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## Pulmonary hypertension and oxidative stress: Where is the link?

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### Abstract

Oxidative stress can be associated with hyperoxia and hypoxia and is characterized by an increase in reactive oxygen (ROS) and nitrogen (RNS) species generated by an underlying disease process or by supplemental oxygen that exceeds the neutralization capacity of the organ system. ROS and RNS acting as free radicals can inactivate several enzymes and vasodilators in the nitric oxide pathway promoting pulmonary vasoconstriction resulting in persistent pulmonary hypertension of the newborn (PPHN). Studies in animal models of PPHN have shown high ROS/RNS that is further increased by hyperoxic ventilation. In addition, antioxidant therapy increased PaO<sub>2</sub> in these models, but clinical trials are lacking. We recommend targeting preductal SpO<sub>2</sub> between 90 and 97%, PaO<sub>2</sub> between 55 and 80 mmHg and avoiding FiO<sub>2</sub> > 0.6–0.8 if possible during PPHN management. This review highlights the role of oxidative and nitrosative stress markers on PPHN and potential therapeutic interventions that may alleviate the consequences of increased oxidant stress during ventilation with supplemental oxygen.

### Keywords

Persistent pulmonary hypertension; Oxidative stress; Free radical; Antioxidant therapy; Nitric oxide; Superoxide anions; Peroxynitrite

## 1. Introduction

Persistent pulmonary hypertension of the newborn (PPHN) is a clinical syndrome that is characterized by failure to achieve or sustain the decline in pulmonary vascular resistance (PVR) at birth, leading to extrapulmonary right-to-left shunting of blood across the patent foramen ovale (PFO) or patent ductus arteriosus (PDA) and severe hypoxemia [1]. Mechanisms that increase PVR in PPHN include high pulmonary vascular tone, abnormal vasoreactivity, remodeled vascular wall structure, and reduced vascular growth [2].

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Declaration of competing interest

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While neonatal lung injury is often a result of a multitude of etiologic factors, oxidative stress has been recognized as a critical factor in the pathophysiology of several neonatal lung diseases associated with PPHN. Oxidative stress is also a common endpoint for multiple events, including inflammation, hypoxia, hyperoxia, and mechanical ventilation, that contribute to sustained lung injury and result in impaired pulmonary hemodynamics and gas exchange [3].

Significance of oxidative stress in various diseases in the neonatal period was first recognized in the 1980's and the term "oxygen radical disease in the newborn" was coined in 1988 [4]. Oxidative stress from various sources, combined with immature antioxidant defenses, may lead to oxidative lung injury in a vulnerable neonate [5].

In this review, we discuss the abnormal fetal-to-newborn transition at birth and the role of oxidative and nitrosative stress in the pathophysiology of PPHN. We briefly discuss the contribution of therapeutic interventions, such as increased  $\text{FiO}_2$ , inhaled nitric oxide (iNO) and mechanical ventilation, on oxidative stress. We finally identify potential therapeutic interventions that may alleviate the consequences of increased oxidant stress during supplemental oxygen ventilation and further discuss the optimal oxygen targets in PPHN.

## 2. Birth and pulmonary vascular transition

At birth the organ of gas exchange changes from the placenta to the lung resulting in major circulatory adjustments. With initial crying, alveolar oxygen tension ( $\text{PAO}_2$ ) increases from fetal levels (~18 mmHg) to postnatal levels (~80–100 mmHg) [6] (Fig. 1). Under normal circumstances, this increase in  $\text{PAO}_2$  triggers a progressive fall in PVR, accompanied by a simultaneous rise in systemic vascular resistance (SVR) once umbilical blood flow ceases. Pulmonary vasodilation and reversal of ductal shunting leads to an 8–10 fold increase in pulmonary blood flow, which is regulated by complex physiologic and biochemical processes with a central role for nitric oxide (NO) [7]. Pulmonary endothelial NO production increases markedly at the time of birth in response to oxygen and exerts its action through soluble guanylate cyclase (sGC) and cyclic guanosine monophosphate (cGMP) [8,9]. Phosphodiesterase type 5 (PDE5) impairs this vasorelaxation by degrading cGMP. In addition, endothelial nitric oxide synthase (eNOS) dysfunction, induced through increased levels of asymmetric dimethyl arginine (ADMA), a competitive endogenous inhibitor of NOS, or by decreased synthesis of the NOS substrate L-arginine results in pulmonary vasoconstriction [10–12]. These enzymes are sensitive to oxidative stress (Fig. 2).

The transition from prenatal to postnatal life causes a significant increase in arterial oxygen tension and the activation of metabolic pathways enabling the newborn's adaptation to the extra-uterine environment. Cellular redox status represents the balance between reductive and oxidant metabolites and is indispensable for cellular growth, differentiation, and preservation of biological functions [13]. Oxidative stress has been defined as the imbalance of pro-and-antioxidants in favor of the former and is the consequence of an excessive free radical production or inability of the antioxidant defense system to neutralize them [14] (Fig. 3). Oxidative stress contributes to cellular and tissue damage and/or dysfunction and

interferes with the normal postnatal decline in the PVR/SVR ratio causing the transitional circulation to persist leading to PPHN.

### 3. Oxidative stress and PPHN

ROS is a generic term for an ample variety of oxidants derived from molecular oxygen. They are part of a family of reactive species, including among others reactive nitrogen, sulfur, carbon, and selenium species that undergo reduction and oxidation reactions. Consequent to these reactions, biological macromolecules such as proteins, DNA, or lipids withstand oxidative modifications that contribute to redox signaling and biological function. However, excessive concentration of ROS reacts non-specifically with macromolecules, further generating other reactive species with potentially toxic effects [15].

The pulmonary vasculature undergoes morphological changes in PPHN that can be mediated by oxidative stress [9]. Past research has shown the expression of various oxidative stress markers changes in the lungs and pulmonary vasculature of animals and humans with PPHN.

#### 3.1. Oxidative and nitrosative stress (Figs. 2–4)

ROS are produced within different cellular substructures: plasma membrane, cytosol, peroxisomes, mitochondria, lysosomes, and endoplasmic reticulum [16]. The extracellular face of the plasma membrane is a major cellular site of the anion superoxide ( $O_2^{\cdot-}$ ) and hydrogen peroxide ( $H_2O_2$ ) generation via NADPH oxidases (NOXs) under physiological conditions [17]. Moreover, ROS are also generated from metabolic redox reactions mostly by the mitochondrial oxidative phosphorylation process in the electron transport chain, but also by the microsomal cytochrome P450 system and by the immune response [18]. Under physiologic circumstances, dioxygen undergoes a tetravalent reduction to water. However, under pro-oxidant conditions, oxygen is only partially reduced to reactive species (Fig. 4). Superoxide anion ( $O_2^{\cdot-}$ ), hydrogen peroxide ( $H_2O_2$ ), and hydroxyl radical ( $\cdot OH$ ) result from monovalent, divalent, and trivalent reduction of oxygen, respectively [19,20]. The family of superoxide dismutases (SODs) in the cytosol, mitochondria, or extracellular fluid contain transition metals such as Cu, Mn, Zn, or Fe and catalyze the dismutation of the anion superoxide into  $H_2O_2$  and  $O_2$ .  $H_2O_2$  can be directly reduced to water by peroxiredoxins (Prx), glutathione peroxidases (GPX), or catalases (CAT). Alternatively, in the presence of reduced transition metals, especially  $Fe^{2+}$ ,  $H_2O_2$  generates highly reactive hydroxyl ( $OH\cdot$ ) radicals (Fenton chemistry). Of note, no effective antioxidant defenses against hydroxyl radicals are present in biological systems [21].

Nitric oxide (NO) is generated during the breakdown of arginine to citrulline by the NADPH-dependent NO synthase (NOS).  $\cdot NO$  is a relatively stable and highly diffusible free radical.  $\cdot NO$ -mediated pathogenicity depends upon the formation of highly reactive and toxic byproducts, such as peroxynitrite anion ( $ONOO^-$ ), peroxynitrous acid ( $ONOOH$ ), and nitrogen dioxide ( $\cdot NO_2$ ). These are extremely cytotoxic compounds that oxidize membrane lipoproteins, enzymatic and structural proteins, and DNA [21]. The formation of RNS requires the interaction of  $\cdot NO$  with oxidants, such as superoxide radicals, hydrogen peroxide, and transition metals like iron or copper. Of note, the rate constant of the

reaction of the anion superoxide ( $O_2^{\cdot -}$ ) with  $\bullet NO$  is significantly higher than that of the SOD-catalyzed dismutation, and therefore,  $\bullet NO$  kinetically outcompetes SOD for the anion superoxide [22]. In addition, in the presence of carbon dioxide ( $CO_2$ ), peroxynitrite forms highly reactive nitrogen dioxide and carbonate radicals, which directly react with DNA and proteins. In addition, peroxynitrous acid in the presence of hydroxyl radicals ( $\bullet OH$ ) generated through Fenton reaction leads to the formation of nitrogen dioxide, which directly attacks lipids and proteins [23]. Under stressful conditions, an excessive production of ROS and RNS will not only cause direct damage to cellular components, but it also lead to aberrant signaling that results in dysfunction and disease [15].

$H_2O_2$  is a redox signaling agent with no free radical characteristics. Under physiologic conditions,  $H_2O_2$  is produced at a controlled steady-state level and acts as a second messenger amplifying its biological signal through kinase cascades or can be transmitted over long distances by conversion to more stable species, such as lipid peroxides or hydroxynonenal. Signal transduction by  $H_2O_2$  is mediated by a selective and efficient oxidation of specific thiols on specific signaling proteins [24]. However, mechanisms and rates of mitochondrial  $H_2O_2$  release required to exert biological effects are still unknown. Acute and intense bursts of mitochondrial  $H_2O_2$  production, such as during post-ischemia reperfusion, do not appear to be sufficient to reach the cytosol. However, protracted generation of  $H_2O_2$ , such as during persistent hyperoxemia, inflammatory processes with persistent activation of macrophages, can enhance cytosolic oxidant levels, perhaps reflecting an eventual overriding of matrix antioxidant defense systems [25].

### 3.2. Analytical biomarkers of oxidative and nitrosative stress damage

The functional and structural damage caused to cellular components because of their interaction with free radicals is highly dynamic and difficult to assess. To date, no gold standard of oxidative/nitrosative stress damage in preterm infants has been established. A comprehensive approach to the pathophysiology of free radicals would include the assessment of the redox steady state and the damage to biomolecules promoted by oxidative stress. The latter is the most widely employed approach to assess oxidative damage and includes metabolites derived from damage to proteins, DNA, RNA, lipids, and carbohydrates. The analytical methods employed have evolved in recent years and include spectroscopic, colorimetric, fluorometric, enzymatic, immunoassay and most recently hyphenated separation techniques such as liquid or gas chromatography or capillary electrophoresis coupled to tandem mass spectrometry, which are at present the most reliable and applicable to most biofluids (including whole blood, plasma, serum, urine, amniotic fluid, or cerebral spinal fluid) [5]. Among the most widely employed and reliable oxidative damage biomarkers are the byproducts of lipid peroxidation, especially the polyunsaturated fatty acids (PUFA), which are essential components of biological membranes. Free radicals acting upon PUFA generate a burst of hydroperoxides in a radical chain reaction yielding to very labile endoperoxides that subsequently decompose in an ample array of compounds, such as isoprostanes/isofurans, neuroprostanes/neurofurans and dihomio-isoprostanes/dihomio-isofurans), which are derived from arachidonic acid, docosahexanoic acid and adrenic acid, respectively. Isoprostanooids and Isofuranoids are excellent biomarkers because of their stability when compared to other frequently employed

biomarkers such as malondialdehyde. Moreover, it has also been reported that isofuranoids' generation is closely dependent upon the oxygen tension, which is of utmost interest given the relevance of oxygen as a causative agent of severe conditions in the perinatal period [26,27].

Preterm infants' DNA repair mechanisms are ineffective, and therefore, preterm infants are predisposed to long-lasting DNA structural alterations. During fetal-to-neonatal transition and thereafter, oxidative stress has been linked to oxidative alterations of DNA consisting of oxidative modifications of the guanine bases. The guanine base from the deoxyribonucleoside deoxyguanosine (2 dG) is the most susceptible to oxidative modifications yielding to the formation of 8-oxo-2'-deoxyguanosine (8-oxo-dG) also known as 8-hydroxy-2'-deoxyguanosine (8-OHdG). The quotient 8-OHdG/2 dG has been widely employed as a reliable marker of oxidative damage to DNA in preterm infants [28–30].

Free radicals induce modifications in the amino acid side chains causing an alteration of the structure and/or the function of proteins. The formation of protein carbonyl derivatives, which is produced by the direct action of free radicals or by the reaction with oxidized compounds, has been widely employed to assess oxidative stress in preterm infants [31–33]. In addition, the determination of specific byproducts of amino acid oxidation is a valid and widely employed alternative approach. Under physiologic conditions, phenylalanine is metabolized to para-tyrosine (*p*-Tyr) but through non-enzymatic hydroxylation caused by oxygen and nitrogen free radicals, phenylalanine is metabolized to meta-tyrosine (*m*-Tyr), ortho-tyrosine (*o*-Tyr), 3-chlorotyrosine (3-Cl-Tyr), 3-nitrotyrosine (3-NO<sub>2</sub>-Tyr) and 3-nitro-4-hydroxyphenylacetic acid (NHPA). *m*-Tyr and *o*-Tyr reflect the direct action of hydroxyl radicals usually generated by the Fenton reaction in the presence of transition metals upon proteins. On the other hand, 3-Cl-Tyr reflects a byproduct of the reaction of hypochlorous acid originated from myeloperoxidase with *p*-Tyr in neutrophils and monocytes. 3-NO<sub>2</sub>-Tyr is the byproduct of the reaction of *p*-Tyr with nitrogen free radicals, especially peroxyxynitrite; furthermore, NHPA is also employed as a marker of protein nitration [5]. Phenylalanine oxidation byproducts have been widely explored in urine samples of preterm infants. This non-invasive approach has allowed performance of serial determinations in different pathologic conditions [29]. Moreover, recently, a validated analytical method has been employed for the determination of protein oxidation byproducts in amniotic fluid to predict fetal outcome [34].

### 3.3. The relevance of ROS/RNS in neonatal lung disease

The importance of ROS in neonatal lung disease has been clearly demonstrated in animal models of PPHN. Studies in our laboratory utilizing the prenatally ligated ductus arteriosus lamb model have linked mitochondrial oxidative stress to maladaptive changes in NO signaling pathways and the pathogenesis of PPHN [35,36]. Furthermore, activation of phosphodiesterase-5 (PDE5), decreased cGMP-responsiveness to exogenous NO, and decreased expression of eNOS, may promote additional injury through increased vasoconstriction and impaired responsiveness to vasodilators (Fig. 2) [37].

Increased H<sub>2</sub>O<sub>2</sub> in pulmonary arteries is associated with decreased expression of soluble guanylate cyclase and reductions in cGMP levels contributing to pulmonary vasoconstriction and decreased activity of antioxidant - extracellular superoxide dismutase (ecSOD) [35,38].

Reaction of O<sub>2</sub><sup>-</sup> with NO forms ONOO<sup>-</sup> which depletes bioavailable NO and impairs NO-mediated vasorelaxation. ONOO<sup>-</sup> is a potent oxidant that causes vascular dysfunction by non-specific nitration of proteins and results in a significant alteration in their functions. Impaired regulation of free radicals potentially induces vascular injury secondary to their interaction with proteins, DNA, RNA and lipids [39].

**3.4. Cellular and biochemical changes from oxidative stress in PPHN (Fig. 2)**—Normal pulmonary vascular tone is regulated by the interaction of NO and endothelin-1 (ET-1) produced by the vascular endothelium [40, 41]. NO is an endothelium-derived relaxing factor synthesized by the oxidation of L-arginine after activation of eNOS [42]. On the other hand, ET-1 has potent vasoactive properties and is mitogenic for pulmonary vascular smooth muscle cells (SMC) [43]. The pulmonary vasoactive effects of ET-1 are mediated by ET<sub>A</sub> receptors, located on vascular SMC (vasoconstriction) and ET<sub>B</sub> receptors, located on vascular EC (vasodilation) [44].

In PPHN, abnormal regulation of the ET-1 and NO signaling cascades occur where ET-1 levels are elevated and eNOS expression is decreased. Oxidative stress enhances expression of ET-1 resulting in vasoconstriction [45]. In addition, there is an increase in the expression of the genes that induce pulmonary vasoconstriction and a reduction in those that induce vasodilation [46]. Overall ET-1-mediated vasoconstriction with reduced NO-mediated vasodilation associated with oxidative stress contributes to pulmonary hypertension.

## 4. Evidence from animal studies

### 4.1. Hyperoxic resuscitation

Ventilation of lambs with 100% oxygen at birth and in the immediate postnatal period is associated with increased contractility of pulmonary arteries [47–49]. Such increased contractility of pulmonary arteries following resuscitation of asphyxiated lambs with 100% oxygen can be reversed by treatment of the vessels with antioxidants, suggesting that ROS is probably the mediator of such increased contractility [50]. In lambs with PPHN, resuscitation with 100% oxygen impairs the subsequent relaxation response to iNO [51]. These results suggest that although resuscitation with 100% oxygen may transiently enhance pulmonary vasodilation, subsequent response to pulmonary vasodilators is impaired and pulmonary vasoreactivity is enhanced, further contributing to PPHN.

### 4.2. Antenatal ductal ligation lamb model of PPHN

Our laboratory has shown that in fetal lambs, ligation or compression of the ductus arteriosus rapidly induces fetal and neonatal pulmonary hypertension. Similar to autopsy findings in newborns with PPHN [52], these lambs mimic the physiopathologic characteristics including medial hypertrophy within the small pulmonary arteries, complete muscularization of pulmonary arteries, and extension of muscle to non-muscularized



arteries leading to persistently increased PVR, (pulmonary artery pressure (PAP), and right ventricular (RV) hypertrophy.

Evidence from animal models of PPHN supports the theory that oxidative stress plays an important role in the pathogenesis of PPHN. Lambs with PPHN demonstrate both antenatal and postnatal evidence of increased oxidative stress compared to control animals, including increased levels of  $O_2^-$  and  $H_2O_2$  in the pulmonary vasculature and exaggerated ROS production in response to hyperoxia after birth [38,53, 54]. In addition, increased oxidative stress in PPHN lambs is linked to negative effects on NO signaling pathways and aberrant pulmonary vasodilatory responses, which again supports the idea that ROS play an important role in the pathogenesis of PPHN [55]. These lambs exhibit alterations in major components of NO-mediated pulmonary vasodilation, including decreased NOS expression and activity, decreased expression of soluble guanylate cyclase, increased PDE5 expression and activity and increased ET-1 levels [46,56,57]. These alterations may be secondary to increased pulmonary artery  $H_2O_2$ , which decreases eNOS expression and impairs cGMP production [54,58]. Ventilation with 100% oxygen and iNO for 24 h resulted in significantly increased levels of peroxynitrite (3-NT) in pulmonary vasculature, and this signal was quenched by treatment with recombinant human superoxide dismutase (rhSOD) or by ventilation with lower concentrations of oxygen [9].

#### 4.3. Ovine model of aortopulmonary shunt

Pulmonary hypertension can be created in lambs by antenatal placement of an aortopulmonary shunt to increase blood flow resulting in mechanical shear stress [59], oxidative stress, and vascular remodeling [60]. In this model, the predominant cause of superoxide anions appears to be NADPH oxidase [60].

#### 4.4. Rodent model of PPHN

A study by Xu et al. found that increased oxidative stress contributes to pulmonary hypertension development in rodents [61]. In normal conditions, extracellular SOD is expressed in high concentrations in the lungs and is responsible for removing extracellular superoxide anions. This study showed that absence of SOD resulted in significantly worse pulmonary hypertension in hypoxic SOD knockout mice and MCT rats with a SOD loss-of-function gene mutation.

In another study in rats, Wang et al. examined the effects of  $17\beta$ -estradiol and 2-methoxyestradiol on the oxidative stress-hypoxia inducible factor-1 (OS-HIF-1) pathway with hypoxia-induced pulmonary hypertension [62]. Hypoxic rats had a significant increase in oxidative stress levels as indicated by increased serum ROS levels, decreased serum SOD, and decreased manganese SOD (MnSOD) levels. Furthermore, MnSOD mRNA and protein levels were decreased in the lung tissue.

In a separate study, rats treated with a high dose of iron dextran demonstrated increased vasoconstriction and vascular hyper-reactivity of pulmonary arteries and reduced NO, which were reversed by antioxidant therapy [63].

#### 4.5. In vitro studies

Pulmonary artery smooth muscle cells (PASMC) isolated from the ductal ligation PPHN lamb model had elevated cytosolic ROS, increased Nox4 expression (NADPH oxidase isoform), and decreased extracellular superoxide dismutase (ecSOD) activity relative to control PASMC. Nox4 RNA knockdown attenuated cytosolic ROS levels and elevated ecSOD activity in these cells [64].

In addition, PASMCs from this PPHN lamb model had increased basal PDE5 activity, decreased cGMP-responsiveness to NO, and increased mitochondrial matrix oxidant stress compared to control PASMC. Mitochondrially targeted catalase decreased PDE5 activity at baseline and after hyperoxia in PPHN PASMC. Similarly, catalase treatment of PPHN lambs ventilated with 100% O<sub>2</sub> decreased PDE5 activity and increased cGMP in the PA [36]. We speculate that PPHN-induced mitochondrial superoxide is converted to H<sub>2</sub>O<sub>2</sub> by MnSOD and crosses mitochondrial membranes thereby contributing to elevated cytosolic ROS.

Rao et al. have shown that ROS generated from using xanthine/xanthine oxidase stimulates vascular smooth muscle DNA synthesis and increases cell number, suggesting that altered ROS generation and scavenging also contribute to pulmonary vascular remodeling in PPHN [65]. Increased vascular remodeling results from disturbed development and enhanced proliferation of PASMC, leading to hyperplasia and hypertrophy of the vascular smooth muscle layer, narrowing vascular lumen, and increasing PVR.

### 5. Antioxidant defense and antioxidant therapy

The antioxidant defense system in newborns, especially premature infants, is underdeveloped [5]. At term, the antioxidant system starts maturing to help transition from the relatively hypoxic fetal environment to the high oxygen tension extrauterine environment after birth [66].

PPHN and hyperoxia elevate pulmonary ROS via distinct mechanisms, suggesting that ROS scavengers may be effective in limiting the oxidant stress in PPHN infants ventilated with oxygen. In the past, the attempts to therapeutically target these antioxidant systems have yielded inconsistent results [67]. Antioxidant therapy developments have been limited by biochemical properties like compound half-life, poor cell penetrance, and difficulty targeting intracellular organelles [68].

#### 5.1. Therapeutic interventions against oxidative stress in PPHN

Oxygen free radicals act on the NO pathway reducing cGMP and promoting pulmonary vasoconstriction. Use of antioxidant therapy may improve systemic oxygenation. Antioxidants regulate vascular signaling pathways by scavenging free radicals. Enzyme systems donate an electron to molecular oxygen to generate superoxide anion (O<sub>2</sub><sup>-</sup>), and SODs generate the non-radical ROS H<sub>2</sub>O<sub>2</sub> through dismutation of superoxide. H<sub>2</sub>O<sub>2</sub> is diffusible across membranes and is scavenged by enzymes including catalase and glutathione peroxidase.



Previously, our laboratory has shown that intratracheal administration of antioxidants, such as the NADPH oxidase inhibitor apocynin and recombinant human SOD (rhSOD), decreases ROS, increases eNOS expression, and normalizes tetrahydrobiopterin levels after ventilation with 100% O<sub>2</sub> for 24 h in neonatal lamb models of PPHN [69,70]. Intratracheal recombinant human SOD (rhSOD) also reduces ONOO<sup>-</sup> mediated protein nitration, decreases PDE5 activity, and increases cGMP in the pulmonary arteries of ventilated PPHN lambs [71,72]. Recombinant human SOD mitigated the increased PA contractility and lung isoprostanes levels and decreased the enhanced lung 3-nitrotyrosine fluorescence observed with iNO therapy [71]. Intratracheal catalase has shown to improve oxygenation by improving NO-mediated vasodilation, increasing lung ecSOD activity, and decreasing PA superoxide levels and PA PDE5 activity in the ductal ligation model of PPHN [36].

Glucocorticoids are commonly used in the management of PPHN. In studies on the PPHN lamb model, antenatal betamethasone and post-natal hydrocortisone are associated with improved oxygenation by increasing superoxide dismutase activity and reducing oxidant stress [73,74].

Clinically, antioxidant therapies have not been thoroughly evaluated. We speculate that the antioxidant therapies will need to be precisely targeted at a cellular and subcellular level to be most effective in the treatment of ROS-induced neonatal pulmonary hypertension. Clinical trials of antioxidant therapies in iNO-resistant PPHN are warranted.

## 6. Oxygen therapy, optimal oxygenation, and oxidative stress markers in PPHN

The hallmark of PPHN is labile hypoxemia, and providing adequate oxygenation forms the mainstay of PPHN therapy. Oxygen is a double-edged sword, as hypoxia (<45 mm Hg) increases PVR by hypoxic pulmonary vasoconstriction and contributes to the pathophysiology of PPHN (Fig. 5). On the other hand, hyperoxia (>80–100 mmHg) does not further decrease PVR and instead results in free radical injury [75]. Among term infants with PPHN, avoiding hypoxia and hyperoxia by titrating supplemental oxygen to maintain saturations in the low to mid 90%<sub>s</sub> (with alarm limits at 90 and 97%) seems to be a reasonable approach [76]. Kapadia et al. have demonstrated that the incidence of hypoxic-ischemic encephalopathy (HIE) increases if neonates with perinatal asphyxia had PaO<sub>2</sub> > 100 mmHg in the first postnatal hour [77]. In the ovine ductal ligation model of PPHN, maintaining oxygen saturation in the 90–97% range results in low PVR [51]. In the same model, as mentioned previously, resuscitation with 100% O<sub>2</sub> does not enhance pulmonary vasodilation compared to 21% and 50% O<sub>2</sub> but impairs the subsequent response to iNO [51]. We speculate that even brief exposure to 100% oxygen at birth increases free radical generation that can impair effectiveness of iNO. Similar increases in oxidative stress have been observed in term human neonates after resuscitation with 100% oxygen [78].

In a lamb model of perinatal asphyxia with meconium aspiration syndrome (MAS) and PPHN, initiation of resuscitation with 21% oxygen followed by titration to achieve the target SpO<sub>2</sub> recommended by the Neonatal Resuscitation Program resulted in higher pulmonary blood flow compared to 21% oxygen ventilation without titration [79]. In the same model

of MAS and PPHN, pulmonary blood flow and O<sub>2</sub> delivery to the brain were higher with SpO<sub>2</sub> targeted in the 95–99% range. However, the 90–94% target range is associated with significantly lower FiO<sub>2</sub> and lower lung oxidative stress [80]. Based on these results, we recommend maintaining preductal oxygen saturation in the low to mid-90% during management of PPHN, with preductal PaO<sub>2</sub> levels between 55 and 80 mmHg.

## 7. Future directions

The role of oxidative stress in PPHN is complex. Though the supplemental oxygen is life-saving therapy for infants with PPHN, it increases oxidant stress through production of free radicals. Further high-quality evidence generated from randomized trials is required to guide oxygen therapy and target levels. Antioxidant therapies have been successfully studied in transitional animal models, but there are limited and inconsistent data in clinical practice. There is an urgent need to conduct randomized clinical studies targeting the oxidative stress signaling systems in infants with PPHN.

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## Abbreviations

<b>ADMA</b>	asymmetric dimethyl arginine
<b>PPHN</b>	Persistent pulmonary hypertension of the newborn
<b>PVR</b>	Pulmonary vascular resistance
<b>NO</b>	Nitric Oxide
<b>cGMP</b>	Cyclic guanosine monophosphate
<b>PDE5</b>	Phosphodiesterase type 5
<b>eNOS</b>	Endothelial nitric oxide synthase
<b>ROS</b>	Reactive oxygen species
<b>NOX</b>	NADPH oxidase isoforms
<b>O<sub>2</sub><sup>•-</sup></b>	Superoxide anion
<b>H<sub>2</sub>O<sub>2</sub></b>	Hydrogen peroxide
<b>OH</b>	Hydroxyl radical
<b>OONO<sup>-</sup></b>	Peroxynitrite
<b>SOD</b>	Superoxide dismutase
<b>PASMC</b>	Pulmonary artery smooth muscle cells

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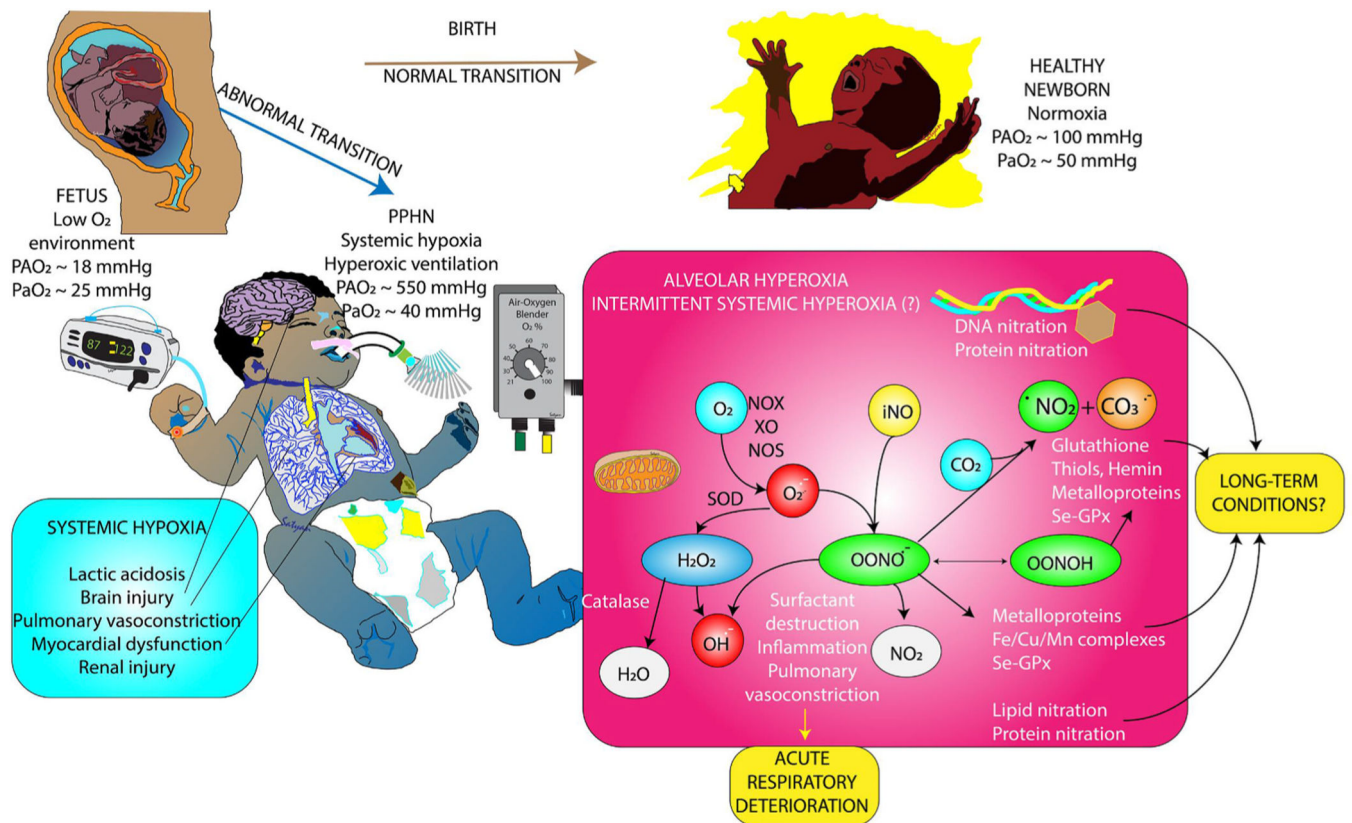
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**Practice Points.**

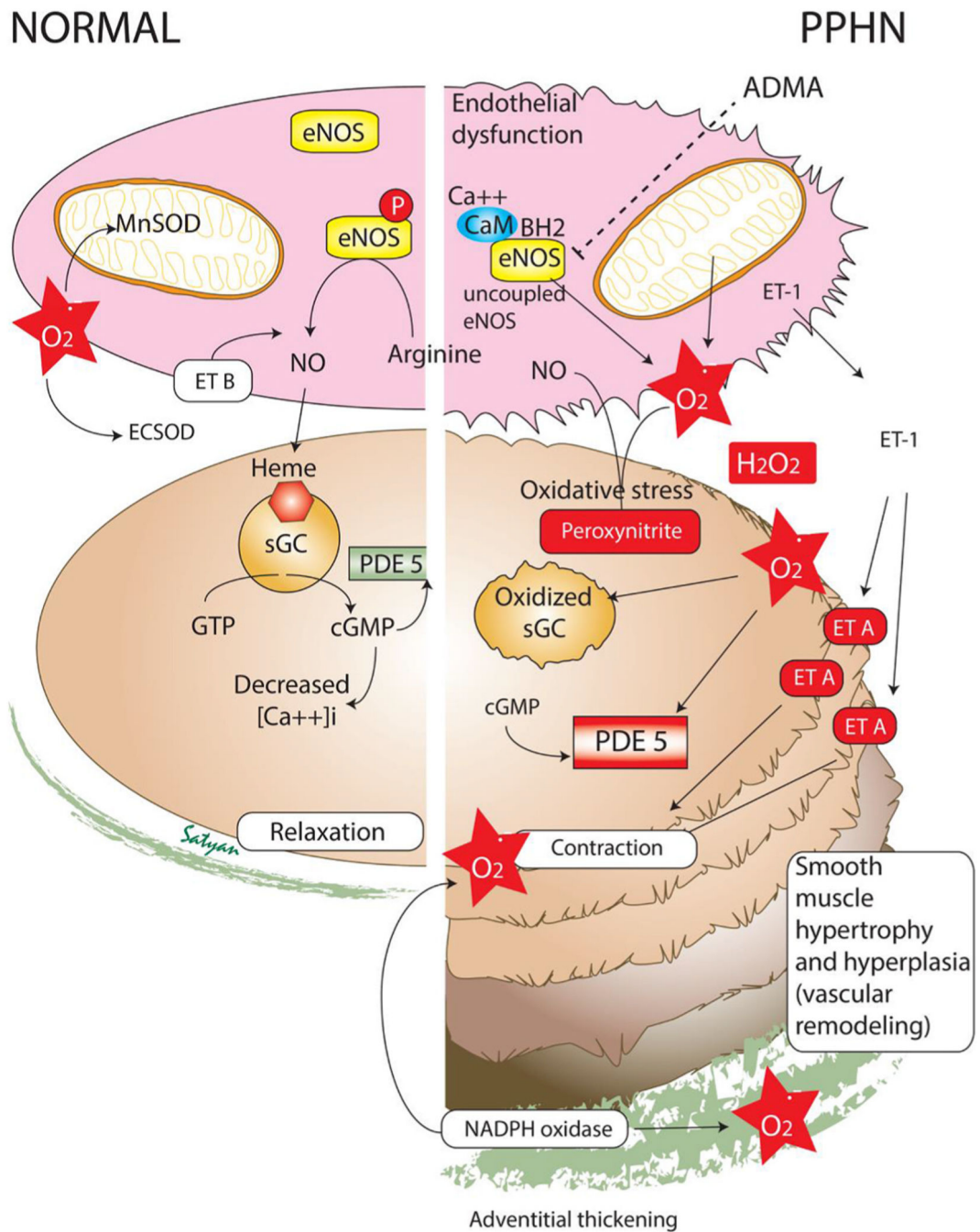
- Oxidative stress is defined as the imbalance of pro-and-antioxidants in favor of the former, and can be associated with hyperoxia and hypoxia.
- Oxygen-derived free radicals compete with NOS for nitric oxide thus promoting pulmonary vasoconstriction and contributing to persistent pulmonary hypertension of the newborn
- To date no gold standard test of oxidative/nitrosative stress damage in preterm infants has been established; However, isoprostanoids and isofuranoids are highly reputed biomarkers of oxidative stress due to their stability and correlation with oxygenation levels.
- A reliable marker of oxidative damage to DNA in preterm infants is ratio of 8-hydroxy-2'-deoxyguanosine/deoxyribonucleoside deoxyguanosine (8OHdG/2 dG).
- Based on animal studies, we recommend a target preductal SpO<sub>2</sub> between 90 and 97%, PaO<sub>2</sub> between 55 and 80 mmHg and avoid FiO<sub>2</sub> > 0.6–0.8 (if possible) during PPHN management.

**Research gaps.**

- Clinical trials using antioxidants in PPHN
- Randomized trials evaluating optimal oxygen targets in PPHN



**Fig. 1.** Normal and abnormal transition at birth and role of oxidative stress in persistent pulmonary hypertension of the newborn (PPHN). The fetus lives in a state of hypoxemia with low arterial ( $PaO_2$ ) and alveolar oxygen ( $PAO_2$ ) tension. Normal transition at birth results in room air ventilation and a modest increase in  $PaO_2$  and  $PAO_2$  leading to pulmonary vasodilation and establishment of lungs as the site of gas exchange. When transition is abnormal, pulmonary vasodilation does not occur resulting in persistence of high pulmonary arterial pressures and right-to-left extrapulmonary shunts leading to systemic hypoxemia despite alveolar hyperoxia. Alveolar hyperoxia leads to oxidative stress and release of reactive oxygen species. See text for details. Copyright Satyan Lakshminrusimha.



**Fig. 2.** Alterations in biochemical pathways in pulmonary vascular endothelial and smooth muscle cells in normal and persistent pulmonary hypertension of the newborn (PPHN). Endothelial dysfunction, smooth muscle hyperplasia and hypertrophy and adventitial thickening are common in PPHN. Increased oxidative stress with high levels of superoxide anions ( $O_2^-$ ) alters several enzymes in the nitric oxide (NO) pathway increasing the risk of vasoconstriction. ADMA - asymmetric dimethyl arginine; eNOS – endothelial nitric oxide synthase; ET – endothelin; SOD – superoxide dismutase; MnSOD – Manganese,

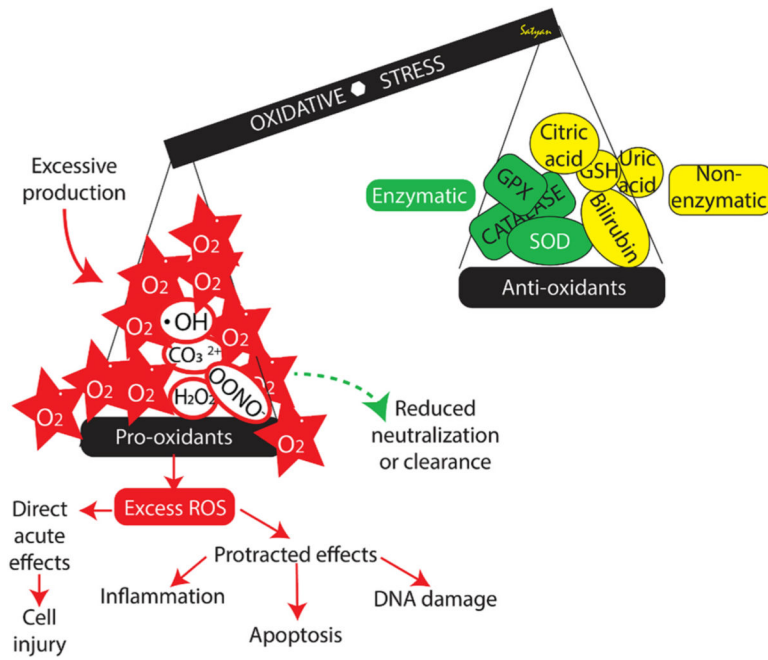
mitochondrial superoxide dismutase; EC-SOD – extracellular superoxide dismutase; sGC – soluble guanylate cyclase; PDE 5 – phosphodiesterase 5; GTP – guanosine triphosphate; cGMP – cyclic guanosine monophosphate; NO – nitric oxide; CaM – calmodulin; Modified from Polin and Fox Fetal and Neonatal Physiology, 6th edition, copyright Satyan Lakshminrusimha (with permission).

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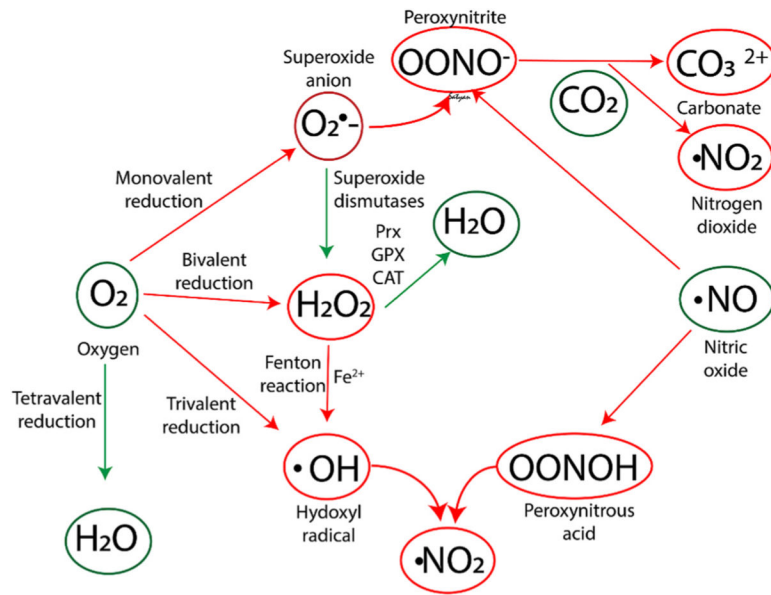
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**Fig. 3.** Definition of oxidative stress and the imbalance between pro-oxidants and antioxidants. Excess reactive oxygen species (ROS) can have acute and chronic effects. Please see text for details. Copyright Satyan Lakshminrusimha.





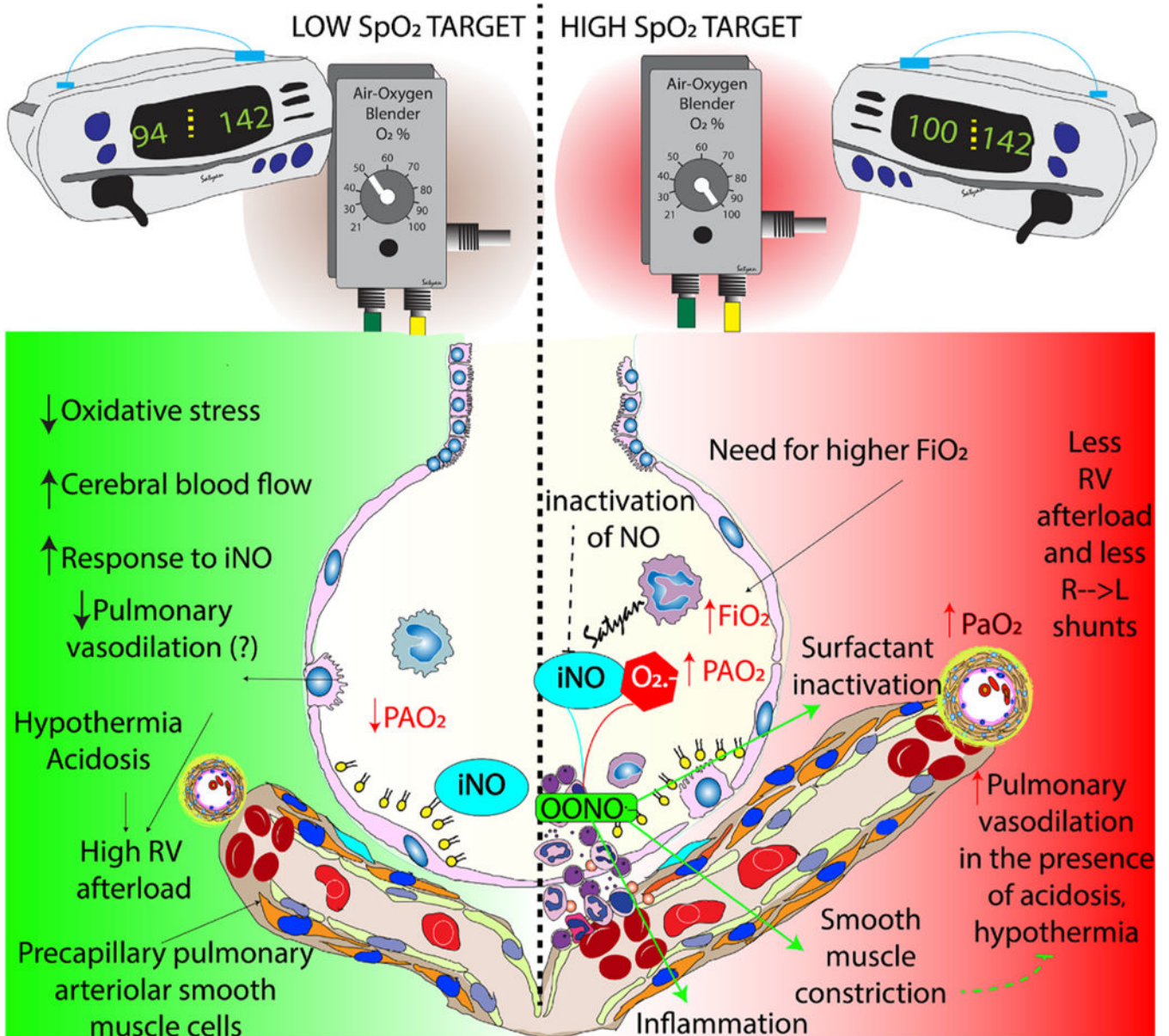
**Fig. 4.** Biochemical reactions involved in oxidative and nitrosative stress. Prx - peroxiredoxins, GPX - glutathione peroxidases (GPX) and CAT - catalases. Please see text for details. Copyright Satyan Lakshminrusimha.

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**Fig. 5.** Benefits and risks of low and high oxygen saturation by pulse oximeter (SpO<sub>2</sub>) targets. Productal SpO<sub>2</sub> in the mid-90s (90–97% - green zone) results in lower oxidative stress, increased cerebral blood flow and better response to inhaled nitric oxide (iNO). However, in the presence of hypothermia and acidosis, low SpO<sub>2</sub> target may not be adequate to promote optimal pulmonary vasodilation resulting in high right ventricular afterload. High SpO<sub>2</sub> targets (~100%) require higher inspired (FiO<sub>2</sub>) and alveolar (PAO<sub>2</sub>) increasing the risk of oxidative stress. Such targets may transiently improve pulmonary vasodilation especially in the presence of hypothermia or acidosis but there is a higher risk of reactive oxygen species formation leading to inactivation of inhaled nitric oxide (iNO), surfactant inactivation and inflammation. Copyright Satyan Lakshminrusimha.