perialveoli mesenchymal tissue (7), increasing surface area-to-tissue volume ratios, and reduced gas diffusion distances (50). Combined with an associated increase in lung compliance (20, 35), these changes greatly increase the gas exchange potential of the lung (10, 20). As the lungs of preterm infants are often surfactant deficient (4), exogenous surfactant is also commonly administered to increase lung compliance and oxygenation (6, 19, 43). Surfactant administration during lung aeration also greatly increases the uniformity of lung aeration, which enhances the distribution of ventilation, increases lung compliance, and reduces the risk of regional overdistension and volutrauma (39).

Respiratory support in the delivery room should include a distending pressure applied to the airways during expiration (32, 51). Previous studies have shown that PEEP, in combination with an appropriate tidal volume, is required to effectively recruit a FRC (40, 49), resulting in an increase in oxygenation (36). Ventilation with PEEP opposes lung collapse and prevents liquid reflooding into the distal airways during expiration (40, 47). This allows gas exchange to continue throughout the respiratory cycle, which is otherwise restricted to the brief period of inflation (40). PEEP also increases lung compliance by reducing the inflation pressures required to aerate the distal gas exchange units. However, it is unknown how antenatal glucocorticoids and prophylactic surfactant interact with PEEP to enhance FRC accumulation after birth.

This study aimed to determine the relative contribution and interaction between antenatal glucocorticoids, prophylactic surfactant, and PEEP on lung aeration and FRC accumulation in preterm rabbits using phase contrast (PC) X-ray imaging. We hypothesized that both betamethasone and prophylactic surfactant would have separate positive effects on FRC recruit-

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Table 1. *Peak inflation pressure required to achieve a set tidal volume of 10 ml/kg at 7 min after ventilation onset*

	Vehicle				Betamethasone			
	Saline		Surfactant		Saline		Surfactant	
PEEP level	*n*	PIP	*n*	PIP	*n*	PIP	*n*	PIP
0 cmH_2O	6	34.2 ± 1.2[a]	6	29.8 ± 1.6[b]	5	24.5 ± 3.3[cb]	6	22.4 ± 1.9[c]
3 cmH_2O	5	29.4 ± 0.9[b]	7	25.7 ± 1.4[b]	7	26.6 ± 2.2[b]	6	20.8 ± 1.0[c]

Values are expressed as means ± SE, and values that do not share a common letter are significantly different from one another, $P < 0.05$. PEEP, positive end expiratory pressure; PIP, peak inflation pressure.

ment and dynamic lung compliance (C_{DL}) in both the presence and absence of ventilation with PEEP. To observe the potential additive effects of surfactant and betamethasone on FRC recruitment in response to PEEP, we used a relatively low PEEP level (3 cmH_2O) to avoid obscuring these additive effects.

METHODS

Experimental Procedure

All animal procedures were approved by the SPring-8 Animal Care and Monash University's School of Biomedical Science's Animal Ethics Committees. Experiments were conducted in experimental hutch 3 of beamline 20B2 in the Biomedical Imaging Centre at the SPring-8 synchrotron in Japan, as previously described (15, 40, 41).

Fifteen pregnant New Zealand white rabbits were divided into two groups and received either intramuscular injections of 0.1 mg/kg betamethasone (Celestone Chronodose; $n = 8$) or saline ($n = 7$) at 48 and 24 h prior to delivery. At 28 days gestation, does were anesthetized using Rapinovet (12 mg/kg bolus iv propofol; Schering-Plough Animal Health, Boxmeer, Netherlands) and intubated, and anesthesia was maintained by inhalation (1.5–4%; isoflurane, Delvet, Seven Hills, Australia). Rabbit kittens were partially delivered (one at a time) by cesarean section, sedated (0.1 mg ip; Nembutal, Abbott Laboratories) and intubated (see Table 1 for kitten numbers per group).

Prior to delivery, both the betamethasone and vehicle-treated groups were further subdivided equally into two groups. In one group, the endotracheal (ET) tube (18G) was preloaded with 0.05 ml surfactant (Curosurf; Chiesi Pharmaceuticals, Parma, Italy; ~100 mg/kg), whereas in the other group, the ET tube was preloaded with 0.05 ml saline. The ET tube was occluded prior to insertion into the trachea and remained occluded until it was connected to the ventilator. All kittens were then fully delivered and placed, head out, in a prewarmed (40°C), water-filled plethysmograph, which was located within the path of the X-ray beam, as previously described (15, 41).

Following placement in the plethysmograph, the ET tube was connected to a custom-made, time-cycled, pressure-controlled ventilator that initiated image acquisition synchronously with each inflation (23). Each of the four groups of kittens were further subdivided into two groups and ventilated with either a PEEP of 3 cmH_2O (3PEEP) or without PEEP (0PEEP). Thus, we had a total of eight groups, and all kittens were equally divided between the four postnatal treatment groups (+/− surfactant and +/− PEEP) within a single litter (Fig. 1). Kitten numbers for each group are presented in Table 1.

All kittens were ventilated with air for 7 min at a rate of 24 inflations/min. Ventilation commenced with a peak inflation pressure (PIP) of 35 cmH_2O and was adjusted to maintain a tidal volume (V_T) of 10 ml/kg; in a 30-g kitten, this equates to a V_T of 0.3 ml. Airway pressures and lung gas volumes (from the plethysmograph) were digitally recorded (PowerLab; ADInstruments, Sydney, Australia) simultaneously with the PC X-ray image acquisition. The plethysmograph was calibrated prior to experimentation by measuring the pressure increase associated with the addition of 1 ml of water; the lowest level of sensitivity is ±0.015 ml. At the completion of the experiment, all animals were humanely killed with an overdose of Nembutal (100 mg/kg) administered intravenously (doe) or intraperitoneally (kittens).

PC X-Ray Imaging

Kittens were positioned ~210 m downstream of the X-ray source, which was passed through a Si(111) monochromator to provide a monochromatic (24 keV) X-ray beam, as previously described (15, 22, 24). The detector (Hamamatsu EM-CCD, C9100-02, and Hamamatsu CCD, C4742-95HR) was positioned a further 2 m downstream of the kittens. The EM-CCD camera had an effective pixel size of 32.2 μm^2 and an active field of view of 32.3 × 32.3 mm^2. The CCD camera had an effective pixel size of 16.2 μm and an active field of view of 64.8 × 43.2 mm^2. Detector sensitivity differences were accounted for during image analysis.

A sequence of seven images was collected during each respiratory cycle, at 250-ms intervals. Inflation onset triggered the opening of the downstream shutter to irradiate the kitten and, following a brief delay (~20 ms), image acquisition using an exposure time of 50 ms.

Image Analysis

Lung gas volumes at FRC were measured throughout the experimental period from the PC X-ray images using a phase retrieval analysis, as previously described (21). This technique is more accurate than water plethysmography for measuring FRC, as it avoids the small

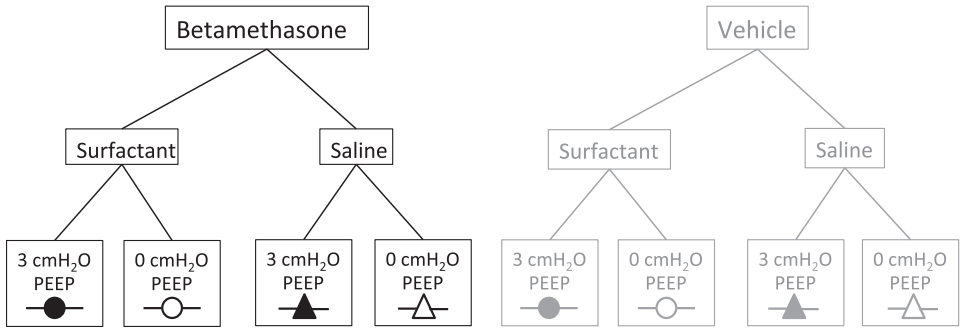


Fig. 1. A flow diagram of the different treatments and experimental groups used in this study. The symbol displayed in each of the experimental groups depicts the same group used in each of the following figures.

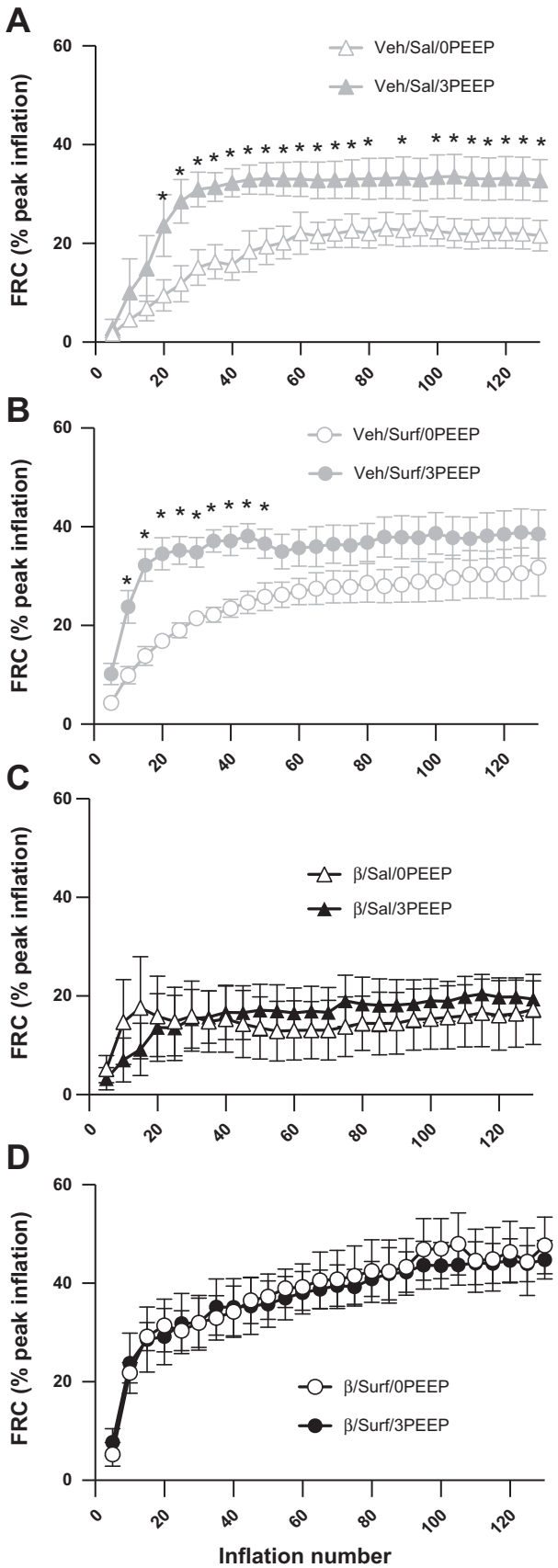


Fig. 2. Changes in functional residual capacity (FRC) during the first 130 inflations, comparing the effect of 3 cmH_2O of positive end-expiratory pressure (3PEEP) vs. no PEEP (0PEEP): with both betamethasone and surfactant (*A*), with surfactant (Surf), but without betamethasone (*B*); with betamethasone (β), but without surfactant (*C*), with both betamethasone and surfactant (*D*). *Significantly different between groups ($P < 0.05$).

effects of buoyancy changes in the kitten caused by lung aeration. The lung gas volumes at FRC from each respiratory cycle were then normalized to the maximum lung gas volume achieved during peak inflation for each kitten to account for differences in body weight and variations between detectors. As such, the FRC curves for each pup are expressed as the percentage of the volume measured at the maximum peak inflation, which was typically within the first 20 inflations.

Physiological Analysis

To assess V_T recruitment in each group of pups, we measured the change in compliance over the first 20 inflations following ventilation onset; after this initial recruitment, the PIP remained relatively constant. As the pressure was adjusted to achieve a set V_T of 10 ml/kg, and some groups required higher inflation pressures than others to achieve this volume, we measured breath-by-breath changes in dynamic lung compliance (C_{DL}). The V_T were obtained from the plethysmograph recording and airway pressures from the ventilator. We also measured the PIP required to achieve a set V_T of 10 ml/kg at the end of the 7-min ventilation period.

Statistical Analysis

A three-way ANOVA was initially performed to demonstrate significant differences between groups. The data were then subdivided, and two-way repeated-measures ANOVAs followed by Holm-Sidak post hoc tests (GraphPad Prism, San Diego, CA) were used to determine significant differences in C_{DL} and FRC between two variables at each inflation (Figs. 2–7). FRC and C_{DL} values are presented as the means ± SE of each group at each inflation. Statistical significance is defined as $P < 0.05$.

RESULTS

Animal Data

Real-time PC X-ray images and simultaneous plethysmograph recordings were collected from 48 preterm rabbit kittens (delivered from 14 does) ventilated from birth (see Table 1 for numbers of kittens per group); all viable kittens were used. The average kitten weight was 31.1 ± 0.8 g and there were no significant differences in body weights between groups ($P > 0.35$). However, when all kittens exposed to betamethasone were grouped together, their mean body weight (29.3 ± 1.2 g) was significantly less than the weight of kittens not treated with betamethasone (32.6 ± 1.1 g, $P = 0.04$).

Functional Residual Capacity

Effects of PEEP. In the absence of betamethasone and surfactant, ventilation with 3 cmH_2O PEEP (3PEEP) significantly enhanced FRC recruitment compared with ventilation with 0 cmH_2O PEEP (0PEEP) (Fig. 2*A*). Surfactant did not alter the effect of 3PEEP on FRC recruitment (Fig. 2*B*), although FRC was recruited more quickly with both 3PEEP and surfactant (Fig. 3*B*). Without PEEP, surfactant had a marginal effect on FRC recruitment, and although it tended to

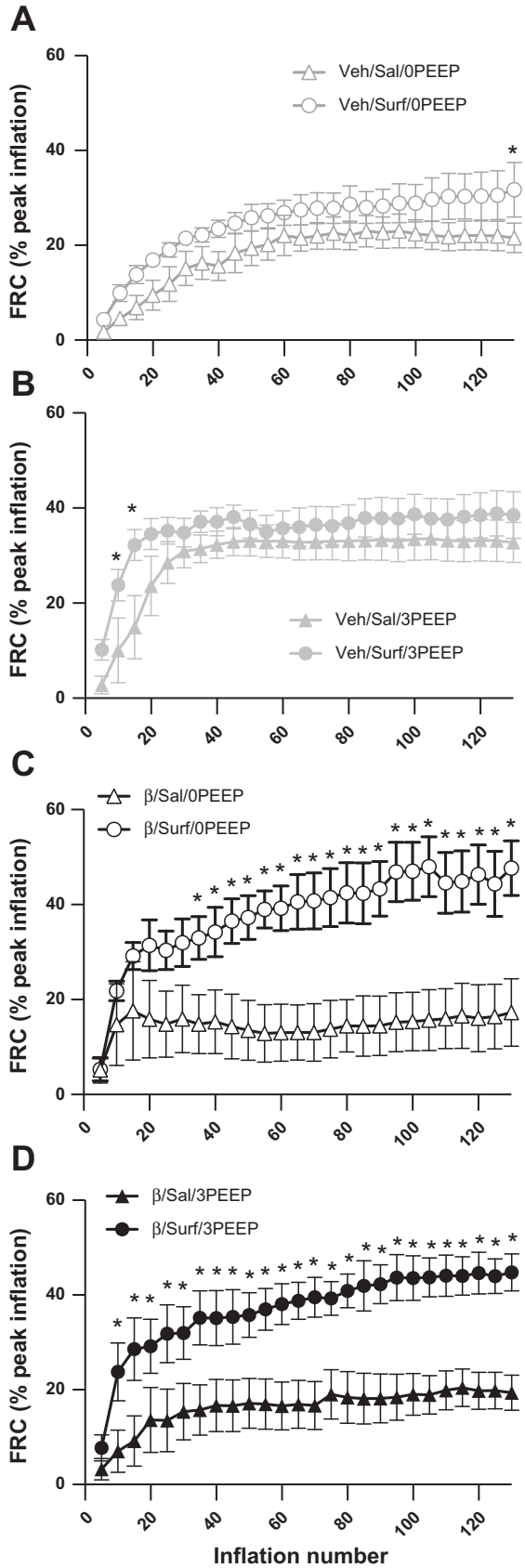


Fig. 3. Changes in functional residual capacity (FRC) during the first 130 inflations, comparing the effect of saline (Sal) vs. surfactant (Surf): without both betamethasone and a positive end-expiratory pressure (PEEP) (*A*), with 3PEEP, but without betamethasone (*B*), with betamethasone (β), but with no PEEP (*C*), with both betamethasone and 3PEEP (*D*). *Significantly different between groups ($P < 0.05$).

be higher in surfactant-treated kittens, this only reached significance at the end of the ventilation period (Fig. 3*A*).

Antenatal betamethasone abolished the benefit of 3PEEP (compared with 0PEEP) on FRC recruitment both with (Fig. 2*D*) and without (Fig. 2*C*) surfactant. In the absence of surfactant, betamethasone resulted in the lowest FRCs measured following stabilization, irrespective of PEEP level (Beta/Saline/0PEEP and Beta/Saline/3PEEP), and these FRC values were not different from kittens that received no treatment (Veh/Saline/0PEEP) (Figs. 2*C* and 4*A*). In the absence of surfactant, betamethasone significantly reduced FRC recruitment in response to 3PEEP (Fig. 4*B*).

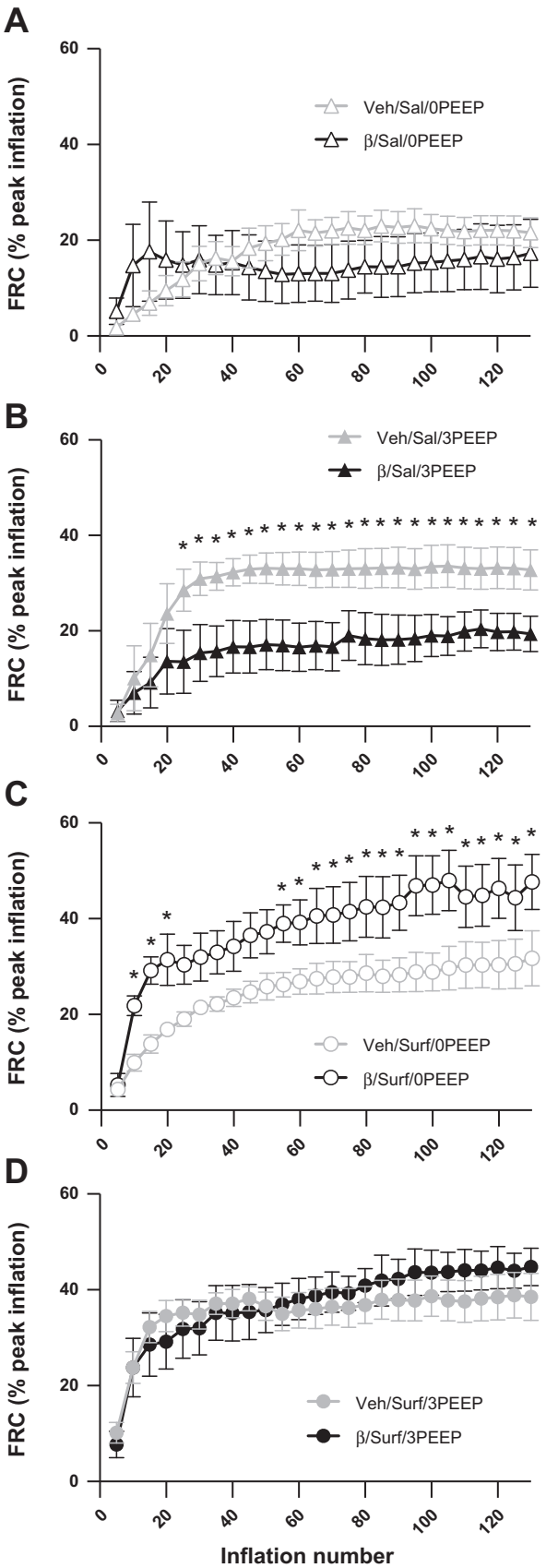
Effects of prophylactic surfactant. In the absence of betamethasone, surfactant had a small, mostly nonsignificant, effect on FRC recruitment during ventilation with both 0PEEP (Fig. 3*A*) and 3PEEP (Fig. 3*B*). As a result, the positive effect of 3PEEP ventilation on FRC recruitment was not influenced by surfactant in the absence of antenatal betamethasone. However, antenatal betamethasone and surfactant had a marked, significant effect on FRC recruitment irrespective of whether the kittens were ventilated with 0PEEP (Fig. 3*C*) or 3PEEP (Fig. 3*D*). Interestingly, surfactant abolished the negative effect of betamethasone on FRC recruitment both in the presence and absence of ventilation with 3PEEP (Fig. 3, *C* and *D*). As a result, the combined effect of antenatal betamethasone and surfactant resulted in the highest FRC values (following stabilization), irrespective of whether the kittens were ventilated with 0PEEP or 3PEEP (Figs. 2*D*, 3*C*, 3*D*, 4*C*, 4*D*).

Effects of antenatal betamethasone. Without surfactant, betamethasone significantly reduced the effect of 3PEEP on FRC recruitment (Fig. 4*B*), resulting in FRCs that were similar in kittens ventilated with 0PEEP (Beta/Sal/0PEEP) and 3PEEP (Beta/Sal/3PEEP). Furthermore, in the absence of both surfactant and PEEP, betamethasone tended to reduce FRC below the FRC in pups receiving no surfactant and no PEEP (Veh/Sal/0PEEP vs. Beta/Sal/0PEEP; Fig. 4*A*). While surfactant greatly enhanced the effect of betamethasone on FRC recruitment in kittens ventilated with both 0PEEP (Fig. 3*C*) and 3PEEP (Fig. 3*D*), betamethasone only had an effect on surfactant-treated kittens ventilated with either 0PEEP (Fig. 4*C*) but not 3PEEP (Fig. 4*D*).

Dynamic Lung Compliance (C_{DL})

To achieve a set V_T of 10 ml/kg, ventilation commenced in all kittens with a starting PIP of 35 cmH_2O, which was altered over subsequent inflations to achieve 10 ml/kg. The change in C_{DL} (V_T/PIP-PEEP) over the first 20 inflations was calculated to indicate V_T recruitment taking into account the delivered PIP.

Effects of PEEP. Without betamethasone and surfactant, 3PEEP significantly increased C_{DL}, compared with ventilation with 0PEEP, from *inflation 11* after ventilation onset (Fig. 5*A*). Without betamethasone, surfactant markedly enhanced the ef-



fect of 3PEEP (vs. 0PEEP) on C_{DL}, mainly by increasing C_{DL} over the first 20 inflations (Fig. 5*B*). The effect of surfactant was additive to the effect of PEEP, significantly increasing C_{DL} in both the absence (0PEEP, Fig. 6*A*) and presence (3PEEP, Fig. 6*B*) of PEEP. Without surfactant, betamethasone abolished the effect of 3PEEP (vs. 0PEEP) on the increase in C_{DL} (Fig. 5*C*). However, unlike the effect of betamethasone on FRC recruitment, the effect of betamethasone on C_{DL} resulted from a significant increase in kittens ventilated with 0PEEP (Fig. 7*A*), with no effect in kittens ventilated with 3PEEP (Fig. 7*B*). Similarly, in combination, surfactant and betamethasone markedly reduced the effect of 3PEEP vs. 0PEEP on C_{DL} (Fig. 5*D*) by selectively enhancing C_{DL} in kittens ventilated with 0PEEP (Fig. 7, *C* and *D*).

Effects of surfactant. Without betamethasone, surfactant significantly increased C_{DL} from the first inflation, irrespective of the PEEP level (Figs. 6, *A* and *B*). The effect of surfactant was independent of, and additive to, the effect of PEEP, resulting in a marked increase in C_{DL} in kittens receiving surfactant and ventilation with 3PEEP. Similarly, surfactant significantly enhanced C_{DL} in betamethasone-treated kittens, during ventilation with both 0PEEP (Fig. 6*C*) and 3PEEP (Fig. 6*D*). This effect of surfactant on C_{DL} was additive to the combined effects of both betamethasone and PEEP. As a result, the highest mean C_{DL} values achieved by *inflation 20* across all groups were in the betamethasone- and surfactant-treated groups ventilated with 3PEEP (0.91 ± 0.04 ml·kg^{-1}·cmH_2O^{-1}). This was consistent with the finding that these kittens required the lowest PIP level (20.8 ± 1.0 cmH_2O) to achieve a set V_T of 10 ml/kg. Indeed, surfactant was found to have the greatest impact on C_{DL} as demonstrated by a significant effect, irrespective of the other treatments (Figs. 6, *A–D*).

Effects of betamethasone. Without surfactant, betamethasone significantly increased C_{DL} in kittens ventilated with 0PEEP from inflation 4 (Fig. 7*A*), but in kittens ventilated with 3PEEP, betamethasone had no effect on C_{DL} (Fig. 7*B*). In surfactant-treated kittens, betamethasone also increased C_{DL} in kittens ventilated with both 0PEEP (from *inflation 4*) and 3PEEP (after *inflation 10*), although the effect of betamethasone was less in kittens ventilated with 3PEEP.

PIP at the End of the Ventilation Period

The PIP required to maintain a set V_T of 10 ml/kg at the end of the ventilation period was significantly higher in kittens that received no betamethasone or surfactant and were ventilated at 0PEEP (34.2 ± 1.2 cmH_2O). The lowest PIPs required were in betamethasone and surfactant-treated kittens, irrespective of the PEEP level used (Table 1).

DISCUSSION

This study has investigated the interactions between three of the most common treatments provided to very preterm infants

Fig. 4. Changes in functional residual capacity (FRC) during the first 130 inflations, comparing the effect of an antenatal vehicle (Veh) vs. betamethasone (β) treatment: without both surfactant and a positive end-expiratory pressure (PEEP) (*A*), with 3PEEP, but without surfactant (*B*), with surfactant (Surf), but with no PEEP (*C*), and with both surfactant and 3PEEP (*D*). *Significantly different between groups ($P < 0.05$).

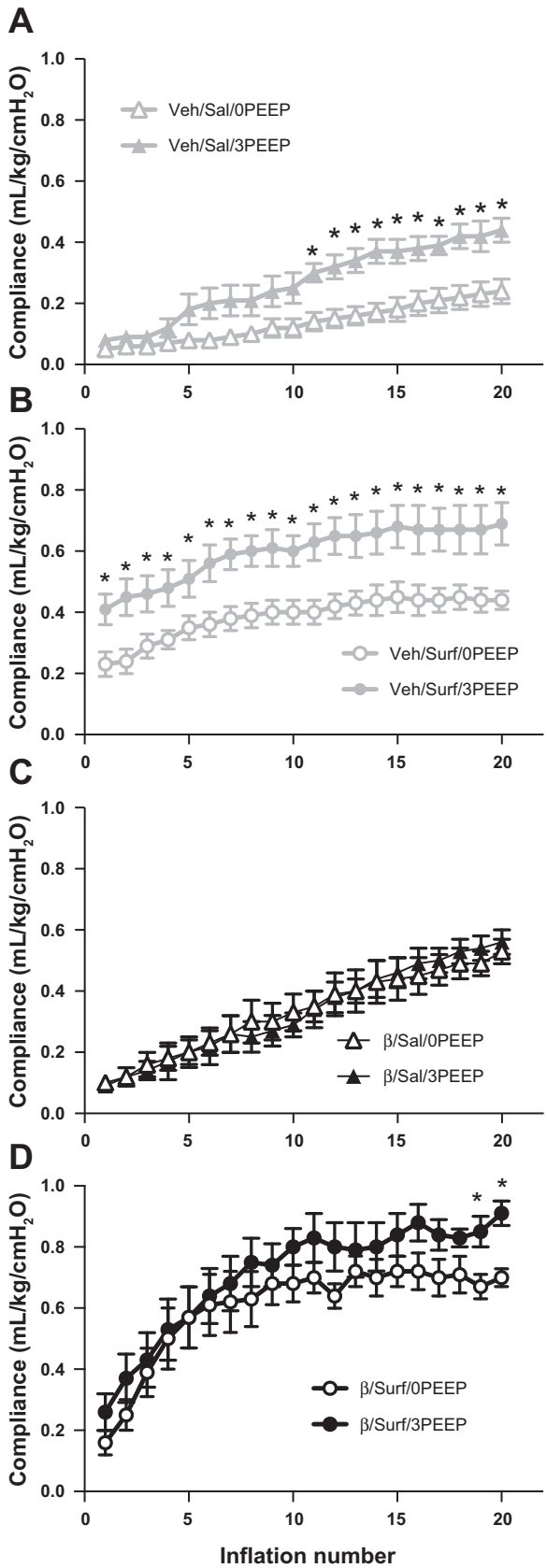
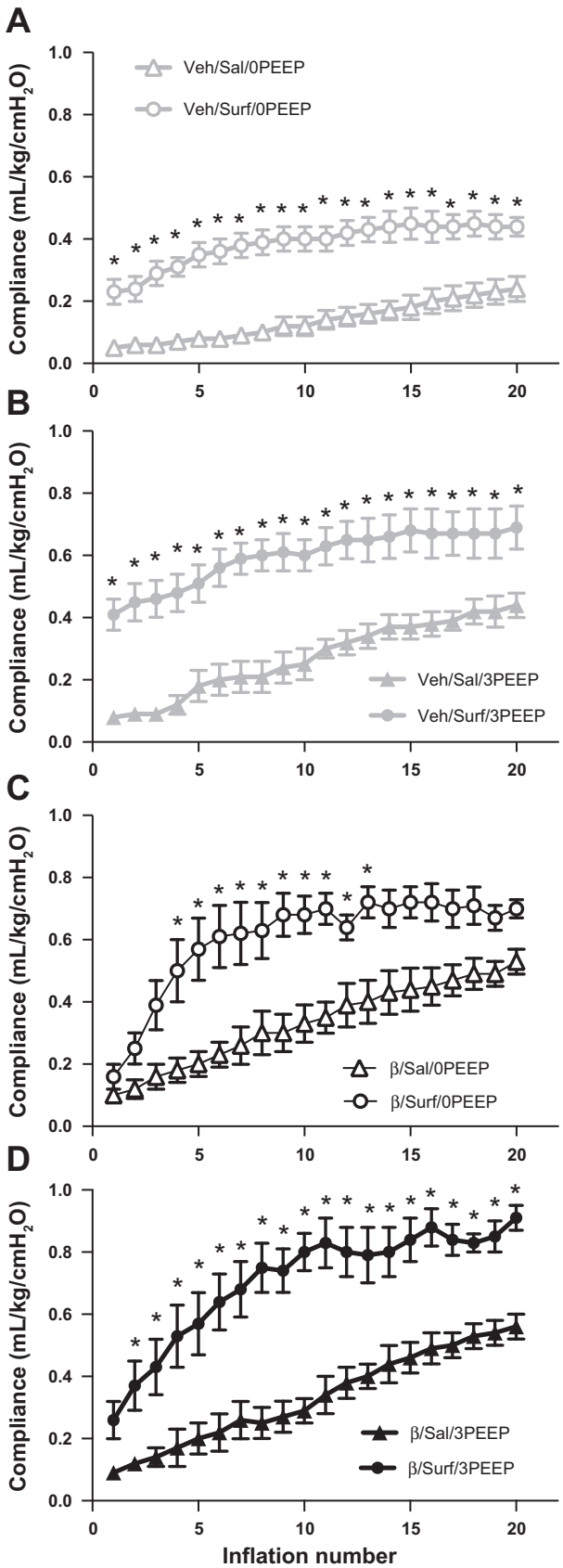


Fig. 5. Changes in dynamic lung compliance over the first 20 inflations, comparing the effect of 3 cmH_2O of positive end-expiratory pressure (3PEEP) vs. no PEEP (0PEEP): without both betamethasone and surfactant (*A*); with surfactant (Surf), but without betamethasone (*B*); with betamethasone (β), but without surfactant (*C*); with both betamethasone and surfactant (*D*). *Significantly different between groups ($P < 0.05$).

on FRC recruitment and changes in C_{DL} immediately after birth in anesthetized, intubated, preterm rabbits. We hypothesized that PEEP would enhance FRC recruitment and C_{DL} and that both antenatal betamethasone and prophylactic surfactant would enhance and have independent additive effects to PEEP. While this hypothesis proved correct for surfactant, betamethasone alone was found to have a negative effect on FRC recruitment. Indeed, without surfactant, betamethasone abolished the positive effect of 3PEEP on FRC recruitment and tended to reduce FRC values below those recorded in pups receiving no treatment and no PEEP. As a result, following stabilization, betamethasone treatment in the absence of surfactant and PEEP was associated with the lowest FRC of all treatment groups (Beta/Sal/0PEEP group; Fig. 2*C*).

Our finding that antenatal betamethasone treatment alone had a negative impact on FRC recruitment is surprising and occurred despite a significant positive effect on C_{DL}, independently of surfactant and PEEP (Fig. 7*A*). With regard to the effects of antenatal betamethasone on FRC recruitment, most previous studies have reported increases in FRC in preterm newborns (11, 25), whereas other studies have reported no significant difference in FRC (26, 27). However, previous studies that have measured an increase in FRC following antenatal corticosteroids either also gave surfactant (11), ventilated with higher PEEP levels, or made measurements hours after birth and not during transition (25). As we also found that antenatal betamethasone significantly increased C_{DL} independently of surfactant and PEEP (Fig. 7*A*), clearly, the betamethasone treatment was effective. This finding is consistent with previous reports that antenatal betamethasone treatment increases fetal lung compliance (20, 35), largely due to major alterations in distal airway structure. These include, reductions in perialveolar tissue volumes, increased lung lumen-to-tissue volume ratios (8, 13, 37), accelerated alveoli septal wall thinning (33, 37), and, in some species, an increase in alveoli number (10). Although the measured effects of antenatal betamethasone on FRC appear contrary to the overall beneficial effects observed clinically, it is important to remember that this study is focused on the factors regulating lung aeration during the immediate newborn period. As such, the beneficial effects of antenatal betamethasone may dominate later in the newborn period, particularly after lung liquid has been cleared from the lung tissue (9, 28).

It is possible that the effects of antenatal betamethasone on increasing fetal lung compliance and reducing perialveolar tissue volumes can explain the negative impact of betamethasone alone on FRC recruitment during lung aeration. Increasing lung compliance before birth leads to a greater retention of liquid within the airways (48), resulting in larger lung liquid volumes at birth. Thus, betamethasone-treated kittens may have had greater volumes of airway liquid to clear, particularly as they were delivered by cesarean section (48). Secondly, although the liquid present in the



airways at birth is rapidly (seconds/minutes) cleared into perialveolar tissue, clearance of this liquid from the tissue is much slower (hours) (9, 46). As a result, airway liquid clearance transiently (~4 h) increases perialveolar tissue pressures (to ~6 cmH_2O) (28, 29), which provides a hydrostatic pressure for liquid to reenter the airways at rest (15). Thus, a glucocorticoid-mediated reduction in perialveolar tissue volumes, means that the same or a larger volume of airway liquid must enter a smaller perialveolar tissue compartment. Depending upon the compliance of this compartment, this must further increase perialveolar tissue pressures, resulting in a greater potential for liquid to reenter the airways and reduce FRC.

The concept that antenatal glucocorticoids result in an increase perialveolar tissue pressures following airway liquid clearance at birth is consistent with our finding that betamethasone abolished the effect of 3PEEP on FRC recruitment (Fig. 2, *A* and *C*); it had a similar effect on C_{DL} (Fig. 5, *A* and *C*), likely through a similar mechanism. We have previously shown that PEEP is essential for establishing and maintaining a FRC at birth (40, 47), as demonstrated in Fig. 2*A*, and is also known to increase C_{DL} (Fig. 5*A*). PEEP provides a hydrostatic pressure within the airways at end-expiration that opposes liquid reentry into the airways, which would otherwise reduce FRC (40). As such, PEEP assists in keeping the lung in a more compliant region of the pressure-volume curve between inflations, thereby increasing its C_{DL}.

In the context of this study, we found that 3 cmH_2O of PEEP was sufficient to oppose liquid reentry into the airways in the absence of betamethasone (Fig. 2*A*), but not in the presence of betamethasone (Fig. 2*C*). It is possible that a higher level (>3 cmH_2O) of PEEP is needed to oppose the potentially higher perialveolar tissue pressures driving liquid reentry in these kittens. We deliberately used a low PEEP level (3 cmH_2O) to avoid obscuring the effect of betamethasone and surfactant treatment on FRC development and to measure any additive effects that may be present. Indeed, a PEEP of 3 cmH_2O was insufficient to induce any further increase in FRC in the betamethasone- and surfactant-treated pups, which we expected to have the greatest FRC recruitment. This is likely because the FRC recruited with betamethasone and surfactant alone was already high and among the highest recorded for all groups. As such, although 3PEEP tended to increase FRC further, it was not statistically significant.

In the absence of betamethasone, surfactant had a minor additive effect to PEEP on FRC recruitment (Fig. 3, *A* and *B*), with PEEP having the dominant effect. This is consistent with our previous findings (39). However, with betamethasone, surfactant had a highly significant positive effect on FRC recruitment, irrespective of the PEEP level (Fig. 3, *C* and *D*), which reversed the negative effect of betamethasone. This was a surprising result that is difficult to explain. As the effects of betamethasone and surfactant on FRC recruitment were not

Fig. 6. Changes in dynamic lung compliance over the first 20 inflations, comparing the effect of saline (Sal) vs. surfactant (Surf): without both betamethasone and a positive end-expiratory pressure (PEEP) (*A*); with 3PEEP, but without betamethasone (*B*); with betamethasone (β), but with no PEEP (*C*); with both betamethasone and 3PEEP (*D*). Values indicated by an asterisk are significantly different between groups (*$P < 0.05$).

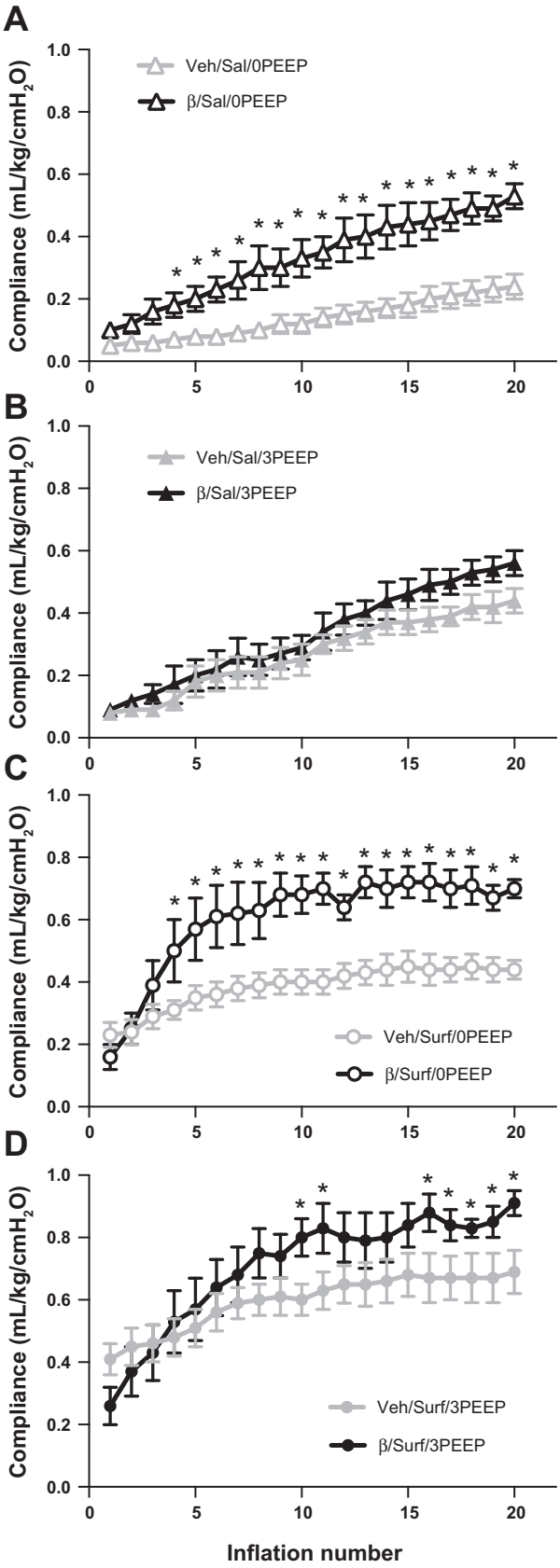


Fig. 7. Changes in dynamic lung compliance over the first 20 inflations, comparing the effect of an antenatal vehicle (Veh) vs. betamethasone (β) treatment: without both surfactant and a positive end-expiratory pressure (PEEP) (*A*); with 3PEEP, but without surfactant (*B*); with surfactant (Surf), but with no PEEP (C); with both surfactant and 3PEEP (*D*). *Significantly different between groups ($P < 0.05$).

additive (Fig. 4, *C* and *D*), it appeared that the primary effect of surfactant was to counter the negative effects of betamethasone. It is possible that high surface tension within the liquid lining the concave aspect of the alveolar surface reduces the hydrostatic pressure within this liquid layer. This would increase the pressure gradient between the perialveolar tissue and lung lumen, leading to a greater tendency for liquid to reenter the airways. On the other hand, reducing this surface tension with surfactant will tend to reduce this pressure gradient and the potential for liquid reentry (39).

Glucocorticoid exposure has been shown to upregulate surfactant synthesis and secretion (3, 17, 18, 42, 45) and increase surfactant protein expression (5, 18). However, these findings are not consistently reported (30, 34, 44), which is likely due to variations in timing and glucocorticoid dose (5). Similarly, the actions of endogenous glucocorticoids on type II alveolar epithelial cell differentiation (12) and surfactant protein synthesis are not consistent with a major role for glucocorticoids in stimulating type II cells and surfactant synthesis near term. If antenatal betamethasone treatment stimulated surfactant production, we would have expected FRC recruitment to be comparable in betamethasone/saline-treated kittens and surfactant-treated kittens without betamethasone. However, this was not the case, suggesting that the betamethasone treatment did not stimulate a notable increase in surfactant synthesis and/or secretion in this group.

The mean body weight of all kittens treated with antenatal betamethasone was significantly less than that of vehicle-treated kittens. A reduction in fetal body weight associated with antenatal betamethasone treatment in experimental models has been reported previously, and the reductions we detected are consistent with these previous reports (16, 31). To correct for biological variations in kitten size, all volumes were expressed and analyzed as the percentage of the maximum peak inflation, which was typically found to be within the first 20 inflations.

In summary, this study has shown significant interactions between antenatal betamethasone, surfactant, and PEEP on FRC recruitment and increases in C_{DL}. Although we hypothesized that both betamethasone and surfactant would both have positive additive effects to PEEP on FRC recruitment, this only held true for surfactant. In contrast, betamethasone alone abolished the positive effect of low PEEP levels (3PEEP) on FRC recruitment. Thus, it is possible that a higher PEEP level is required to establish and maintain an FRC following antenatal betamethasone treatment unless surfactant is also provided. While the mechanism is unknown, it is possible that the maturational changes in lung structure induced by betamethasone treatment leads to larger airway liquid volumes and to a smaller perialveolar tissue compartment at birth. Having to accommodate larger volumes of liquid within a smaller compartment will further increase perialveolar tissue pressures leading to a greater potential for liquid to reenter the airways and reduce FRC immediately after birth.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.R.C., S.B.H., A.B.t.P., B.A.A., M.J.W., R.A.L., A.F.L., and M.J.K. performed experiments; J.R.C., A.B.t.P., M.J.W., L.K., A.F.L., and M.J.K. analyzed data; J.R.C., S.B.H., A.B.t.P., M.J.W., and M.J.K. interpreted results of experiments; J.R.C. and M.J.K. prepared figures; J.R.C., M.J.W., L.K., and M.J.K. drafted manuscript; J.R.C., S.B.H., A.B.t.P., B.A.A., M.J.W., L.K., R.A.L., C.J.M., A.F.L., and M.J.K. edited and revised manuscript; J.R.C., S.B.H., A.B.t.P., B.A.A., M.J.W., L.K., R.A.L., C.J.M., A.F.L., and M.J.K. approved final version of manuscript; S.B.H., A.B.t.P., R.A.L., and C.J.M. conception and design of research.

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