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High-frequency oscillatory ventilation during physiological-based cord clamping attenuates inflammation in preterm lambs

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ABSTRACT

Background Adequate lung aeration at birth is essential for a successful transition to extrauterine life. Premature infants are especially vulnerable to injury caused by mechanical ventilation, particularly when exposed to excessive tidal volumes during initial respiratory support.

Objective To evaluate whether high-frequency oscillatory ventilation (HFOV) initiated at birth facilitates lung aeration and reduces lung inflammation and injury, compared with conventional mechanical ventilation (CMV) in preterm lambs.

Design Preterm lambs (126±1 days' gestation, term ~148 days) were instrumented to assess blood flow, pressure, oxygenation and blood gases. Lambs were allocated to HFOV (n=6), CMV (n=7) or unventilated control (UVC, n=8) groups. In ventilated groups, respiratory support was initiated during physiological-based cord clamping (PBCC). Lung aeration was assessed by lung ultrasound (LUS) in HFOV lambs. Postmortem lung tissues underwent histological and molecular analyses of inflammation and injury.

Results HFOV achieved effective respiratory stabilisation using significantly lower tidal volumes compared with CMV (1.7±0.4 vs 6.2±1.5 mL/kg, p<0.0001). LUS confirmed rapid lung aeration following HFOV. Histological analyses revealed significantly fewer CD45-positive and CD163-positive inflammatory cells in HFOV lungs compared with CMV (p<0.001 and p<0.05, respectively). Gene expression profiling demonstrated lower expression of key inflammatory and injury markers in HFOV versus CMV lambs, with some levels approaching those observed in UVC (p<0.05).

Conclusions HFOV initiated during PBCC supports efficient lung aeration while minimising lung inflammation and injury in preterm lambs. These findings suggest that HFOV may reduce lung inflammation during initial respiratory stabilisation in preterm neonates.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Excessive tidal volumes during preterm resuscitation increase lung inflammation and injury.

⇒ Whether high-frequency oscillatory ventilation (HFOV) delivered during physiological-based cord clamping at preterm birth reduces lung inflammation and injury compared with conventional mechanical ventilation (CMV) is not known.

WHAT THIS STUDY ADDS

⇒ HFOV was able to effectively stabilise the preterm newborn at birth using significantly lower tidal volumes during physiological-based cord clamping.

⇒ It also showed lower key molecular and histological measures of lung inflammation compared with CMV, including reduced leucocytes and macrophages in lung tissue.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Initiating respiratory support during delayed cord clamping with HFOV reduces lung inflammation and injury compared with CMV, which may represent a new way to improve respiratory outcomes in preterm infants.

INTRODUCTION

The transition from fetal to postnatal life requires effective lung aeration to initiate gas exchange and cardiovascular adaptation. In preterm infants, this process is often impaired by pulmonary immaturity, weak inspiratory effort and underdeveloped respiratory musculature, leading to respiratory distress. A primary concern during respiratory support is ventilator-induced lung injury (VILI), predominantly driven by excessive tidal volumes (V_T), which can overdistend immature alveoli, triggering an inflammatory cascade that exacerbates lung damage.[1–4]

While non-invasive ventilation is preferred when feasible, many preterm infants still require invasive support during initial stabilisation. High-frequency oscillatory ventilation (HFOV) has been proposed as a potential lung-protective alternative to conventional mechanical ventilation (CMV), owing to its capacity to deliver very low V_T at high frequencies, thereby reducing alveolar overdistension, minimising atelectasis and promoting alveolarisation.[5–8] Although HFOV has been extensively used in the treatment of respiratory distress syndrome and as a rescue strategy for severe respiratory failure,[9 10] its

application during deferred or physiological-based cord clamping (PBCC) has not been fully explored.

PBCC is defined as deferring cord clamping until after lung aeration and cardiopulmonary stabilisation has been obtained.[11 12] Studies in preterm lambs demonstrate that PBCC improves the cardiopulmonary transition compared with immediate cord clamping and reduces hypoxia,[13 14] particularly if the duration between cord clamping and ventilation onset is prolonged.[15] Lung aeration lowers pulmonary vascular resistance and enhances pulmonary blood flow, facilitating the pulmonary vasculature to gradually assume the role of the placenta in providing oxygenated blood and preload to the left heart.[16] The role of HFOV in the setting of PBCC is not known.

This study compares the effects of HFOV and CMV in preterm lambs, using PBCC. We hypothesised that HFOV would promote effective lung aeration in liquid-filled immature lungs and reduce early lung inflammation and injury compared with CMV during the transitional period after birth.

METHODS

Ethics approval

All experimental procedures were approved by the Monash Medical Centre-A Animal Ethics Committee (MMCA2023/05) and conducted in accordance with the Australian National Health and Medical Research Council guidelines.[17] Methodological reporting is per the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.[18]

Experimental groups

Prior to surgery, lambs were allocated to one of three groups:

1. Unventilated control (UVC, n=8): delivered and immediately euthanised without instrumentation or respiratory support (injury control group).
2. CMV (n=7): received immediate respiratory support using CMV.
3. HFOV (n=6): received immediate respiratory support using HFOV.

The UVC and CMV lambs were used from a previous study[19] in keeping with the principle of reduction.

Instrumentation and delivery

At 126±1 (SD) days' gestation (range: 123–127 days, term ~148 days), glucocorticoid-treated ewes were anaesthetised and surgery was performed for placement of flow probes around the left main pulmonary artery, right carotid artery and vascular catheters into the contralateral carotid artery and jugular vein as described previously.[19] A 4.0 mm cuffed endotracheal tube was inserted and lung fluid was passively drained. A transcutaneous oxygen saturation (SpO_2) probe (Masimo Radical 4, California, USA) was attached to the right forelimb. For UVC lambs, fetal blood was sampled, and euthanasia was immediately performed via intravenous sodium pentobarbitone (100 mg/kg, Lethobarb, Virbac, Australia) into the umbilical vein.

Ventilation strategy

Following instrumentation, lambs were dried and placed prone atop a warm water bottle for thermal support. Ventilation commenced on the intact umbilical cord with 100% fraction of inspired oxygen (FiO_2). HFOV lambs were ventilated using an SLE5000 ventilator (Inspiration Healthcare, UK) at a frequency of 12 Hz, mean airway pressure (MAP) 18 mbar and inspiratory-to-expiratory ratio of 1:2. The amplitude was manually adjusted by a dedicated operator to achieve initial V_T: 1.5 mL/kg. CMV lambs were ventilated (Babylog 8000+, Dräger, Germany) in volume-guarantee (VG) mode targeting a V_T of 7 mL/kg, positive end-expiratory pressure of 5 cmH_2O, respiratory rate of 60 breaths/min, inspiratory time of 0.5 s and a bias flow rate of 10 L/min. An upper peak inflation pressure of 40 cmH_2O was used.

After 10 min of ventilation—deemed an appropriate time for lambs to achieve lung aeration and cardiopulmonary stability, and the maximum time used in a PBCC trial[11]—the umbilical cord was clamped. The lambs were then weighed and moved to a radiant warmer. MAP and FiO_2 were titrated to maintain preductal SpO_2 between 90% and 95%, and rate and V_T were adjusted based on serial arterial blood gas measurements to achieve permissive hypercapnia. In the HFOV group, frequency was fixed at 12 Hz for the duration of the experiment to ensure protocol consistency and reduce intersubject variability. V_T was adjusted manually in discrete increments of 0.5 mL/kg in response to partial pressure of carbon dioxide ($PaCO_2$) trends. Continuous intravenous anaesthesia was maintained using Alfaxan (5–15 mg/kg/hour, Jurox, Australia). At 60 min, lambs were euthanised via intravenous pentobarbitone sodium.

Lung ultrasound (HFOV group)

Lung ultrasound (LUS) was performed using a Philips Compact Extreme Ultrasound machine (CX-50, Koninklijke Philips, The Netherlands) with a high-frequency L12-3 linear probe. Anterior lung fields were scanned in the supine position initially using a depth of 4 cm and gain set to 70. Images were acquired in the longitudinal plane and scored using the system described by Brat *et al*.[20]

Physiological data acquisition

Physiological parameters, including pulmonary blood flow, arterial pressure, heart rate and oxygenation, were continuously recorded using LabChart software (ADInstruments, Australia). Blood gas data were combined with ventilation parameters to calculate oxygenation indices (figure 2).[21]

Tissue collection and processing

At postmortem, the left lung was excised and snap-frozen in liquid nitrogen for molecular analysis. The right lung was inflation-fixed via tracheal instillation of 10% formalin at 20 cmH_2O. Fixed lungs were sectioned into 0.5 cm slices, and three 1.5 cm^2 samples were randomly selected from different lobes for paraffin embedding. Sections (5 µm) were mounted onto slides for histological and immunohistochemical analysis. Three sections from the right upper lobe, two from the middle lobe and four from the lower lobe were analysed. Five random fields of view per section were analysed.

H&E staining

Sections of the right lung were processed and then stained with H&E for morphometric analysis, including mean linear intercept (Lm) and airspace/tissue percentages, as recommended by the American Thoracic Society.[19 22]

Immunohistochemistry

Sections of the right lung were prepared as described previously[23] and labelled with 1:200 Leucocyte Common Antigen (CD45, Bio-Rad, Cat#: MCA2220GA) and 1:400 Scavenger receptor cysteine-rich type-1 protein M130 (CD163, OriGene Cat#: SM2160P). Positive cells were visualised using light microscopy (Olympus, Tokyo, Japan) at 40× magnification and analysed with cellSens imaging software (V.2.3, Olympus). CD45-positive

Table 1 Baseline fetal and arterial blood gas characteristics

	UVC	CMV	HFOV
Group characteristics			
Number (n)	8	7	6
Sex (% male)	37.5	85.7	33.3
Twins (n)	4	4	4
GA (days) (range)	126±1 (123–127)	125*±1 (123–126)	126±1 (125–126)
Lung liquid drained (mL)	–	57.8±19.7	64.6±35.8
Body weight (kg)	3.5±0.5	3.2±0.2	3.6±0.4
Lung weight (g)	111.5±30.5	116.5±18.5	132.3±18.1
Baseline arterial blood gas characteristics			
pH	7.29±0.08	7.24±0.10	7.26±0.02
$PaCO_2$ (mm Hg)	59.8±14.4	55.6±9.6	57.5±3.1
PaO_2 (mm Hg)	25.3±13.5	27.9±14.7	19.9±4.1
SaO_2 (mm Hg)	53.8±29.3	62.5±25.9	49.3±14.8
Lactate (mmol/L)	3.7±1.2	6.6±4.7	4.7±1.2
Haemoglobin (g/L)	115.0±7.0	128.0±12.0	131.7±19.3

Data are presented as mean±SD.
*P=0.0367 vs HFOV.
CMV, conventional mechanical ventilation; GA, gestational age; HFOV, high-frequency oscillatory ventilation; $PaCO_2$, partial pressure of carbon dioxide; PaO_2, partial pressure of oxygen; SaO_2, arterial oxygen saturation; UVC, unventilated control.

and CD163-positive cells were manually quantified using Fiji/ImageJ (V.2.14.0, LOCI, Wisconsin, USA) and expressed as total and relative to total cell counts.

Molecular analysis

High-throughput real-time quantitative PCR using Fluidigm Access Array System Technology (Fluidigm, California, USA) and TaqMan primers (Thermo Fisher Scientific, Massachusetts, USA) was undertaken to quantify messenger RNA (mRNA) expression of 20 curated genes (online supplemental table 1). Levels of mRNA are expressed as fold-change from UVCs. Gene expression was normalised to the ribosomal protein S18 (*RPS18*) gene.[24]

Statistical analysis

The primary outcome of the study was lung inflammation, assessed by molecular and histological assessments. A sample size of six per group was determined to be sufficient to detect significant differences based on previous studies.[25 26] Investigators could not be blinded to the group due to the nature of the studies. Statistical analyses were conducted using GraphPad Prism (GraphPad Software, California, USA). Data are presented as mean±SD or mean±SEM. Depending on data distribution and design, comparisons were made using one-way analysis of variance (ANOVA), two-way ANOVA with repeated measures or mixed effects modelling (time and experimental group as factors). Tukey or Šídák post hoc tests were applied where appropriate. A p value of <0.05 was considered statistically significant.

RESULTS

Baseline characteristics

A total of 21 lambs were included in the study. Baseline characteristics and arterial blood gas measurements taken before lung liquid drainage are summarised in table 1. The mean gestational age was 1 day less in CMV lambs compared with HFOV lambs (p=0.0367), with no other significant differences between groups.

Ventilation and lung ultrasound

Respiratory stabilisation was achieved with significantly lower V_T in HFOV lambs (CMV: 6.2±1.5 vs HFOV: 1.7±0.4 mL/kg; p<0.0001), while MAP was higher (CMV: 17.4±1.7 vs HFOV: 18.7±1.0 cmH_2O; p=0.0046). FiO_2 was not different between groups after cord clamping (CMV: 77.3±13.0% vs HFOV: 79.4±20.1%). CMV lambs received a peak inflation pressure of 34.8±5.8 cmH_2O while HFOV lambs received a mean amplitude of 29.1±8.3 mbar.

LUS performed immediately after ventilation onset demonstrated early lung aeration in HFOV lambs (figure 1). Within

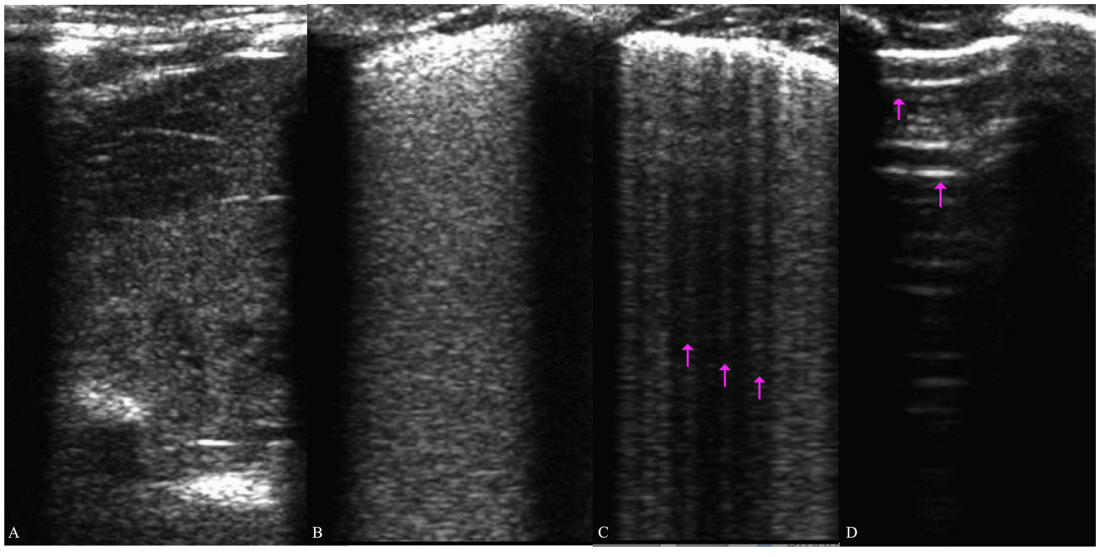

Figure 1 Lung ultrasound image of high-frequency oscillatory ventilation lambs: (A) before start ventilation, extended consolidations. (B) Alveolar pattern (defined as the presence of crowded and coalescent B lines with or without consolidations limited to subpleural space), image from a lamb with 1 min of ventilation. (C) Interstitial pattern (defined as the presence of ≥3 B lines (pink arrows), well-spaced), image from a lamb with 8 min of ventilation. (D) Normal aerated lung (defined by the presence of only A lines (pink arrows)), image from a lamb with 20 min of ventilation.

20 min, half of the HFOV lambs exhibited normal aeration (three scored 0, two scored 1, one scored 2), and by the end of the experiment, all but one had fully aerated anterior lung fields, with one lamb displaying an interstitial pattern (figure 1).

Blood gas status and oxygenation

HFOV lambs had higher $PaCO_2$ levels between 12 and 30 min of ventilation (p=0.006, figure 2), while pH remained comparable between groups. Lactate levels were higher in CMV lambs at 45 and 60 min (p<0.05, figure 2), whereas bicarbonate (HCO_3^-) levels were higher in HFOV lambs at 60 min (p=0.0109). CMV lambs had higher arterial oxygen saturation (SaO_2) at 6 and 9 min (p=0.004) and greater arterial oxygen tension (PaO_2) at 6 min (p=0.0449); no differences were observed thereafter. SpO_2 levels were comparable between groups, with slight elevations in CMV lambs at 3–4 min and in HFOV lambs at 60 min (p<0.05). Oxygenation index (OI) was higher in HFOV lambs during the first 6 min of ventilation and the alveolar-arterial oxygen gradient ($AaDO_2$) from cord clamping until 15 min (p<0.05). The PaO_2/FiO_2 ratio was

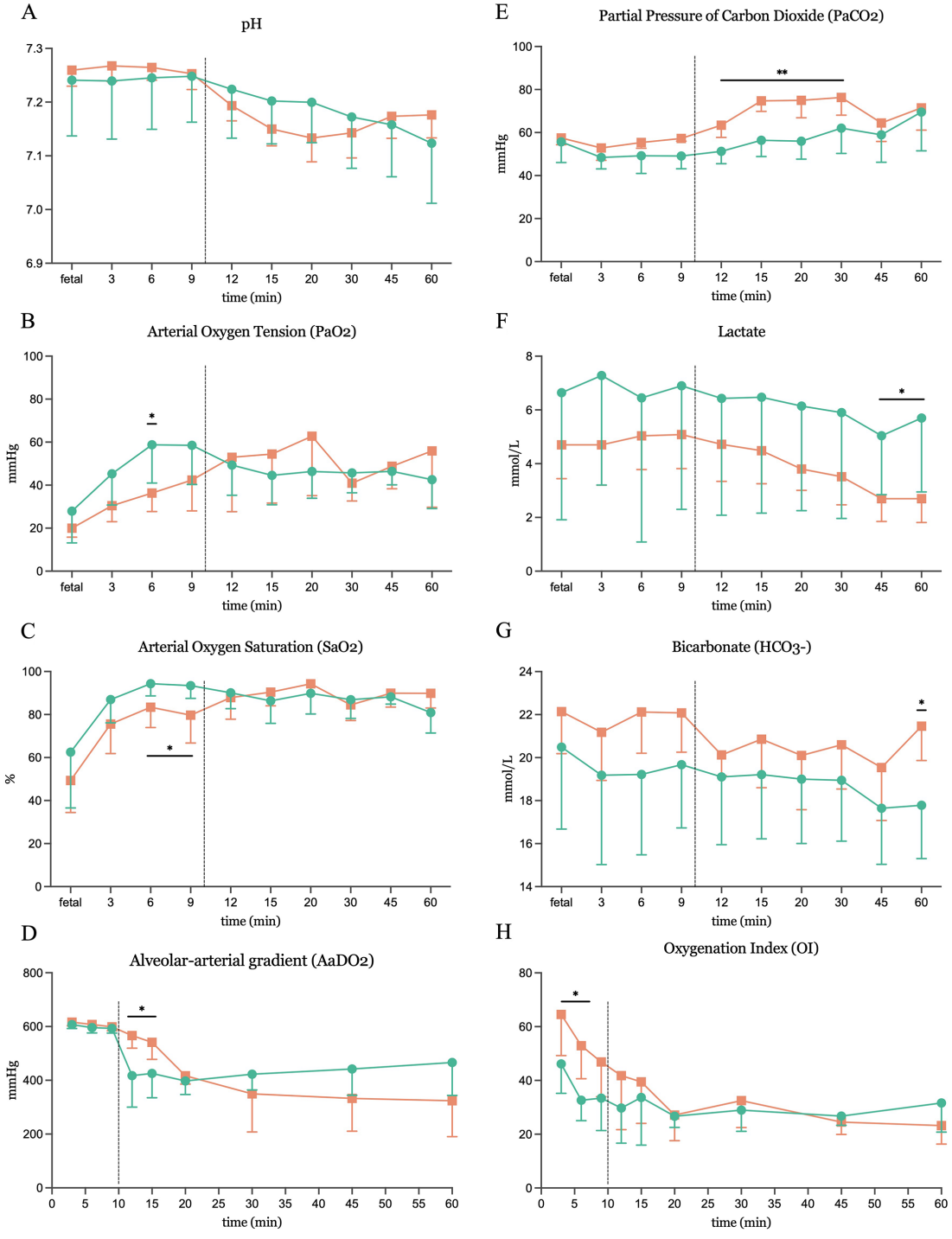

Figure 2 Blood gases and oxygenation over time: (A) pH, (B) PaO_2, (C) SaO_2, (D) $AaDO_2$, (E) $PaCO_2$, (F) lactate, (G) SpO_2, (aH) OI. Conventional mechanical ventilation is represented by green circles and high-frequency oscillatory ventilation by orange squares. The dotted line represents the timing of cord clamping. Data are presented as mean±SD. *P<0.05, **p<0.01.

higher in HFOV lambs after 45 min, persisting until the end of the experiment (p=0.02).

Physiological data

CMV lambs had higher peak systolic pulmonary blood flow between 13–18 and 35–40 min and increased end-diastolic pulmonary blood flow during the final 20 min (p<0.05, online supplemental figure 1). Mean pulmonary blood flow was higher in CMV lambs at 50 min (p=0.041). Mean, systolic and diastolic blood pressures were higher in HFOV lambs (p=0.001, p=0.0354 and p=0.0008, respectively). Heart rate was similar between groups.

Histological evaluation

The number of CD45-positive cells was significantly higher in CMV lambs compared with UVC and HFOV lambs overall, and within each individual lung lobe (figure 3; p<0.0001). HFOV lambs had increased CD45-positive cells compared with UVC in the middle lobe (p<0.05). When adjusted for total cell count, CMV lambs had the highest proportion of CD45-positive cells compared with UVC across all lung regions (p<0.0001) and compared with HFOV in the right upper, middle and total lung. HFOV lambs had higher adjusted CD45-positive cells than UVC lambs in the right middle, lower and total lung (p<0.05).

Both ventilated groups had significantly increased CD163-positive cells across all lung regions compared with UVC (p<0.0001, figure 3). However, CMV lambs had higher CD163-positive cells than CMV lambs overall, and in the lower and middle lobes (p<0.05, p<0.01, p<0.001, respectively).

Overall tissue fraction per field of view was significantly higher in both ventilated groups compared with UVC (p<0.05, online supplemental figure 2), with a more pronounced increase in the lower lung regions (p<0.05). The percentage of airspace per field was reduced in ventilated lambs (p<0.05). Lm did not differ between groups.

Gene expression

mRNA expression of pro-inflammatory cytokines *IL-1A*, *IL-1B*, *IL-6*, *TNFA*, *CCL2*, *TGFB1*, *TP53* and *PTGS2* was higher in CMV lambs compared with UVC and HFOV lambs (p<0.05 for all; figure 4). Despite increased expression in HFOV lambs compared with UVC, it did not reach statistical difference. *IL-8*, *NFKB1* and *BAK1* mRNA levels were increased in CMV compared with UVC (p<0.05). *IFNY*, *CXCL10*, *IL-10*, *BCL2*,

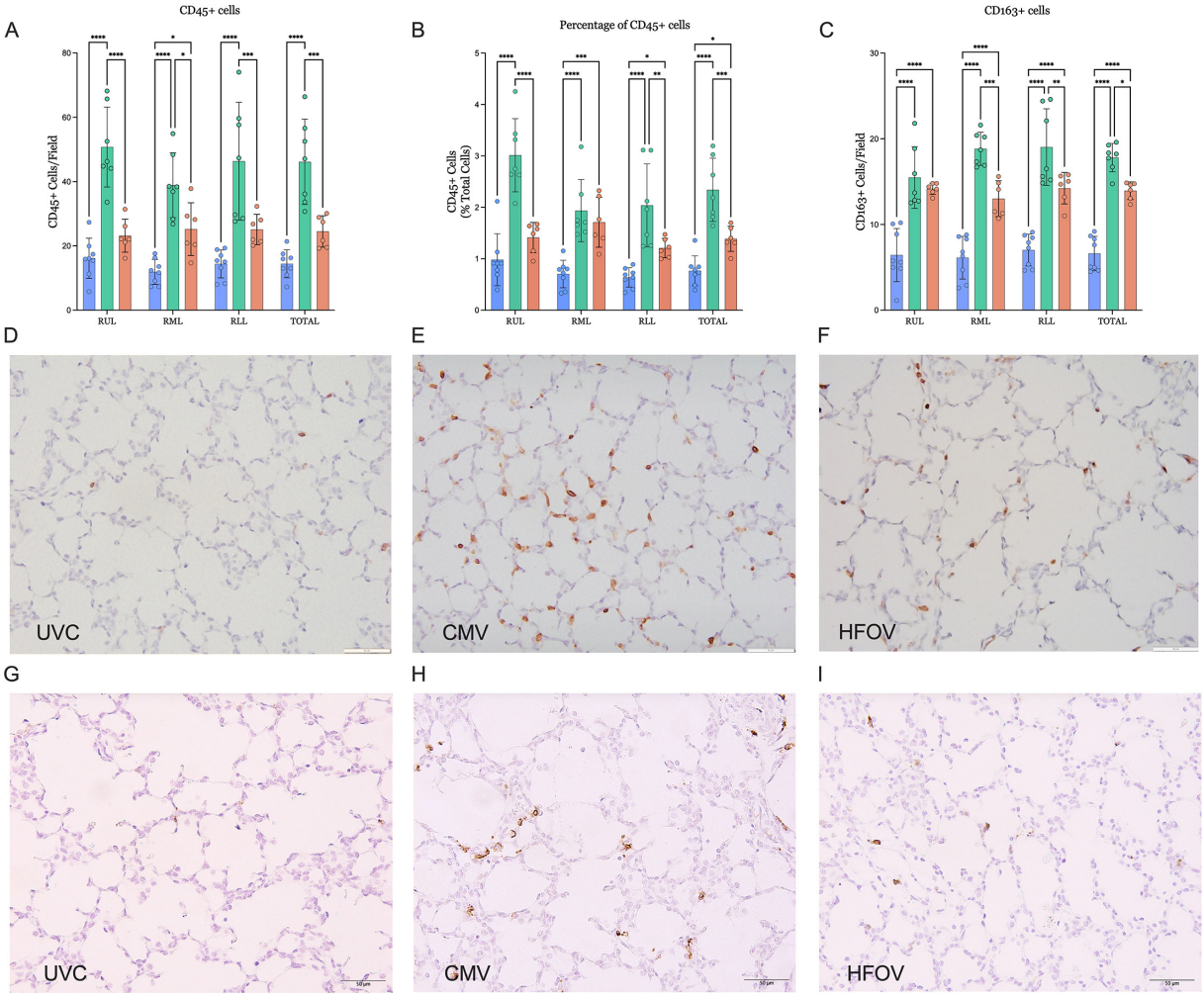

Figure 3 CD45 and C163 analysis: right upper lung (RUL), right middle lung (RML), right lower lung (RLL). Unventilated control (UVC) is represented by blue columns, conventional mechanical ventilation (CMV) by green columns and high-frequency oscillatory ventilation (HFOV) by orange columns. Data are presented as mean±SD. *P<0.05, **p<0.01, ***p<0.001, ****p<0.0001. (A) CD45+ cells, (B) percentage of CD45 relative to total cell count, (C) CD163+ cells, (D–F) representative CD45-stained lung sections from UVC, CMV and HFOV, respectively and (G–I) representative CD163-stained lung sections from UVC, CMV and HFOV, respectively. Scale bar=50 µm.

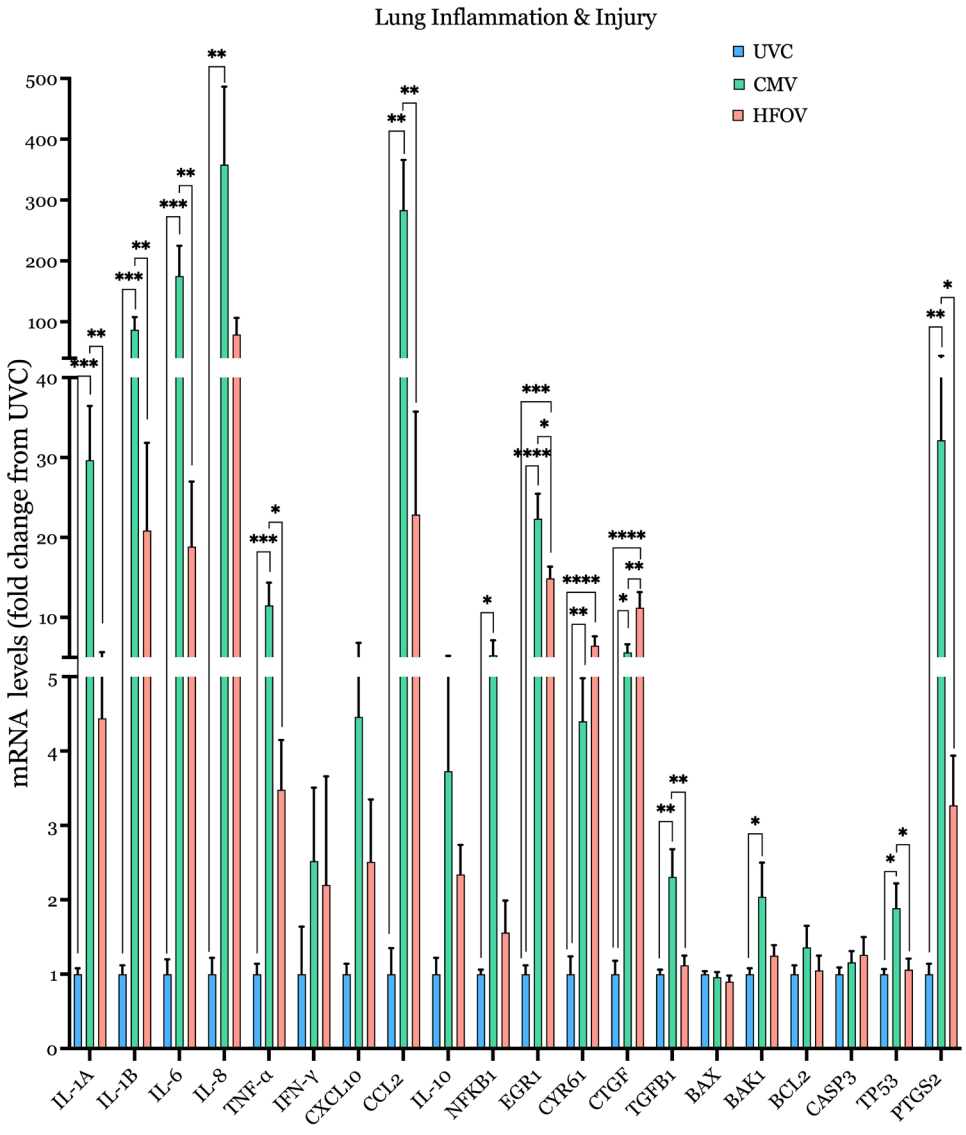

Figure 4 Gene expression: interleukin-1 alpha (*IL-1A*), interleukin-1 beta (*IL-1B*), interleukin-6 (*IL-6*), interleukin-8 (*IL-8*), tumour necrosis factor alpha (*TNFA*), interferon gamma (*IFNY*), interferon gamma-induced protein 10 (*CXCL10*), monocyte chemotactic protein 1 (*CCL2*), interleukin-10 (*IL-10*), nuclear factor kappa B subunit 1 (*NFKB1*), early growth response protein 1 (*EGR1*), cysteine-rich angiogenic inducer 61 (*CYR61*), connective tissue growth factor (*CTGF*), transforming growth factor beta 1 (*TGFB1*), BCL2-associated X (*BAX*), BCL2 antagonist/killer 1 (*BAK1*), BCL2 apoptosis regulator (*BCL2*), caspase-3 (*CASP3*), tumour protein p53 (*TP53*), prostaglandin-endoperoxide synthase 2 (*PTGS2*). Unventilated control (UVC) is represented by blue columns, conventional mechanical ventilation (CMV) by green columns and high-frequency oscillatory ventilation (HFOV) by orange columns. Data are presented as mean±SEM. *P<0.05, **p<0.01, ***p<0.001, ****p<0.0001. mRNA, messenger RNA.

BAX and *CASP3* gene expression was not different between groups. *EGR1* mRNA levels were significantly increased in HFOV and CMV lambs compared with UVC lambs. *EGR1* mRNA levels were significantly higher in CMV compared with HFOV lambs (p<0.05). *CYR61* and *CTGF* mRNA levels were significantly increased in CMV and HFOV lambs compared with UVC (p<0.05). HFOV lambs had significantly higher *CTGF* mRNA levels compared with CMV (p<0.001).

DISCUSSION

Effective and gentle respiratory support is critical for preterm infants to ensure a smooth transition to extrauterine life while minimising lung inflammation and injury. We hypothesised that HFOV, when applied immediately after birth, would facilitate lung aeration and attenuate early inflammatory and injury response compared with CMV. Our findings demonstrated that HFOV was able to recruit the liquid-filled lung at birth and initiate the cardiovascular transition similarly to CMV. Importantly, HFOV reduced lung inflammation compared with CMV, indicated by reduced inflammatory cells (CD45 and CD163) and pro-inflammatory gene expression. Taken together, our findings highlight the critical role of V_T in increasing lung inflammation and injury at birth, and the potential for HFOV to reduce lung inflammation at birth.

CMV triggered a robust inflammatory response marked by upregulation of mRNA expression of classical pro-inflammatory cytokines, chemokines (*CCL2*) and key signalling mediators (*NFKB1, TGFB1* and *PTGS2*). These genes are well-established mediators of VILI, contributing to epithelial damage, immune cell recruitment and impaired alveolar development.[27–29] In contrast, HFOV produced only modest changes, with most gene expression not different to UVC, indicating substantially

less inflammatory activation than CMV. Histological assessment supports this contention. HFOV lambs had lower CD45-positive cells across all lung regions compared with CMV lambs, and although higher than UVC, overall inflammation was reduced. CD163-positive cells were highest in CMV lambs compared with both UVC and HFOV, reflecting greater monocyte recruitment and differentiation in response to lung injury, consistent with the robust cytokine response observed in the CMV group.[30 31] Interestingly, genes associated with early extracellular matrix remodelling and angiogenesis (*CYR61*, *CTGF*) were elevated in both ventilated groups, but more prominently in HFOV lambs. This suggests that controlled lung distension may stimulate early reparative responses following mechanical stress.[32 33] Taken together, our findings highlight the critical role of V_T in the pathogenesis of VILI[1–3] and reinforce the importance of reducing V_T at preterm birth. We did not use recruitment manoeuvres or optimised lung-protective ventilation in these studies. Recruitment strategies during HFOV and CMV can alter inflammation and injury.[34–36] Differences in aeration and injury mechanisms, including barotrauma, ergotrauma, rheotrauma and shear forces, may also influence findings.

LUS confirmed early and progressive lung aeration in HFOV lambs in anterior fields, achieved without the need for recruitment manoeuvres and with significantly lower V_T compared with CMV. These findings highlight the potential role of sustained distending pressure in facilitating early lung aeration. By delivering a continuous MAP, HFOV may enable more gradual and uniform clearance of fetal lung fluid while limiting the large breath-to-breath volume swings associated with CMV. Given that rapid volume fluctuations are key drivers of VILI,[37] the minimal inflammation observed in HFOV lambs likely reflects protection from atelectrauma and ergotrauma, particularly at the alveolar level.[38 39]

The transient elevation in $PaCO_2$ observed between 12 and 30 min in HFOV lambs likely stemmed from protocol-imposed constraints; specifically, a fixed 12 Hz frequency throughout the study and manual V_T adjustments in discrete 0.5 mL/kg increments. Nevertheless, pH remained similar between groups, and $PaCO_2$ values eventually aligned with those in the CMV group, indicating effective early gas exchange with HFOV was during early stabilisation. These findings align with clinical evidence supporting HFOV as an effective primary mode for preterm infants with respiratory distress syndrome.[40 41] However, most of the randomised trials evaluating HFOV were conducted decades ago, using outdated ventilator technologies and strategies that differ from current standards, including the absence of VG modes and PBCC. Our findings highlight the need to re-evaluate the potential benefits of HFOV within modern neonatal care practices.

PBCC may improve cardiovascular stability, reduce systemic and cerebral hypoxia and potentially decrease neonatal mortality.[14 42] A key finding was the minimal effect of HFOV on the cardiovascular transition at birth, providing reassurance that high MAPs during PBCC did not impede this process, consistent with recent studies.[43] We did observe a lower end-diastolic pulmonary flow and the emergence of retrograde flow patterns in HFOV lambs from 40 min. This was potentially driven by higher airway pressure, which increased pulmonary vascular resistance and promoted right-to-left shunting through the ductus arteriosus.[44] Despite these changes, overall pulmonary blood flow was similar between groups, indicating maintained cardiac output.

In this study, all lambs were ventilated on the intact umbilical cord using 100% FiO_2. While this contrasts with current guidelines, we recently showed that 100% oxygen during PBCC reduces hyperoxia compared with immediate cord clamping and does not increase oxidative stress markers.[19] Indeed, in our study, none of the lambs exhibited biochemical or clinical evidence of hyperoxia during cord-based ventilation. While recent evidence suggests starting in higher FiO_2 may reduce mortality in preterm infants,[45] these studies were not conducted in the context of PBCC. The optimal starting FiO_2 during PBCC remains unknown and warrants further investigation.

Our study has limitations that warrant consideration. Lambs were delivered via caesarean section under maternal anaesthesia, and sedation was maintained throughout the experiment. Although this model diverges from spontaneous preterm birth in humans, it allowed for rigorous physiological monitoring and control of ventilation variables, providing insights that would be challenging to obtain in clinical settings. The lambs in this study were not randomised to reduce animal use, as the UVC and CMV groups came from an earlier study.[19] The lambs were from different breed years (2023 and 2024), which may impact interpretation of the findings. The CMV group included more males, who typically have lower lung compliance due to surfactant differences.[46 47] A key limitation was the lack of true VG mode during HFOV and protocol constraints like fixed frequency, which likely reduced V_T precision and CO_2 clearance, resulting in clinically suboptimal levels. Future studies will use optimised HFOV settings, including VG and wider frequency and amplitude ranges, to better control gas exchange. LUS was done only in HFOV lambs due to operator availability. The short study duration also limited long-term outcome assessment, although focusing on the immediate postnatal period is justified as it is a critical window for lung injury.

In conclusion, we evaluated the use of HFOV during PBCC in preterm lambs. Our data suggest that HFOV facilitates early lung aeration and effective gas exchange and reduces lung inflammation compared with CMV in the critical first hour of life. These insights may inform future clinical trials and pave the way for incorporating HFOV into delivery room practices for preterm infants.

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Contributors BCB, AWG, CTR, GRP: conceptualisation, methodology, analysis, investigation, resources, writing—original draft, visualisation; EGV, VZ, SBK, HL, AT, DAB, JAW: methodology, analysis, investigation, resources, writing—reviewing and editing; ZJ: methodology, analysis, investigation, resources; RCS, RSP: conceptualisation, methodology, investigation, writing—reviewing and editing; CTR, GRP: conceptualisation, methodology, analysis, investigation, resources, writing—original draft, visualisation, supervision, project administration, funding; guarantor: GRP.

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Competing interests None declared.

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REFERENCES

1 Slutsky AS, Ranieri VM. Ventilator-induced lung injury. *N Engl J Med* 2013;369:2126–36.
2 Hillman NH, Polglase GR, Pillow JJ, *et al*. Inflammation and lung maturation from stretch injury in preterm fetal sheep. *Am J Physiol Lung Cell Mol Physiol* 2011;300:L232–41.
3 Davies IM, Polglase GR. Inflating or Overinflation? New Evidence for Lung Injury at Birth. *Am J Respir Crit Care Med* 2023;208:517–8.
4 Pereira-Fantini PM, Kenna KR, Fatmous M, *et al*. Impact of tidal volume strategy at birth on initiating lung injury in preterm lambs. *Am J Physiol Lung Cell Mol Physiol* 2023;325:L594–603.
5 Mukerji A, Belik J, Sanchez-Luna M. Bringing back the old: time to reevaluate the high-frequency ventilation strategy. *J Perinatol* 2014;34:464–7.
6 Ackermann BW, Klotz D, Hentschel R, *et al*. High-frequency ventilation in preterm infants and neonates. *Pediatr Res* 2023;93:1810–8.
7 Reyburn B, Li M, Metcalfe DB, *et al*. Nasal ventilation alters mesenchymal cell turnover and improves alveolarization in preterm lambs. *Am J Respir Crit Care Med* 2008;178:407–18.
8 Rehan VK, Fong J, Lee R, *et al*. Mechanism of reduced lung injury by high-frequency nasal ventilation in a preterm lamb model of neonatal chronic lung disease. *Pediatr Res* 2011;70:462–6.
9 Cools F, Offringa M, Askie LM. Elective high frequency oscillatory ventilation versus conventional ventilation for acute pulmonary dysfunction in preterm infants. *Cochrane Database Syst Rev* 2015;2015:CD000104.
10 Salvo V, Zimmermann LJ, Gavilanes AW, *et al*. First intention high-frequency oscillatory and conventional mechanical ventilation in premature infants without antenatal glucocorticoid prophylaxis. *Pediatr Crit Care Med* 2012;13:72–9.
11 Knol R, Brouwer E, van den Akker T, *et al*. Physiological versus time based cord clamping in very preterm infants (ABC3): a parallel-group, multicentre, randomised, controlled superiority trial. *Lancet Reg Health Eur* 2025;48:101146.
12 Knol R, Brouwer E, Vernooij ASN, *et al*. Clinical aspects of incorporating cord clamping into stabilisation of preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2018;103:F493–7.
13 Bhatt S, Alison BJ, Wallace EM, *et al*. Delaying cord clamping until ventilation onset improves cardiovascular function at birth in preterm lambs. *J Physiol* 2013;591:2113–26.
14 Polglase GR, Dawson JA, Kluckow M, *et al*. Ventilation onset prior to umbilical cord clamping (physiological-based cord clamping) improves systemic and cerebral oxygenation in preterm lambs. *PLoS One* 2015;10:e0117504.
15 Smolich JJ, Kenna KR, Esler MD, *et al*. Greater sympathoadrenal activation with longer preventilation intervals after immediate cord clamping increases hemodynamic lability at birth in preterm lambs. *Am J Physiol Regul Integr Comp Physiol* 2017;312:R903–11.
16 Polglase GR, Wallace MJ, Morgan DL, *et al*. Increases in lung expansion alter pulmonary hemodynamics in fetal sheep. *J Appl Physiol (1985)* 2006;101:273–82.
17 National Health and Medical Research COuncil. *Australian code for the care and use of animals for scientific purposes*. 8th edn. Canberra: National Health and Medical Research Council, 2013.
18 Percie du Sert N, Hurst V, Ahluwalia A, *et al*. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol* 2020;18:e3000410.
19 Poulos Z, Vandenberg E, Johnson Z, *et al*. Impact of resuscitation with 100% oxygen during physiological-based cord clamping or immediate cord clamping on lung inflammation and injury. *Pediatr Res* 2025.
20 Brat R, Yousef N, Klifa R, *et al*. Lung Ultrasonography Score to Evaluate Oxygenation and Surfactant Need in Neonates Treated With Continuous Positive Airway Pressure. *JAMA Pediatr* 2015;169:e151797.
21 Coleman M. Respiratory and pulmonary physiology. In: Duke J, ed. *Anesthesia secrets*. Philadelphia: Mosby, 2011: 17–23.
22 Hsia CCW, Hyde DM, Ochs M, *et al*. An Official Research Policy Statement of the American Thoracic Society/European Respiratory Society: Standards for Quantitative Assessment of Lung Structure. *Am J Respir Crit Care Med* 2010;181:394–418.
23 Vandenberg EG, Kelly SB, Zahra VA, *et al*. Investigating pulmonary inflammation and injury after progressive systemic inflammation in preterm fetal sheep. *Front Physiol* 2025;16:1542613.
24 Clark EL, Bush SJ, McCulloch MEB, *et al*. A high resolution atlas of gene expression in the domestic sheep (Ovis aries). *PLoS Genet* 2017;13:e1006997.
25 Hillman NH, Moss TJM, Kallapur SG, *et al*. Brief, large tidal volume ventilation initiates lung injury and a systemic response in fetal sheep. *Am J Respir Crit Care Med* 2007;176:575–81.
26 Allison BJ, LaRosa DA, Barton SK, *et al*. Dose-dependent exacerbation of ventilation-induced lung injury by erythropoietin in preterm newborn lambs. *J Appl Physiol (1985)* 2019;126:44–50.
27 Huang T-H, Fang P-H, Li J-M, *et al*. Cyclooxygenase-2 Activity Regulates Recruitment of VEGF-Secreting Ly6C^{high} Monocytes in Ventilator-Induced Lung Injury. *Int J Mol Sci* 2019;20:1771.
28 Chen L, Xia HF, Shang Y, *et al*. Molecular Mechanisms of Ventilator-Induced Lung Injury. *Chin Med J* 2018;131:1225–31.
29 Ma S-F, Grigoryev DN, Taylor AD, *et al*. Bioinformatic identification of novel early stress response genes in rodent models of lung injury. *Am J Physiol Lung Cell Mol Physiol* 2005;289:L468–77.
30 Etzerodt A, Moestrup SK. CD163 and Inflammation: Biological, Diagnostic, and Therapeutic Aspects. *Antioxid Redox Signal* 2013;18:2352–63.
31 Kowal K, Silver R, Sławińska E, *et al*. CD163 and its role in inflammation. *Folia Histochem Cytobiol* 2011;49:365–74.
32 Marini JJ, Kummer RL, Rocco PRM. Does 'sub-threshold' ventilatory stress promote healing after lung injury? *ICMx* 2024;12.
33 Chaqour B, Goppelt-Struebe M. Mechanical regulation of the Cyr61/CCN1 and CTGF/CCN2 proteins. *FEBS J* 2006;273:3639–49.
34 Miedema M, McCall KE, Perkins EJ, *et al*. Lung Recruitment Strategies During High Frequency Oscillatory Ventilation in Preterm Lambs. *Front Pediatr* 2018;6:436.
35 Tingay DG, Pereira-Fantini PM, Oakley R, *et al*. Gradual Aeration at Birth Is More Lung Protective Than a Sustained Inflation in Preterm Lambs. *Am J Respir Crit Care Med* 2019;200:608–16.
36 Polglase GR, Moss TJM, Nitsos I, *et al*. Differential effect of recruitment maneuvres on pulmonary blood flow and oxygenation during HFOV in preterm lambs. *J Appl Physiol (1985)* 2008;105:603–10.
37 Tingay DG, Fatmous M, Kenna K, *et al*. Inflating Pressure and Not Expiratory Pressure Initiates Lung Injury at Birth in Preterm Lambs. *Am J Respir Crit Care Med* 2023;208:589–99.
38 van Kaam AH. Optimal Strategies of Mechanical Ventilation: Can We Avoid or Reduce Lung Injury? *Neonatology* 2024;121:570–5.
39 Kneyber MCJ. Driving Pressure and Mechanical Power: The Return of Physiology in Pediatric Mechanical Ventilation. *Pediatr Crit Care Med* 2021;22:927–9.
40 Ganguly A, Makkar A, Sekar K. Volume Targeted Ventilation and High Frequency Ventilation as the Primary Modes of Respiratory Support for ELBW Babies: What Does the Evidence Say? *Front Pediatr* 2020;8:27.
41 Cools F, Askie LM, Offringa M, *et al*. Elective high-frequency oscillatory versus conventional ventilation in preterm infants: a systematic review and meta-analysis of individual patients' data. *Lancet* 2010;375:2082–91.
42 Ersdal HL, Linde J, Mduma E, *et al*. Neonatal outcome following cord clamping after onset of spontaneous respiration. *Pediatrics* 2014;134:265–72.
43 Sett A, Truong J, Crotty A, *et al*. Dynamic lung aeration after birth does not impede pulmonary blood flow in preterm lambs. *Arch Dis Child Fetal Neonatal Ed* 2025;111:F41–8.
44 Crossley KJ, Allison BJ, Polglase GR, *et al*. Dynamic changes in the direction of blood flow through the ductus arteriosus at birth. *J Physiol* 2009;587:4695–704.
45 Seidler AL, Aberoumand M, Hunter KE, *et al*. Deferred cord clamping, cord milking, and immediate cord clamping at preterm birth: a systematic review and individual participant data meta-analysis. *Lancet* 2023;402:2209–22.
46 Ishak N, Hanita T, Sozo F, *et al*. Sex differences in cardiorespiratory transition and surfactant composition following preterm birth in sheep. *Am J Physiol Regul Integr Comp Physiol* 2012;303:R778–89.
47 De Matteo R, Ishak N, Hanita T, *et al*. Respiratory adaptation and surfactant composition of unanesthetized male and female lambs differ for up to 8 h after preterm birth. *Pediatr Res* 2016;79:13–21.