



BASIC SCIENCE ARTICLE

Effect of various inspired oxygen concentrations on pulmonary and systemic hemodynamics and oxygenation during resuscitation in a transitioning preterm model

Praveen Chandrasekharan¹, Munmun Rawat¹, Sylvia F. Gugino¹, Carmon Koenigsnecht¹, Justin Helman¹, Jayasree Nair¹, Payam Vali² and Satyan Lakshminrusimha²

BACKGROUND: The Neonatal Resuscitation Program recommends initial resuscitation of preterm infants with low oxygen (O_2) followed by titration to target preductal saturations (SpO_2). We studied the effect of resuscitation with titrated O_2 on gas exchange, pulmonary, and systemic hemodynamics.

METHODOLOGY: Twenty-nine preterm lambs (127 d gestation) were randomized to resuscitation with 21% O_2 ($n = 7$), 100% O_2 ($n = 6$), or initiation at 21% and titrated to target SpO_2 ($n = 16$). Seven healthy term control lambs were ventilated with 21% O_2 .

RESULTS: Preductal SpO_2 achieved by titrating O_2 was within the desired range similar to term lambs in 21% O_2 . Resuscitation of preterm lambs with 21% and 100% O_2 resulted in SpO_2 below and above the target, respectively. Ventilation of preterm lambs with 100% O_2 and term lambs with 21% O_2 effectively decreased pulmonary vascular resistance (PVR). In contrast, preterm lambs with 21% O_2 and titrated O_2 demonstrated significantly higher PVR than term lambs on 21% O_2 .

CONCLUSION(S): Initial resuscitation with 21% O_2 followed by titration of O_2 led to suboptimal pulmonary vascular transition at birth in preterm lambs. Ventilation with 100% O_2 in preterm lambs caused hyperoxia but reduced PVR similar to term lambs on 21% O_2 . Studies evaluating the initiation of resuscitation at a higher O_2 concentration followed by titration based on SpO_2 in preterm neonates are needed.

Pediatric Research (2018) 84:743–750; <https://doi.org/10.1038/s41390-018-0085-x>

INTRODUCTION

The optimal concentration of supplemental oxygen during initial resuscitation of premature infants remains controversial. The International Liaison Committee on Resuscitation (ILCOR) recommends initiating resuscitation of preterm newborns (<35 weeks postmenstrual age (PMA)) with a low inspired oxygen concentration ($\leq 30\%$) (strong recommendation, moderate-quality evidence).¹ ILCOR does not recommend the use of high concentration ($\geq 65\%$) of supplemental oxygen.^{1,2} Similarly, the Neonatal Resuscitation Program (NRP) recommends that resuscitation of preterm newborns <35 weeks gestation at birth should be initiated with low concentration of inspired oxygen ($\leq 30\%$), and the oxygen concentration should be titrated to achieve preductal SpO_2 targets.³ The evidence for this recommendation comes from the analysis of seven randomized control trials (RCTs)^{4–10} which have shown no advantage of using high oxygen compared to low oxygen for the critical outcome of mortality (relative risk (RR) 1.48; 95% confidence interval (CI) 0.8–2.73). After these recommendations were published, a large RCT by Oei et al. targeting SpO_2 between 65 and 95% up to 5 min of postnatal life and 85–95% from 5 min until NICU admission has shown that the mortality was high in infants <28 weeks PMA initially resuscitated with 21% O_2 compared to 100% O_2 (RR 3.9 (1.1–13.4); $p = 0.01$ –non-prespecified analysis).¹¹ A retrospective study by Rabi et al. comparing infants ≤ 27 weeks' gestation before and after 2006, when the policy regarding the initial oxygen concentration

for preterm infants in the delivery room was changed from 100 to <100% oxygen and titration to achieve target SpO_2 was published in 2015.¹² This Canadian Neonatal Network study has shown that the adjusted odds ratio (AOR) for the primary outcome of severe neurologic injury or death was higher in the lower oxygen group (AOR 1.36; 95% CI 1.11–1.66) and in those resuscitated with 21% oxygen (AOR 1.33; 95% CI 1.04–1.69), when compared to 100% oxygen. Despite the current evidence, the optimal concentration of supplemental oxygen for initial resuscitation of preterm neonates remains unclear.

The effect of titrating inspired oxygen during resuscitation of a newly born preterm model on gas exchange and hemodynamics is not known. Our objectives were to determine the effect of resuscitating a preterm lamb model with 21% O_2 , 100% O_2 , and titrating supplemental oxygen as per NRP recommended preductal saturations on (i) oxygen saturations, (ii) arterial oxygenation, and (iii) systemic and pulmonary hemodynamics.

METHODOLOGY

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of University at Buffalo. Time-dated pregnant ewes (May Farms, Buffalo Mills, PA) at 127–128 d gestation (preterm equivalent, the term equivalent 147–150 d) were sedated, intubated, and ventilated with 21–30% oxygen and anesthetized with 2% isoflurane. Before delivery, the lambs were

¹Department of Pediatrics, University at Buffalo, Buffalo, NY, USA and ²Department of Pediatrics, UC Davis, Sacramento, CA, USA

Correspondence: Praveen Chandrasekharan (pkchandr@buffalo.edu)

Received: 20 February 2018 Revised: 23 May 2018 Accepted: 25 May 2018

Published online: 2 June 2018

randomized using sequential opaque envelopes (target randomization ratio—1:1:3—21% O₂, 100% O₂, and titrated O₂, respectively—see below). Preterm lambs were partially exteriorized via a cesarean section. They were instrumented while on placental circulation and maternal anesthesia. The ewes did not receive prenatal steroids. Term lambs at 142 d gestation (to reduce the risk of spontaneous labor) were used as controls. The instrumentation of these lambs was as described previously.¹⁶

Monitoring

Preductal saturations were measured by Masimo radical pulse oximeter probe (Masimo, Irvine, CA) that was placed on the right forelimb. We targeted preductal saturations based on current NRP recommendations.³ A respiratory monitor (NM3 Philips, Respiro-nics, MA) with a mainstream end-tidal carbon dioxide detector (ETCO₂) was attached to the tracheal tube.^{13,14} Cerebral saturation was measured by NONIN (SenSmart Model X-100, Plymouth, MN) regional oximetry. The cerebral regional saturations (CrSO₂) were measured in preterm lambs but not in term lambs (controls). The carotid arterial oxygen content was measured, as described previously in preterm lambs.¹⁵ The oxygen extraction was calculated in preterm lambs as a product of left carotid blood flow and the difference in arterial oxygen content (carotid artery) and the venous oxygen content (jugular bulb).¹⁵ Venous gases were not available in term lambs.

Randomization and protocol

After instrumentation and surfactant instillation (Infasurf 3 ml/kg, ONY), the lamb was delivered following early cord clamping. Based on the randomization arm, preterm lambs were resuscitated with 21% O₂ or 100% O₂, regardless of preductal SpO₂. In the targeted oxygen group, the O₂ was initiated at 21% and titrated to maintain oxygen in the NRP specified range as mentioned above. If preductal SpO₂ was outside this range, inspired oxygen was adjusted by 5% every 15 s. Term healthy lambs were ventilated with 21% O₂, irrespective of SpO₂ and served as controls.¹⁶ After the first 30 min, the variable O₂ group was further subdivided into three saturation target ranges to mimic NeOPROM studies (85–89%, 90–94%, and 95–99%—data not shown in this paper).¹⁷ As “positive” and “negative” controls, we ventilated two groups of lambs continuously with 21% oxygen and 100% oxygen irrespective of SpO₂. Our original intention was to study 6 lambs in each group. In the titrated group, we intended to study 18 lambs, but 2 were lost during instrumentation. Because of this reason, the variable group has approximately three times the number of lambs in the 21% and 100% categories.

Ventilation was initiated with the Siemens Servo 300A (Maquet Medical, Salt Lake City, Utah) with a peak inspiratory pressure (PIP) of 30–35 cm H₂O and a PEEP of 5–6 cm H₂O to target a tidal volume of 8–9 ml/kg in preterm lambs. Tidal volume data were not available in the term lambs. Arterial blood gases were drawn at birth, 1, 2, and 5 min after birth followed by every 5 min for the first 30 min. Systemic and pulmonary hemodynamics were continuously recorded using AcqKnowledge Acquisition and Analysis Software (BIOPAC Systems, Goleta, CA).

Sample size and power calculation

Based on previous studies, to detect a difference in pulmonary vascular resistance of 0.15 mmHg/ml/kg/min, we needed six lambs in each group.^{18,19} A power estimate was performed by one-way ANOVA, using statistical analysis software 9.4 (SAS, NC). With the current randomization, the power to detect the difference in PVR is 0.85 with an alpha of 0.05.

Statistics

Categorical variables are presented as numbers and percentages, while continuous variables are presented as means and standard

deviations. Data were analyzed using chi-square, *t*-test, and ANOVA. Statistical significance was set at <5%.

RESULTS

The baseline characteristics of lambs before resuscitation are shown in Table 1. There were a total of 36 lambs in the study: six preterm lambs ventilated with 100% O₂, seven preterm lambs ventilated with 21% O₂, sixteen preterm lambs ventilated with targeting SpO₂ range, and seven term lambs ventilated with 21% O₂. The weight of the term lambs was significantly higher compared to preterm lambs (Table 1).

Saturation

In the first 30 min after birth, use of 21% and 100% O₂ resulted in SpO₂ below and above the NRP recommended target range, respectively. Titrating oxygen in preterm lambs and use of 21% oxygen in term lambs resulted in NRP recommended target saturations (Fig. 1a). Among preterm lambs, the time spent within the target SpO₂, as recommended by NRP was 10% in 100% O₂ group, 47% with the titrated O₂ group, and 30% in 21% O₂ group. Term lambs ventilated with 21% O₂ spent 65% of the first 30 min within the NRP recommended target range (significantly higher than preterm lambs, *p* < 0.001).

FiO₂

The inspired oxygen concentration required to maintain targeted saturations in the titrated FiO₂ preterm group is shown in Fig. 1b (median FiO₂ 0.31 (interquartile range: 0.21–0.40)).

Tidal volume and arterial carbon dioxide

There was no difference in tidal volumes in preterm lambs (Figure S1a). The term lambs had lower PaCO₂ compared to preterm lambs and these values were statistically different (Figure S1b) (*p* < 0.01).

Alveolar oxygenation

The calculated alveolar oxygenation (PAO₂) values with 100% O₂, titrated O₂, and 21% O₂ were 643 ± 24.4, 244 ± 156, and 95 ± 24.2 mmHg and were significantly different (*p* < 0.001) (Figs. 2 and 3). The PAO₂ in term lambs (99 ± 11 mmHg) resuscitated with 21% O₂ was similar to preterm lambs 21% inspired oxygen group.

Arterial oxygenation

Arterial oxygen tension (PaO₂) was the highest with 100% O₂ (256 ± 144 mmHg) and was higher than 21% O₂ group of preterm lambs (39 ± 16 mmHg, *p* < 0.001), titrated oxygen group (60 ± 36 mmHg, *p* < 0.001), and in term controls (37 ± 10 mmHg, *p* < 0.001) in the first 30 min. The PaO₂ was higher in preterm lambs

Table 1. Baseline characteristics and carotid arterial blood gases prior to delivery

Parameters (in means±SD)	Preterm lambs			Term lambs
	100% oxygen (N = 6)	Titrated oxygen (N = 16)	21% oxygen (N = 7)	21% oxygen (N = 7)
Weight (kg)	3.1 ± 0.4	2.9 ± 0.6	2.8 ± 0.4	4.0 ± 0.6*
Female <i>n</i> (%)	3 (50%)	7 (44%)	4 (57%)	3 (43%)
Twins <i>n</i> (%)	2 (33%)	5 (31%)	2 (29%)	2 (29%)
pH	7.15 ± 0.20	7.12 ± 0.12	7.13 ± 0.19	7.2 ± 0.2
PaCO ₂ (mm Hg)	84.9 ± 27.0	83.7 ± 26.5	81.3 ± 24.0	77 ± 20.5

**p* < 0.05, data are presented as mean and SD unless specified

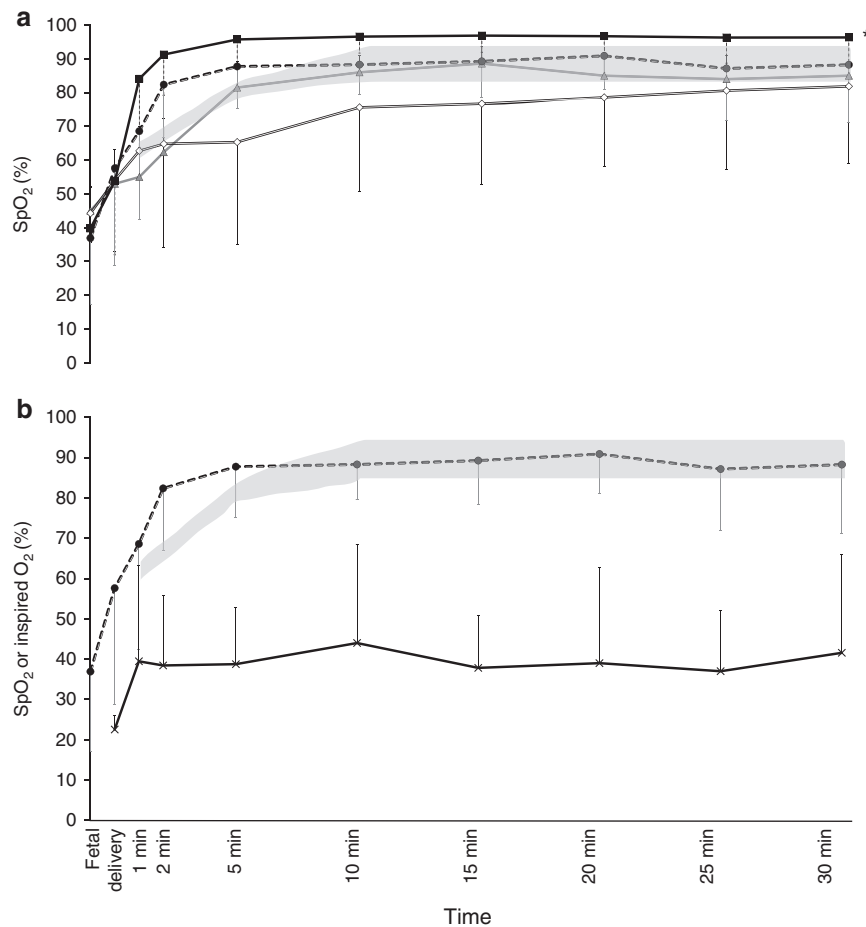


Fig. 1 **a** Changes in saturations (SpO₂) with 100% inspired oxygen preterm lambs (closed square), 21% oxygen preterm lambs (open diamond), titrated oxygen (closed circle), and term 21% oxygen controls (triangle). The shaded area shows the NRP recommended saturation targets. **b** Changes in titrated oxygen (cross) and saturations (closed circle) in the titrated oxygen group. Hyphenated lines/shaded area shows the NRP recommended saturation targets.

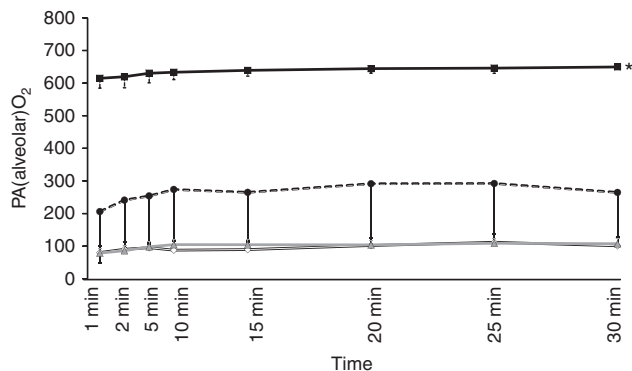


Fig. 2 PAO₂ (alveolar oxygenation) is shown in Fig. 3. A total of 100% inspired oxygen preterm lambs (closed square), 21% oxygen preterm lambs (open diamond), and titrated oxygen (closed circle) were different. * $p < 0.001$ by ANOVA. Use of 21% oxygen in the term (triangle) and preterm lambs showed similar PAO₂.

resuscitated with the titrated O₂ group compared to preterm lambs resuscitated with 21% O₂ ($p = 0.039$) (Fig. 3).

Heart rate

Heart rates were similar in preterm lambs resuscitated with 100% O₂ ($183 \pm 27/\text{min}$), 21% O₂ ($175 \pm 27/\text{min}$), titrated O₂ ($173 \pm 37/\text{min}$), and in term controls ($180 \pm 25/\text{min}$).

Pulmonary blood flow

The pulmonary blood flow (PBF) was the highest in preterm lambs resuscitated with 100% O₂ and was different from preterm lambs resuscitated with 21% O₂ ($p < 0.001$) and titrated oxygen ($p < 0.001$) but was similar to term controls (Fig. 4a).

Carotid blood flow

The carotid blood flow (CBF) was similar in all four groups (Fig. 4b).

Cerebral regional saturation (CrSO₂), carotid arterial oxygen content, and oxygen extraction

The CrSO₂ (Figure S2a) and carotid arterial oxygen content (Figure S2b) were higher and statistically different in the lambs that received 100% O₂ ($p < 0.01$). The oxygen extraction was not different among the preterm lambs (Figure S2c).

Systemic mean blood pressures and pulmonary arterial mean blood pressures

Left-to-right ductal flow is an essential contributor of pulmonary blood flow immediately after birth (Fig. 5).²⁰ The pressure gradient driving net ductal flow is the difference between systemic and pulmonary blood pressures. Mean systemic blood pressure was significantly higher than the pulmonary mean blood pressure (PBP) in term lambs ventilated with 21% O₂ (systemic mean blood pressures (SBP): 80.2 ± 14.4 mmHg, PBP: 48.4 ± 7.2 mmHg, $p < 0.001$), preterm lambs ventilated with 100% O₂ (SBP: 57.8 ± 10.5 mmHg, PBP: 38.4 ± 8.3 mmHg, $p < 0.001$), and in preterm lambs

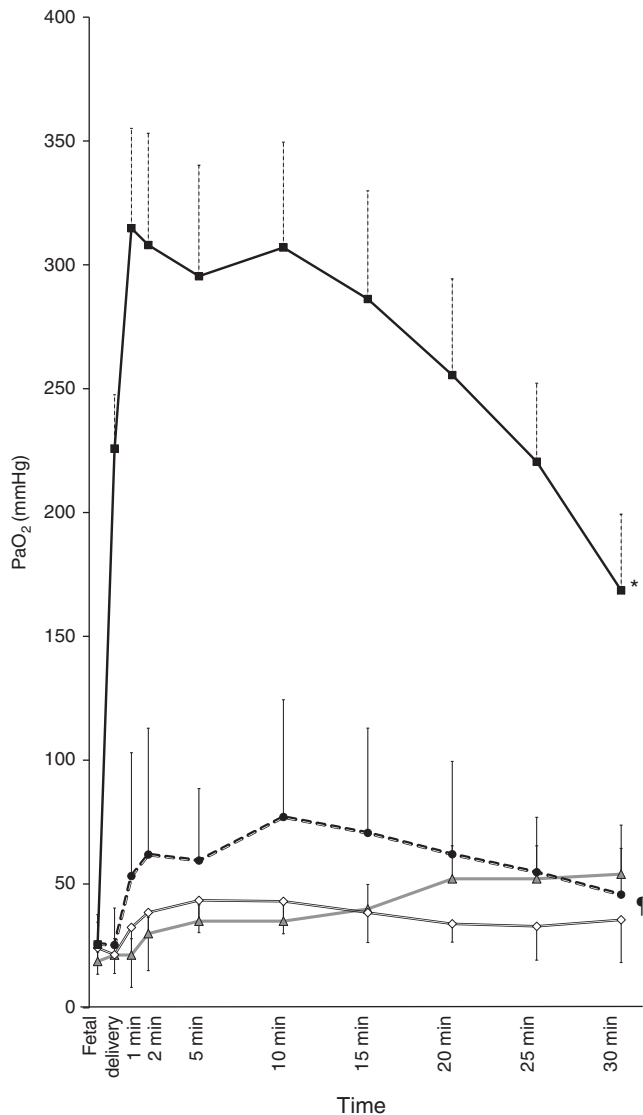


Fig. 3 Arterial oxygen tension (PaO₂) changes with 100% oxygen preterm lambs (closed square), 21% oxygen preterm lambs (open diamond), titrated oxygen (closed circle), and term 21% (triangle) oxygen controls (triangle). *100% preterm oxygen was statistically different from the rest by ANOVA ($p < 0.001$). † The titrated oxygen group was different from 21% preterm lambs by ANOVA ($p < 0.01$).

where the inspired oxygen was titrated to maintain saturations (SBP: 49.4 ± 8.2 mmHg, PBP: 41.5 ± 11.1 mmHg, $p < 0.01$). In contrast, preterm lambs ventilated with 21% O₂ had similar systemic and pulmonary arterial pressures with no gradient across the ductus throughout the first 30 min of the postnatal period (SBP: 49.1 ± 9.0 mmHg, PBP: 47.4 ± 7.4 mmHg, $p = 0.25$ as shown in Fig. 5d). The difference in mean systemic blood pressure and pulmonary arterial pressure over the first 30 min after birth was significantly higher in term lambs ventilated with 21% oxygen and in preterm lambs ventilated with 100% oxygen compared to preterm lambs ventilated with titrated O₂ and 21% oxygen.

Compared to fetal values, pulmonary arterial pressure significantly decreased over the first 30 min in term lambs (21% oxygen) and preterm lambs ventilated with 100% oxygen. There was no significant difference in pulmonary arterial pressure at 30 min after birth when compared to fetal values in preterm lambs ventilated with 21% oxygen. In lambs ventilated with titrated O₂, the difference between fetal pulmonary arterial pressure compared to pressure at 30 min of postnatal age had a p -value of 0.05.

Pulmonary vascular resistance

The PVR was the highest in preterm lambs ventilated with 21% O₂ (1.5 ± 0.9 mmHg/ml/kg/min) and was significantly different from preterm lambs ventilated with 100% O₂ (0.6 ± 0.3 mmHg/ml/kg/min, $p < 0.001$), titrated O₂ (0.9 ± 0.6 mmHg/ml/kg/min, $p < 0.001$), and term lambs ventilated with 21% O₂ (0.7 ± 0.3 mmHg/ml/kg/min, $p < 0.001$) (Fig. 6). The PVR was significantly higher in the titrated oxygen group compared to the 100% O₂ group in preterm lambs ($p = 0.03$). Both these groups were not statistically different from term lambs resuscitated with 21% O₂.

DISCUSSION

Effective ventilation of the lungs with optimal inspired O₂ is the key factor mediating pulmonary transition at birth, leading to an eight-to-tenfold increase in PBF permitting effective gas exchange.^{21–23} In this study, we have demonstrated that ventilation with 21% O₂ in term lambs without lung pathology reduced pulmonary arterial pressure and increased PBF. Ventilation of preterm lambs with 100% O₂ significantly reduced pulmonary arterial pressure, established a significant pressure gradient across the ductus, and marginally increased PBF, similar to that seen in term lambs ventilated with 21% O₂. In contrast, ventilation with 21% O₂ in preterm lambs did not significantly reduce pulmonary arterial pressure over the first 30 min after birth and the increase in PBF was significantly lower than that in term lambs. Interestingly, initiation of ventilation at 21% O₂ and titration of FiO₂ targeting NRP recommended SpO₂ range led to a modest decrease in pulmonary arterial pressure, but the increase in PBF was significantly lower than that achieved in term lambs. To our knowledge, this is the first study evaluating gas exchange, systemic and pulmonary hemodynamics, and cerebral oxygen delivery in a transitioning preterm model targeting the NRP recommended SpO₂ ranges. These differences were achieved with similar tidal volumes and PaCO₂ values among the three groups of preterm lambs, suggesting that these differences were mainly due to variation in oxygen tension (Figure S1).

The supplemental oxygen needed in a preterm neonate requiring assisted ventilation that facilitates the pulmonary vascular transition remains a subject of controversy. It is not known if the target saturation range recommended by NRP is adequate to increase pulmonary blood flow and establish lungs as the site of gas exchange in extremely preterm neonates requiring positive-pressure ventilation. We demonstrate that titrating inspired oxygen resulted in SpO₂ in the desired range and 100% inspired O₂ resulted in saturations above the target range. These results are different from the study in 287 preterm infants by Oei et al.¹¹ In their study, initiation of resuscitation with 21% oxygen and titration resulted in mean SpO₂ below the target range for the first 7–8 min after birth. We speculate that this difference was secondary to (a) all lambs in our study being intubated (and having received the surfactant), compared to only 30% of infants in the Oei et al. study; (b) SpO₂ values were available in the very first minute and O₂ was adjusted immediately after birth; (c) we titrated O₂ by 5% every 15 s as compared to 10% every minute in the clinical study; (d) our lambs were not exposed to antenatal steroids, while 97% of mothers in the Oei et al. study had received antenatal steroids; and finally (e) species differences.

Dawson et al. have published changes in preductal SpO₂ after birth for the term and preterm infants with no supplemental oxygen.^{24,25} The supplemental oxygen required to achieve target SpO₂ was higher (30–67%) in extremely preterm infants <27 6/7 weeks compared to moderate preterm infants less than 28–32 weeks (23–43%). Similarly, Phillipos et al. have shown that infants <28 weeks of gestation requiring PPV who were initially ventilated with 30% oxygen had markedly lower saturations compared to NRP targets.²⁵ With decreasing gestational age, higher FiO₂ is necessary to achieve target oxygen saturations.²⁵

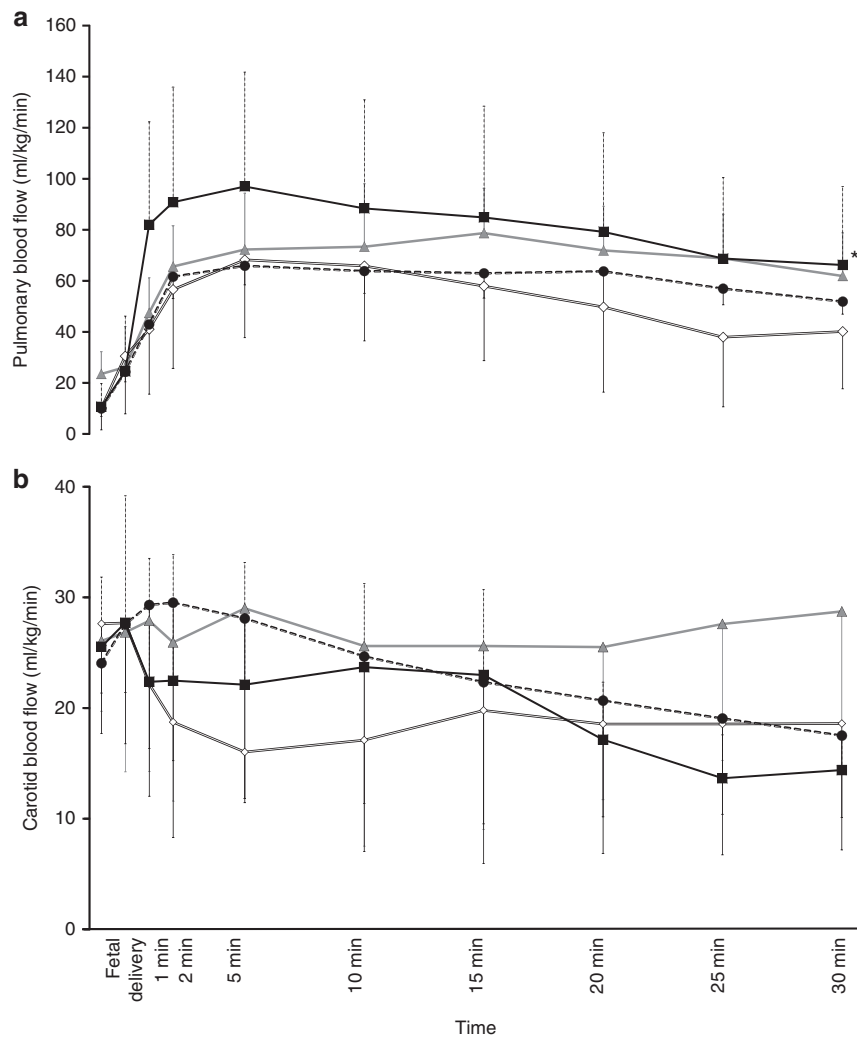


Fig. 4 **a** Left pulmonary arterial blood flow (PBF) changes with 100% oxygen preterm lambs (closed square), 21% oxygen preterm lambs (open diamond), titrated oxygen (closed circle), and term 21% controls (triangle). *100% preterm lambs were different from 21% preterm lambs and the titrated O₂ group. But they were not different from term 21% controls $p < 0.001$ calculated by ANOVA. **b** Left carotid blood flow (CBF) changes with 100% oxygen preterm lambs (closed square), 21% oxygen preterm lambs (open diamond), titrated oxygen (closed circle), and term 21% controls (triangle). The flows were not different.

We found similar differences between term lambs where target SpO₂ could be achieved by ventilation with 21% O₂ but required $44 \pm 4\%$ oxygen to achieve the same target in preterm lambs (equivalent gestational age <28 weeks in humans). Interestingly, the preductal PaO₂ achieved by preterm lambs ventilated with 21% O₂ was similar to that in term lambs. We do not have a physiologic explanation for the difference in SpO₂ between preterm and term lambs ventilated with 21% oxygen, although for a given SpO₂ value, there may be a wide range of PaO₂ values.²⁶ In an individual patient analysis by Oei et al. that included 706 infants, only 11% reached NRP recommended SpO₂ of 80–85% by 5 min.²⁷ Infants who did not reach 80% SpO₂ by 5 min had 2.4 times higher mortality and were 4.5 times more likely to develop severe intraventricular hemorrhage.^{27,28} The data from this study need to be interpreted with caution as it is unclear if the infants were sick and could not achieve >80% SpO₂ in spite of receiving high FiO₂ or if less supplemental oxygen was provided resulting in SpO₂ <80%. Furthermore, a study by Follett et al. concluded that “there was a lag time of approximately 30 s to achieve the FiO₂ at the facemask”. A slower change in oxygen could have yielded different results. Our study was different from

the clinical situation where intubation and surfactant delivery were performed before delivery.

Ventilation with 100% O₂ resulted in supraphysiological PaO₂ (Fig. 3). The PaO₂ in the titrated oxygen group was significantly higher than preterm lambs resuscitated with 21% oxygen. Interestingly, delivery of more oxygen to the brain did not result in higher oxygen extraction (Figure S2) but resulted in higher CrSO₂. Previous studies have shown that resuscitation with higher oxygen can lead to oxidative stress injury, inflammation, and chronic lung disease.^{9,29} Kapadia et al. have shown that when resuscitation was initiated with low oxygen and titrated to target NRP recommended SpO₂, the infants had low cumulative oxygen exposure, lower oxidative stress, and decreased respiratory morbidities.⁵ Continuous use of 100% O₂ may lead to hyperoxic injury and may cause the arrest of lung development.^{30–32} In an asphyxiated term lamb model, we have shown that use of 100% oxygen increases oxidative stress.³³ Previously, we have also shown that oxidized glutathione activity correlated with PaO₂ in a preterm lamb model.³⁴ In a prospective clinical study by Castillo et al., maintaining saturations between 85 and 93% resulted in PaO₂ levels of 40–80 mmHg, 87% of the time in a neonate.³⁵ In our

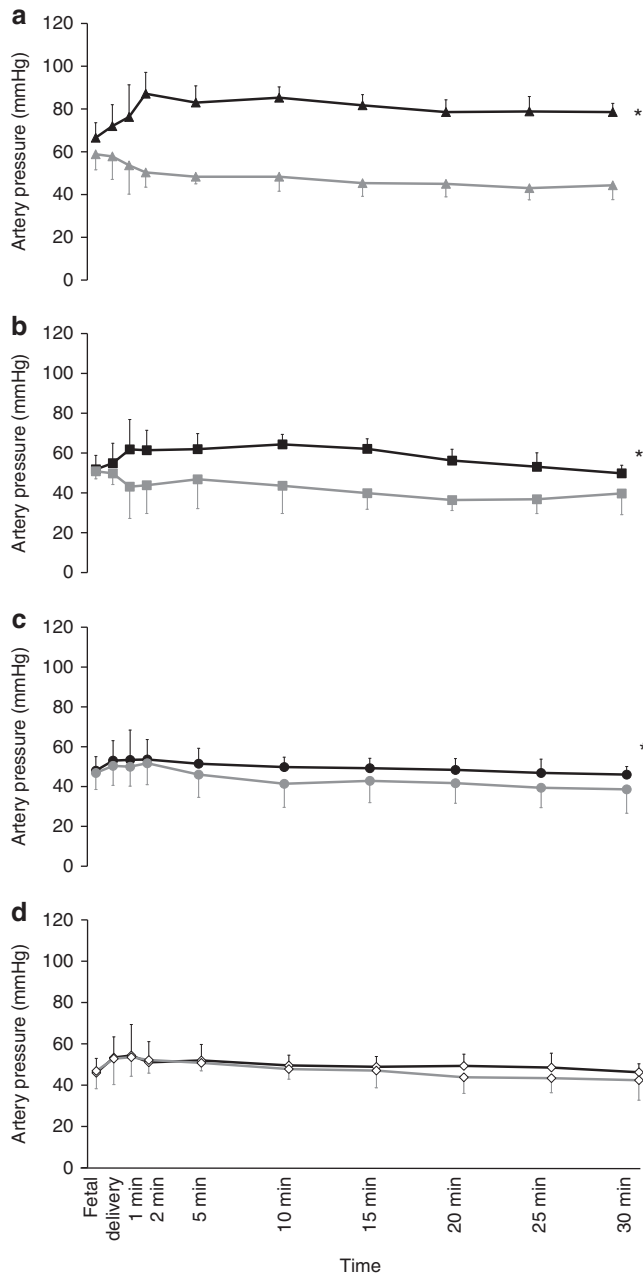


Fig. 5 Systemic and pulmonary arterial mean pressures in term 21% oxygen (a), preterm 100% oxygen (b), preterm titrated oxygen (c), and preterm 21% oxygen (d). a Black and gray triangles represent the mean systemic and mean pulmonary arterial pressures in term lambs ventilated with 21% inspired oxygen (controls). b Black and gray squares represent the mean systemic and mean pulmonary arterial pressures in preterm lambs resuscitated with 100% oxygen. c Black and gray circles represent the mean systemic and mean pulmonary arterial pressures in preterm lambs resuscitated with oxygen titrated to achieve target SpO₂. d Open black and gray diamonds represent the mean systemic and mean pulmonary arterial pressures in preterm lambs resuscitated with 21% inspired oxygen concentration. **p* < 0.001 by ANOVA—the difference between systemic and pulmonary pressures.

study, titrating oxygen to maintain SpO₂ in the target range resulted in an average PaO₂ of 54 mmHg, while the continuous use of 21% oxygen resulted in an average PaO₂ of 35 mmHg during the first 30 min after birth. A PaO₂ of at least 45 mmHg is required for successful pulmonary vascular transition.^{16,19,33,36} To

achieve a delicate balance between hyperoxia and hypoxia, maintaining SpO₂ in the target range suggested by NRP may be safe until more evidence is available in preterm infants.

At birth with the establishment of respiration, PVR decreases, PBF increases, and the ductal shunt from right to left changes to left to right.^{37,38} Heart rate is the single most important predictor of adequate ventilation.³ Since our lambs were intubated and ventilated immediately after birth, we did not find any difference in the heart rate between the groups. In contrast, Oei et al. demonstrated a lower heart rate in preterm infants resuscitated initially with 21% O₂ when compared to 100% O₂.¹¹ We observed a higher PBF in preterm lambs ventilated with 100% O₂ similar to that seen in term lambs ventilated with 21% oxygen. Although PAO₂ and PaO₂ achieved by ventilation with 21% oxygen were similar in preterm and term lambs, the increase in pulmonary blood flow was significantly lower in preterm lambs. We speculate that this difference might be secondary to lung disease, ventilation–perfusion mismatch, the lower sensitivity of pulmonary vasculature to oxygen, and/or due to the differences in the pressure gradient across the ductus.

The differences in systemic and pulmonary blood pressures were strikingly different in preterm lambs ventilated with different oxygen concentrations (Fig. 5). The gradient across the ductus (the difference in systemic and pulmonary pressures) in term lambs ventilated with 21% oxygen was higher than preterm lambs ventilated with 21% O₂ or titrated FiO₂ and similar to preterm lambs ventilated with 100% O₂. The difference in pulmonary and systemic pressures reached a statistically significant difference in the titrated oxygen group but not in 21% preterm lambs.

Why is the pressure gradient across the ductus important in the golden hour of resuscitation, especially in preterm infants? Once the cord is clamped, systemic blood pressure increases and with ventilation, there is a significant decrease in PVR. The increase in the right ventricular output is not the sole contributor to increased blood flow to the lung after birth.^{38,39} The change in the shunt in the ductus that switches from left to right contributes to the right ventricular output immediately after birth. This transition is a critical process that helps sustain and stabilize PBF, pulmonary venous return, and thus maintaining the left ventricular output.^{20,37,39} Our study highlights the effect of using various oxygen concentrations in this critical process.

The drop in PVR was the highest with the use of 100% O₂ in preterm lambs and was similar to 21% oxygen use in term lambs. Use of 21% O₂ in preterm lambs resulted in no change in PVR by 30 min of postnatal age compared to fetal values. Titrating oxygen to maintain target SpO₂ leads to a drop in PVR by 57% at 30 min of life compared to the fetal PVR. The decrease in PVR, in this case, can be directly related to the PaO₂ achieved by various oxygen concentrations during resuscitation. We have previously shown that in an asphyxiated model with lung disease, use of 21% O₂ may not be adequate to achieve pulmonary vasodilation secondary to low PaO₂ levels, although in a term model without lung disease, use of 21% O₂ did result in a significant decrease in PVR.^{16,19}

The suboptimal increase in PBF with the initiation of ventilation with 21% oxygen and titration to achieve target SpO₂ (when compared to 100% O₂ ventilation) is a concern. Recent evidence from Oei et al. study shows that extremely preterm infants (<28 weeks' gestation) had a higher mortality when ventilation was initiated with 21% O₂ as compared to 100% O₂, further emphasizing the importance of this finding.¹¹ In the same study, the most common cause of death in preterm infants resuscitated initially with 21% O₂ was respiratory failure (which may potentially be secondary to RDS complicated by impaired pulmonary vascular transition). It is important to recognize that the results in <28 weeks' gestation of preterm infants in this study were not part of a prespecified analysis and that the sample size was small. However, increased mortality with lower oxygen supplementation

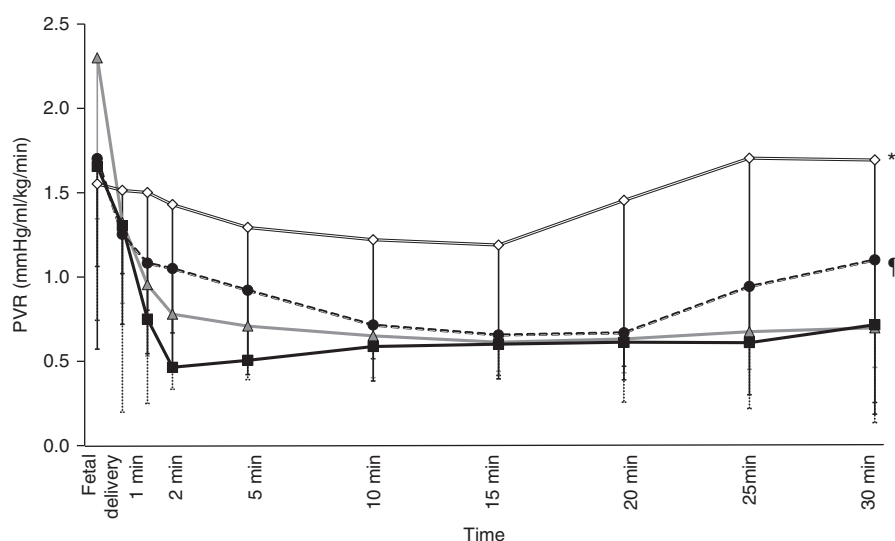


Fig. 6 Pulmonary vascular resistance (PVR) changes in preterm lambs resuscitated with 100% oxygen (closed squares), 21% oxygen (open diamonds), and titrated inspired oxygen to maintain target SpO₂ (closed circles). Term lambs ventilated with 21% inspired oxygen (controls—triangles) are shown by a hyphenated line. * 21% preterm oxygen was statistically different from the rest by ANOVA ($p < 0.001$).

is a concern. These findings are similar to the retrospective Canadian study that showed higher mortality and evidence of higher neurologic injury in preterm infants resuscitated with <100% oxygen.¹² We speculate that inadequate pulmonary vascular transition in preterm infants resuscitated with lower FiO₂ may potentially play a role in mediating higher respiratory morbidity.

Studies in postnatal preterm infants have shown that 100% O₂ inhalation results in ~15% reduction in cerebral blood flow.^{40,41} Exposure to oxygen at birth results in prolonged cerebral vasoconstriction in preterm infants.⁶ However, a recent study in stable preterm infants did not demonstrate cerebral vasoconstriction following hyperoxia.⁴² The relationship between hyperoxia and cerebral blood flow is complex and are elegantly summarized in a recent editorial.⁴³

Cerebral vasoconstriction following 100% oxygen may potentially protect the brain from hyperoxic injury. However, in our study, we found no reduction in CBF in spite of 100% oxygen resuscitation in preterm lambs. The cerebral saturations and the carotid arterial oxygen content were the highest with 100% O₂ resuscitation, resulting in increased delivery of oxygen, but oxygen extraction was similar between the three groups of preterm lambs. Premature neonates are at a risk of hyperoxic brain injury, especially when exposed to supraphysiological oxygen levels compared to their fetal environment. Gerstner et al. have shown that hyperoxia could lead to maturation-dependent cell death in the developing white matter.⁴⁴ The lack of reduction in cerebral blood flow despite high PaO₂ levels suggests that the risk of cerebral hyperoxia is considerably higher in preterm infants initially resuscitated with 100% oxygen and may contribute to brain injury. We acknowledge that species differences and similar PaCO₂ levels between groups in our study may account for some of the variations among studies. Based on these findings, resuscitators should exercise caution and avoid initiating resuscitation with 100% oxygen in preterm infants, and support the currently planned study comparing 60% and 30% oxygen for initial resuscitation of preterm infants ((PRESOX) (NCT01773746)).

There are several limitations to this study. Ventilating these lambs with an endotracheal tube and prophylactic surfactant helped achieve targeted saturations early and would have been different if bag and mask ventilation was initiated. The time spent in the prespecified saturation range in the titrated O₂ group was

only 47%, while 30% were above that range and the remainder of the 23% were below that range. We found it practically difficult to maintain saturations in the 5% range in the first 5 min. During the 5–30-min period, the target range broadens to 85–95% and targets were achievable. Improved time spent within the SpO₂ range may have altered the pulmonary hemodynamics in these lambs. We performed immediate cord clamping in our study and did not evaluate the effect of placental transfusion during initial ventilation.^{3,45,46} The ductal flow in this study was not measured/analyzed. None of these lambs had bradycardia and did not require chest compressions or epinephrine.^{47,48} All these experiments were conducted in a controlled setting with personnel available to titrate oxygen every 15 s, that may be different in a real-life scenario in a busy delivery room.⁴⁹ We did not study the initiation of ventilation with 65–100% oxygen, followed by decreasing FiO₂ based on SpO₂—this may be an important arm that requires further study. We continued ventilating these infants for 6 h and did not obtain tissues at the end of the 30-min period. Hence, oxidative stress markers are not available at the end of 30 min to evaluate the injury.

CONCLUSION

Titration of oxygen starting from 21% O₂ successfully achieved target saturations. Continuous use of 100% and 21% oxygen resulted in saturations above and below the target range. Use of 100% oxygen resulted in better pulmonary transition, as evidenced by the significant difference in systemic and pulmonary hemodynamics, compared to use of 21% oxygen in preterm lambs, but was similar to term controls ventilated with 21% O₂. Ventilation with 100% O₂ in preterm lambs caused hyperoxia and did not increase oxygen delivery to the brain, but reduced PVR similar to term lambs on 21% O₂. Initiation of resuscitation with low oxygen (21%) and titration based on NRP recommended target saturations, resulted in significantly lower increase in PBF, compared to term lambs and may not be adequate. In the future, studies evaluating the effect of resuscitating preterm infants with higher initial oxygen concentration (60–100%) and titrating down targeting saturations are needed. We eagerly await additional studies such as the “Study of Room Air Versus 60% Oxygen for Resuscitation of Premature Infants (PRESOX) (NCT01773746)” for more insight.

FUNDING

1R01HD072929 to S.L. and Salary support from University at Buffalo—Dr. Henry C. and Bertha H. Buswell Grant to P.C.

ADDITIONAL INFORMATION

Supplementary Information accompanies this paper at <https://doi.org/10.1038/s41390-018-0085-x>.

Competing interests: The authors declare that they have no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Perlman, J. M., et al. Neonatal Resuscitation Chapter C 2015 Part 7: Neonatal Resuscitation: 2015 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science With Treatment Recommendations. *Circulation* **132**, S204–S241 (2015).
2. Wyckoff, M. H. et al. Part 13: Neonatal Resuscitation: 2015 American Heart Association Guidelines Update for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. *Circulation* **132**, S543–S560 (2015).
3. American Academy of Pediatrics & American Heart Association. *Textbook of Neonatal Resuscitation (NRP)* 7th edn, p. 326 (2016).
4. Armanian, A. M. & Badiee, Z. Resuscitation of preterm newborns with low concentration oxygen versus high concentration oxygen. *J. Res. Pharm. Pract.* **1**, 25–29 (2012).
5. Kapadia, V. S. et al. Resuscitation of preterm neonates with limited versus high oxygen strategy. *Pediatrics* **132**, e1488–e1496 (2013).
6. Lundstrom, K. E., Pryds, O. & Greisen, G. Oxygen at birth and prolonged cerebral vasoconstriction in preterm infants. *Arch. Dis. Child Fetal Neonatal Ed.* **73**, F81–F86 (1995).
7. Rabi, Y., Singhal, N. & Nettel-Aguirre, A. Room-air versus oxygen administration for resuscitation of preterm infants: the ROAR study. *Pediatrics* **128**, e374–e381 (2011).
8. Rook, D. et al. Resuscitation of preterm infants with different inspired oxygen fractions. *J. Pediatr.* **164**, 1322–1326 e1323 (2014).
9. Vento, M. et al. Preterm resuscitation with low oxygen causes less oxidative stress, inflammation, and chronic lung disease. *Pediatrics* **124**, e439–e449 (2009).
10. Wang, C. L. et al. Resuscitation of preterm neonates by using room air or 100% oxygen. *Pediatrics* **121**, 1083–1089 (2008).
11. Oei, J. L., et al. Targeted oxygen in the resuscitation of preterm infants, a randomized clinical trial. *Pediatrics* **139** (2017).
12. Rabi, Y. et al. Outcomes of preterm infants following the introduction of room air resuscitation. *Resuscitation* **96**, 252–259 (2015).
13. Chandrasekharan, P., et al. Continuous capnography monitoring during resuscitation in a transitional large mammalian model of asphyxial cardiac arrest. *Pediatr. Res.* **81**, 898–904 (2017).
14. Chandrasekharan, P. K. et al. Continuous end-tidal carbon dioxide monitoring during resuscitation of asphyxiated term lambs. *Neonatology* **109**, 265–273 (2016).
15. Smolich, J. J., Soust, M., Berger, P. J. & Walker, A. M. Indirect relation between rises in oxygen consumption and left ventricular output at birth in lambs. *Circ. Res.* **71**, 443–450 (1992).
16. Rawat, M. et al. Neonatal resuscitation adhering to oxygen saturation guidelines in asphyxiated lambs with meconium aspiration. *Pediatr. Res.* **79**, 583–588 (2016).
17. Askie, L. M. et al. NeOProM: Neonatal Oxygenation Prospective Meta-analysis Collaboration study protocol. *BMC Pediatr.* **11**, 6 (2011).
18. Dupont, W. D. & Plummer, W. D. Jr. Power and sample size calculations. A review and computer program. *Control Clin. Trials* **11**, 116–128 (1990).
19. Lakshminrusimha, S. et al. Pulmonary hemodynamics in neonatal lambs resuscitated with 21%, 50%, and 100% oxygen. *Pediatr. Res.* **62**, 313–318 (2007).
20. Crossley, K. J. et al. Dynamic changes in the direction of blood flow through the ductus arteriosus at birth. *J. Physiol.* **587**, 4695–4704 (2009).
21. Konduri, G. G. & Kim, U. O. Advances in the diagnosis and management of persistent pulmonary hypertension of the newborn. *Pediatr. Clin. North Am.* **56**, 579–600 (2009). [Epub ahead of print, October 7, 2017].
22. Lakshminrusimha, S. & Keszler, M. Persistent pulmonary hypertension of the newborn. *Neoreviews* **16**, e680–e692 (2015).
23. Lang, J. A. et al. Increase in pulmonary blood flow at birth: role of oxygen and lung aeration. *J. Physiol.* **594**, 1389–1398 (2016).
24. Dawson, J. A. et al. Defining the reference range for oxygen saturation for infants after birth. *Pediatrics* **125**, e1340–e1347 (2010).
25. Phillippos, E. et al. Oxygen saturation and heart rate ranges in very preterm infants requiring respiratory support at birth. *J. Pediatr.* **182**, 41–46 e42 (2017).
26. Lakshminrusimha, S., Manja, V., Mathew, B. & Suresh, G. K. Oxygen targeting in preterm infants: a physiological interpretation. *J. Perinatol.* **35**, 8–15 (2015).
27. Oei, J. L., et al. Outcomes of oxygen saturation targeting during delivery room stabilisation of preterm infants. *Arch. Dis. Child Fetal Neonatal Ed.* (2017), [Epub ahead of print, October 7, 2017].
28. Oei, J. L., Wright, I. M. & Saugstad, O. D. Authors' response. *Pediatrics* **139** (2017).
29. Vento, M. et al. Resuscitation with room air instead of 100% oxygen prevents oxidative stress in moderately asphyxiated term neonates. *Pediatrics* **107**, 642–647 (2001).
30. Coalson, J. J., Winter, V. & deLemos, R. A. Decreased alveolarization in baboon survivors with bronchopulmonary dysplasia. *Am. J. Respir. Crit. Care Med* **152**, 640–646 (1995).
31. Jobe, A. H. & Bancalari, E. Bronchopulmonary dysplasia. *Am. J. Respir. Crit. Care Med* **163**, 1723–1729 (2001).
32. Warner, B. B., Stuart, L. A., Papes, R. A. & Wispe, J. R. Functional and pathological effects of prolonged hyperoxia in neonatal mice. *Am. J. Physiol.* **275**, L110–L117 (1998).
33. Lakshminrusimha, S. et al. Pulmonary hemodynamics and vascular reactivity in asphyxiated term lambs resuscitated with 21 and 100% oxygen. *J. Appl. Physiol.* (1985) **111**, 1441–1447 (2011).
34. Patel, A. et al. Exposure to supplemental oxygen downregulates antioxidant enzymes and increases pulmonary arterial contractility in premature lambs. *Neonatology* **96**, 182–192 (2009).
35. Castillo, A. et al. Pulse oxygen saturation levels and arterial oxygen tension values in newborns receiving oxygen therapy in the neonatal intensive care unit: is 85% to 93% an acceptable range? *Pediatrics* **121**, 882–889 (2008).
36. Lakshminrusimha, S. et al. Pulmonary arterial contractility in neonatal lambs increases with 100% oxygen resuscitation. *Pediatr. Res.* **59**, 137–141 (2006).
37. Polglase, G. R. et al. Respiratory support for premature neonates in the delivery room: effects on cardiovascular function and the development of brain injury. *Pediatr. Res.* **75**, 682–688 (2014).
38. Rudolph, A. M. Fetal and neonatal pulmonary circulation. *Am. Rev. Respir. Dis.* **115**, 11–18 (1977).
39. Kluckow, M. Low systemic blood flow and pathophysiology of the preterm transitional circulation. *Early Hum. Dev.* **81**, 429–437 (2005).
40. Leahy, F. A., Cates, D., MacCallum, M. & Rigatto, H. Effect of CO₂ and 100% O₂ on cerebral blood flow in preterm infants. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* **48**, 468–472 (1980).
41. Nijijima, S., Shortland, D. B., Levene, M. I. & Evans, D. H. Transient hyperoxia and cerebral blood flow velocity in infants born prematurely and at full term. *Arch. Dis. Child* **63**, 1126–1130 (1988).
42. Thing, M., Sorensen, L. C. & Pryds, O. Transient hyperoxia does not affect regional cerebral tissue oxygen saturation in moderately preterm or term newborns. *Acta Paediatr.* **104**, 657–662 (2015).
43. Saugstad, O. D. Hyperoxia and cerebral vasoconstriction in healthy newborns. *Acta Paediatr.* **104**, 645–646 (2015).
44. Gerstner, B. et al. Hyperoxia causes maturation-dependent cell death in the developing white matter. *J. Neurosci.* **28**, 1236–1245 (2008).
45. Hooper, S. B. et al. The timing of umbilical cord clamping at birth: physiological considerations. *Matern Health Neonatol. Perinatol.* **2**, 4 (2016).
46. Katheria, A. C., Truong, G., Cousins, L., Oshiro, B. & Finer, N. N. Umbilical cord milking versus delayed cord clamping in preterm infants. *Pediatrics* **136**, 61–69 (2015).
47. Vali, P., et al. Evaluation of timing and route of epinephrine in a neonatal model of asphyxial arrest. *J. Am. Heart Assoc.* **6** (2017), [Epub ahead of print, February 18, 2017].
48. Vali, P. et al. Hemodynamics and gas exchange during chest compressions in neonatal resuscitation. *PLoS ONE* **12**, e0176478 (2017).
49. Oei, J. L., Saugstad, O. D. & Vento, M. Oxygen and preterm infant resuscitation: what else do we need to know? *Curr. Opin. Pediatr.* **30**, 192–198 (2018).