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Optimal oxygenation and role of free radicals in PPHN

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Abstract

Effective ventilation of the lungs is essential in mediating pulmonary vasodilation at birth to allow effective gas exchange and an increase in systemic oxygenation. Unsuccessful transition prevents the increase in pulmonary blood flow after birth resulting in hypoxemia and persistent pulmonary hypertension of the newborn (PPHN). Management of neonates with PPHN includes ventilation of the lungs with supplemental oxygen to correct hypoxemia. Optimal oxygenation should meet oxygen demand to the tissues and avoid hypoxic pulmonary vasoconstriction (HPV) while preventing oxidative stress. The optimal target for oxygenation in PPHN is not known. Animal models have demonstrated that PaO_2<45mmHg exacerbates HPV. However, there are no practical methods of assessing oxygen levels associated with oxidant stress. Oxidant stress can be due to free radical generation from underlying lung disease or from free radicals generated by supplemental oxygen. Free radicals act on the nitric oxide pathway reducing cGMP and promoting pulmonary vasoconstriction. Antioxidant therapy improves systemic oxygenation in an animal model of PPHN but there are no clinical trials to support such therapy. Targeting preductal SpO_2 between 90-97% and PaO_2 at 50-80 mmHg appears prudent in PPHN but clinical trials to support this practice are lacking. Preterm infants with PPHN present unique challenges due to lack of antioxidant defenses and functional and structural immaturity of the lungs. This review highlights the need for additional studies to mitigate the impact of oxidative stress in the lung and pulmonary vasculature in PPHN.

Preface: appropriate clinical application of oxygen for the infant with PPHN

Introduction

A 39-week gestation infant with meconium aspiration syndrome (MAS), hypoxic-ischemic encephalopathy (HIE) and cyanosis is transferred to the regional perinatal center. During transport, the baby's oxygen saturations (SpO_2) were labile and fluctuating between 78 to 94%. Hence, inspired oxygen was increased to 100%. An umbilical arterial blood gas obtained soon after arrival to the neonatal intensive care unit (NICU) demonstrated a pH of

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7.12, $PaCO_2$ of 64 mmHg, PO_2 of 42 mmHg with a base deficit of 8 mEq/L. The infant was placed on high frequency oscillatory ventilation (HFOV) and treated with surfactant and inhaled nitric oxide (iNO). Umbilical arterial PaO_2 increased to 180 mmHg with SpO_2 of 99% with these measures.

The clinical conundrum described in this vignette is commonly encountered in the NICU. Perinatal distress and HIE increase oxidative stress. [1] Hyperoxic ventilation increases oxygen toxicity to the lung. Despite hyperoxic ventilation, systemic hypoxemia results in inadequate oxygen delivery to systemic tissues. Difficulties in titrating inspired oxygen result in intermittent systemic hyperoxia and hypoxemia exacerbating oxidative stress. The clinician faces dilemma in an attempt to achieve the goals of supplemental oxygen therapy: (a) reduce pulmonary vascular resistance (PVR), (b) increases oxygen delivery to the tissues and (c) minimize formation of free radicals.

This review discusses the use of supplemental oxygen to overcome the abnormal fetal to newborn transition at birth evident in PPHN, methods to determine oxygen saturation and optimal oxygenation, and sources and targets of reactive oxygen species (ROS) generation in experimental PPHN and due to oxygen exposure. We then discuss oxidant stress in pulmonary hypertension (PH) associated with prematurity, and identify potential therapeutic targets that may improve the efficacy of antioxidants to alleviate the consequences of increased oxidant stress during supplemental oxygen ventilation for term and preterm infants with PPHN.

The fetal to newborn transition.

At birth, the lung adapts to replace the placenta as the organ of gas exchange. This is facilitated by a dramatic decrease in pulmonary vascular resistance, regulated by complex physiological and biochemical processes with a central role for NO, resulting in an 8-10 fold increase in pulmonary blood flow [2]. eNOS converts L-arginine and molecular oxygen to L-citrulline and the vasodilator NO using O_2 as well as electrons from NADPH. NO stimulates vasorelaxation by activating soluble guanylate cyclase (sGC) to generate cGMP, while phosphodiesterase type 5 (PDE5) impairs vasorelaxation by degrading cGMP (figure 1). The activity of eNOS is regulated by protein phosphorylation as well as by the availability of substrate and several cofactors including calcium-calmodulin, HSP90, and tetrahydrobiopterin (BH_4). Mechanisms that inhibit eNOS activity or attenuate downstream NO signaling can induce vasoconstriction.

Abnormal vascular transition at birth:

In conditions such as birth asphyxia, [1] MAS, [3] [4] intrauterine stress, [5] maldevelopment leading to alveolar and vascular hypoplasia (e.g., congenital diaphragmatic hernia – CDH) [6] [7] and maternal ingestion of non-steroidal anti-inflammatory drugs (NSAIDs) [8] during late pregnancy (by constriction of ductus arteriosus), the increase in pulmonary blood flow soon after birth may not occur. [9, 10] In these conditions, the high pulmonary vascular resistance (PVR) characteristic of the fetal lung persists into the neonatal period leading to PPHN. PPHN is characterized by extrapulmonary right-to-left

shunting resulting in labile hypoxemia. [9] Management of neonates with PPHN includes ventilation of the lungs with supplemental oxygen to correct hypoxemia and use of pulmonary vasodilators such as inhaled nitric oxide (iNO).

Delivery room management of term infants at risk for PPHN:

The diagnosis of PPHN is often made in the postnatal period. However, infants with an antenatal diagnosis of CDH, presence of meconium stained amniotic fluid and perinatal asphyxia can be identified at birth to be at risk for PPHN. [11] Current guidelines recommend initiation of resuscitation for all term newborn with 21% oxygen and titrating inspired oxygen based on preductal SpO_2. [12] The Canadian guidelines for management of CDH recommend supplemental oxygen to achieve $SpO_2 \geq 85\%$. [13] The European guidelines recommend initiation of ventilation with $FiO_2 < 1.0$ and titrated to achieve preductal SpO_2 between 80 and 95%. [14] Investigators at Children's Hospital of Philadelphia have demonstrated that reducing initial FiO_2 from 1.0 to 0.5 did not result in an untoward events. [15]

Stuides in lambs without PPHN have shown that use of 100% oxygen for initial ventilation results in a greater decrease in PVR compared to 21% oxygen but impairs subsequent vasodilator response to iNO and systemic acetylcholine. [16] Lambs with PPHN induced by antenatal ductal ligation showed similar degree of decrease in PVR with ventilation with 21%, 50% and 100% oxygen. [17] After the first 30 minutes, all the three groups of lambs were ventilated with 50% oxygen and treated with iNO. Prior exposure to 100% oxygen impaired vasodilator response to iNO. [17] An increase in pulmonary arterial superoxide anions is observed in asphyxiated lambs after 30 min of ventilation with 100% oxygen. [18] Resuscitation with 100% oxygen was associated with increased pulmonary arterial contractility to norepinephrine, a phenomenon that was reversed with prior treatment with superoxide dismutase. [18] We speculate that even brief exposure to 100% oxygen at birth increases free radical generation that can impair effectiveness of iNO. Similar increase in oxidative stress has been observed in human term neonates after resuscitation with 100% oxygen. [19] In an ovine model of MAS with PPHN, initiation of resuscitation with 21% oxygen followed by titration to achieve target SpO_2 recommended by the Neonatal Resuscitation Program [12] resulted in higher pulmonary blood flow compared to 21% oxygen ventilation only. [20] These results suggest that initial resuscitation of term infants at risk for PPHN with 21-50% oxygen with titration to achieve target SpO_2 recommended by Neonatal Resuscitation Program is probably a safe practice and may potentially enhance subsequent response to pulmonary vasodilators such as iNO.

Assessment of oxygenation in PPHN:

Site of measurement of oxygenation status:

Traditional management of PPHN assessed oxygenation by measurement of postductal PaO_2 using umbilical arterial catheters and calculation of oxygenation index (OI) using the formula – OI = Mean airway pressure (cm H_2O) × FiO_2 × 100 + Postductal PaO_2. [21, 22] The primary determinant of PVR is the oxygen level in the precapillary pulmonary arteriole [23] which is influenced by alveolar oxygen (PAO_2). [24] Clinically systemic arterial

oxygenation is used as a surrogate of alveolar oxygenation and in parenchymal lung disease with ventilation perfusion mismatch and high alveolar-arterial oxygen gradients (A-a DO_2), systemic PaO_2 levels will not accurately reflect alveolar or pre-pulmonary capillary arteriolar oxygen levels (figure 2). In patients with right-to-left ductal shunting, assessment of preductal oxygenation using a right radial arterial line or right upper limb pulse oximetry might be a more accurate strategy in the management of PPHN. [25]

Targets for preductal PaO_2 in the management of PPHN in term infants:

The optimal lower limit for PaO_2 in the management of PPHN should meet oxygen demand to the tissues and avoid hypoxic pulmonary vasoconstriction (figure 3). In healthy newborn calves, the limit of PaO_2 below which hypoxic pulmonary vasoconstriction occurs appears to be 45 mmHg. [26] Similar values were observed in our laboratory newborn lambs with PPHN induced by meconium aspiration and control lambs without lung disease. [27] However, the upper limit of optimal oxygenation is not clear and is probably related to toxicity and oxidative stress. While measures of oxidative stress are measured in experimental settings, we do not have a practical method of assessing oxygen toxicity in the neonatal intensive care unit. Increasing PaO_2 above 100 mmHg does not result in enhanced pulmonary vasodilation. [17] Hypoxemia (<45 mmHg) causes pulmonary vasoconstriction and normoxia (50-80 mmHg) results in pulmonary vasodilation but, hyperoxia (PaO_2 > 100 mmHg) does not result in additional pulmonary vasodilation but can lead to oxidative stress. [28] Kapadia et al have demonstrated that the incidence of HIE increases if neonates with perinatal asphyxia had PaO_2 > 100 mmHg in the first hour of postnatal period. [29] Exposure to prolonged hyperoxic ventilation and high oxygenation indices at the time of randomization to iNO in clinical trials is associated with a higher incidence of ECMO/death. [30] [31] In lambs with PPHN induced by antenatal ligation of ductus arteriosus, exposure to 100% oxygen at birth impairs vasodilator response to iNO. [17] These associations with hyperoxic ventilation, high OI and impaired response to iNO can partly be explained by generation of ROS in the pulmonary vasculature (figure 1). Based on these results, we recommend targeting preductal PaO_2 between 50 and 80 mmHg during the management of PPHN in term infants.

Targets for oxygen saturation by pulse oximetry (SpO_2) in PPHN:

Non-invasive and continuous assessment of oxygenation is typically done using pulse oximeters. There are very few published guidelines for optimal SpO_2 targets in term infants with PPHN. The Canadian [13] and European [14] guidelines for the management of PPHN associated with congenital diaphragmatic hernia (CDH) recommend a preductal SpO_2 targets between 85 to 95% and 80 to 95% respectively. Postductal SpO_2 targets > 70% are considered adequate during management of CDH patients in the absence of lactic acidosis. The American Thoracic Society (ATS) guidelines for Pediatric Pulmonary Hypertension recommend SpO_2 targets between 92-95% for the management of pulmonary hypertension associated with bronchopulmonary dysplasia (BPD). [32] In lambs with PPHN induced by antenatal ductal ligation, preductal SpO_2 targets of 90-97% are associated with low PVR. [17] Based on these published guidelines and preclinical data, we recommend preductal SpO_2 target of 90 to 97% and postductal target of >70% (in the absence of lactic acidosis) during the management of acute phase of PPHN in term infants (figure 3).

Oxygen-hemoglobin dissociation curve – relationship between SpO_2 and PaO_2: Several factors such as type of hemoglobin, pH and body temperature can shift the oxygen-hemoglobin dissociation curve. [33] It is not uncommon to observe a preterm neonate admitted from the delivery room have a preductal SpO_2 of 90% with an umbilical arterial PaO_2 in the high 30s and low 40s (mmHg). Preductal SpO_2 is an important determinant of oxygen content of arterial blood (CaO_2) and oxygen delivery to essential organs such as brain and heart. [25, 27] However, it may be prudent to periodically check an arterial blood gas to assess PaO_2 (see paragraph below).

Asphyxia, therapeutic hypothermia and PPHN: Birth asphyxia is a common cause of oxidative stress [34] and is often associated with PPHN. [1] Therapeutic hypothermia either by selective head cooling or whole body cooling is standard of care in the management of moderate to severe hypoxic-ischemic encephalopathy (HIE) in term infants. PPHN is associated with approximately a fourth of patients undergoing whole body hypothermia for moderate to severe HIE. [3] Due to a shift in oxygen-hemoglobin dissociation curve to the left during hypothermia, higher preductal SpO_2 targets of 95-98% may be necessary to achieve PaO_2 of 50 to 80 mmHg during whole body hypothermia. [35] Providing supplemental oxygen right from birth is unlikely to benefit these patients at risk for HIE and PPHN. Data from asphyxiated lambs demonstrates that hyperoxic ventilation at birth increases PaO_2 but the decrease in PVR by 30 minutes is similar to that achieved with normoxic ventilation. [28] In addition, following perinatal asphyxia, infants with hyperoxemia on their first blood gas (PaO_2>100 mmHg) were more likely to develop moderate to severe HIE compared to infants with normoxemia. [29] The same study demonstrated that among infants with moderate to severe HIE, hyperoxemia on admission was more likely to be associated with abnormal brain MRI findings.

Free radicals in PPHN: Free radicals are important vascular signaling molecules that regulate pulmonary vascular tone and function. Multiple enzymatic oxidase systems contribute to the production of free radicals in the vessel wall, and each system has specific roles in vascular physiology (figure 1). In the endothelium, mitochondria, xanthine oxidase, and NADPH oxidases (Nox) generate free radicals, while an uncoupling of endothelial nitric oxide synthase (eNOS) can also contribute [36, 37]. In vascular smooth muscle, Nox isoforms and mitochondria are significant sources of free radicals [38-42]. Antioxidants regulate vascular signaling pathways by scavenging free radicals. Enzyme systems donate an electron to molecular oxygen to generate superoxide anion ($O_2^{\cdot -}$), and superoxide dismutases (SODs) generate the non-radical reactive oxygen species (ROS) H_2O_2 through dismutation of superoxide. H_2O_2 is diffusible across membranes via aquaporins and is thus an important intracellular and intercellular signaling molecule. H_2O_2 is scavenged by enzymes including catalase and glutathione peroxidase. Superoxide can also react with other radicals such as nitric oxide ($^{\cdot}NO$) to form peroxynitrite ($ONOO^{\cdot -}$), thus depleting bioavailable NO and impairing NO-mediated vasorelaxation. ONOO is an extremely reactive oxidant that can promote further vascular dysfunction by non-specific nitration of proteins resulting in a significant alteration in their functions. Impaired regulation of free radicals potentially induces vascular injury due to their interaction with proteins, DNA, RNA and lipids [43, 44].

Experimental PPHN and ROS.—In fetal lambs, ligation or compression of the ductus arteriosus rapidly induces fetal and neonatal pulmonary hypertension. Similar to newborns that die of PPHN, these lambs have medial hypertrophy within the small pulmonary arteries, complete muscularization of normally partially muscularized pulmonary arteries, and extension of muscle to non-muscularized arteries. The endothelium and vascular smooth muscle of PPHN pulmonary arteries display elevated levels of superoxide [45, 46] and H_2O_2 [47, 48]. NADPH oxidase (Nox) enzymes are membrane proteins that transfer electrons from NADPH to molecular oxygen, producing ROS intracellularly or extracellularly depending on the isoform and subcellular location of the enzyme. The Nox2 isoform is expressed in cells comprising the vascular wall and increased Nox2 subunit expression correlates with increased superoxide levels and impaired pulmonary vasorelaxation in both lamb and piglet models of neonatal pulmonary hypertension [45, 49]. Furthermore, extracellular SOD (ecSOD or SOD3) activity is decreased in PPHN pulmonary arteries [50] exacerbating the effects of Nox2-derived superoxide. The Nox4 isoform has been shown to generate both superoxide and H_2O_2 depending upon the stimulus and cell type [51], and increased Nox4 expression correlates with increased H_2O_2 in PPHN pulmonary arteries [48].

As shown in figure 4, PPHN lambs exhibit alterations in major components of NO-mediated pulmonary vasodilation including decreased NOS expression and activity [52], decreased expression of soluble guanylate cyclase [53], increased PDE5 expression and activity [54], and increased ET-1 levels [55]. These alterations may be due, in part, to increased pulmonary artery H_2O_2, which potentially contributes to decreased eNOS expression [56], impaired cGMP production [57], elevated PDE5 activity [57] and decreased ecSOD activity [50]. Peroxynitrite formation is elevated in PPHN lambs [58], and inhibits NOS activity via mechanisms that include decreased association with HSP90 [59, 60]. eNOS becomes a source of ROS when the enzyme becomes uncoupled, resulting in incomplete reduction of molecular oxygen with the formation of superoxide. Uncoupled eNOS is evident in pulmonary arteries isolated from PPHN lambs [61] and may be a consequence of increased Nox activity [62, 63]. The role of superoxide in impaired NO-mediated pulmonary vasodilation in PPHN is demonstrated by studies showing improved NO-mediated relaxation in isolated pulmonary arteries treated with SOD or superoxide scavengers [64]. The negative impact of elevated H_2O_2 in PPHN is confirmed by similar studies showing improved relaxation to exogenous NO in PPHN pulmonary arteries treated with catalase [47]. Overall these data highlight the positive feedback mechanisms leading to increased ROS and impaired NO-mediated vasodilation in PPHN.

In vitro studies.—Pulmonary artery smooth muscle cells (PASMC) isolated from PPHN lambs exhibit elevated cytosolic ROS, increased Nox4 expression and decreased ecSOD activity relative to control PASMC [48]. Nox4 siRNA knockdown attenuates cytosolic ROS levels and elevates ecSOD activity in these cells [48]. PPHN PASMC also display elevated mitochondrial matrix ROS, associated with increased activity of the mitochondrial SOD MnSOD, increased PDE5 activity and decreased cGMP-responsiveness to NO [65]. These data raise the possibility that PPHN-induced mitochondrial superoxide is converted to H_2O_2 by MnSOD and crosses mitochondrial membranes thereby contributing to elevated cytosolic

ROS. Increasing evidence indicates cross talk between the mitochondria and Nox isoforms [66]. ROS stimulate vascular SMC growth suggesting that altered ROS generation and scavenging also contribute to pulmonary vascular remodeling in PPHN [67, 68].

Pulmonary artery endothelial cells (PAEC) isolated from PPHN lambs also exhibit elevated ROS associated with increased expression of Nox2 and Nox4 and impaired angiogenesis [69]. Further studies indicate that uncoupled eNOS and decreased MnSOD (or SOD2) activity also contribute to elevated ROS and impaired angiogenesis in PPHN PAEC [59, 70].

Oxygen exposure and ROS.—Chronic exposure to hyperoxia (60% O_2 and greater) induces oxidant stress, pulmonary hypertension and vascular remodeling in newborn rats [71] and mice [72]. Increased lung Nox1 expression was reported in neonatal mice on postnatal day 7 after 24h exposure to 75% O_2 from birth [73]. Furthermore, ROS generation and lung injury is attenuated in Nox1- but not in Nox2-deficient adult mice following exposure to 100% O_2 for 72h [74]. Conversely, lung Nox2 and Nox4 expression is increased in neonatal mice following exposure to 75% O_2 for 7d [75]. In PASMC isolated from control lambs, exposure to 95% O_2 for 24h increases cytosolic ROS and increases PDE5 activity [57]. Furthermore, exposure to 30 minutes of 95% O_2 increases mitochondrial but not cytosolic ROS while overexpression of mitochondrial catalase attenuates PDE5 activity after 24h [76]. Together these data suggest that acute hyperoxia elevates mitochondrial ROS, which results in elevated cytosolic ROS upon prolonged exposure. Chronic hyperoxia exposure activates PDE5 in PASMC and may have additional consequences for NO signaling as highlighted above.

Antioxidant therapy.—As described above, PPHN and hyperoxia elevate pulmonary ROS via common and distinct mechanisms and suggest that ROS scavengers may be effective in limiting the oxidant stress in PPHN infants ventilated with oxygen. In neonatal lamb models of PPHN, intratracheal administration of antioxidants decreases ROS, increases eNOS expression and normalizes tetrahydrobiopterin levels after ventilation with 100% O_2 for 24h [77, 78]. Intratracheal recombinant human SOD (rhSOD) also reduces ONOO-mediated protein nitration (figure 5) [58], decreases PDE5 activity and increases cGMP in the pulmonary arteries of ventilated PPHN lambs [79]. Similarly, Intratracheal catalase improves oxygenation, increases lung ecSOD activity, decreases PA superoxide levels, decreases PA PDE5 activity and increases PA cGMP in ventilated PPHN lambs [50, 65]. These data suggest that antioxidant therapy may increase oxygenation in PPHN lambs by improving NO-mediated vasodilation at multiple points in the signaling pathway.

Using the same PPHN lamb model, both antenatal betamethasone [80] and postnatal hydrocortisone significantly improved oxygenation, in part by increasing superoxide dismutase activity and reducing oxidant stress [81, 82] However, it is important to note that clinical trials of antioxidant therapy for neonatal pulmonary diseases have had only limited success [83]. This may be due to the timing of treatment relative to disease progression, the subcellular location of ROS in different diseases, and the involvement of ROS in normal cell signaling. It is likely that 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.

The preterm lung:

The preterm lung is meant to develop under hypoxic conditions that favor rapid parenchymal and pulmonary vascular growth, which means that exposure to even ambient levels of oxygen after birth produces relative hyperoxia. [84] Various factors that promote oxidative stress in preterm infants can be associated with development of pulmonary hypertension (figure 6). The controversies surrounding oxygen use and pulmonary hypertension in preterm infants can be discussed under three sub-headings – delivery room and immediate postnatal management, early acute preterm PPHN and bronchopulmonary dysplasia (BPD) with pulmonary hypertension.

Delivery room and postnatal management:

Oxygen supplementation during delivery room resuscitation of extremely preterm infants has been a subject of controversy and is reviewed in a recent manuscript. [85] Extremely preterm infants with poor respiratory effort at birth are unable to establish functional residual capacity (FRC) or achieve physiologically appropriate SpO_2 and/or heart rate by 5 minutes after birth and are at high risk of negative outcomes. [86] [87] Several multi-center randomized trials attempting to minimize oxidative stress in this extremely premature population by (a) promoting placental transfusion through delayed cord clamping [88] or umbilical cord milking [89], (b) limiting inspired oxygen [90] or targeting lower SpO_2 [91] and establishing FRC through sustained inflation [92] have failed to demonstrate benefit. Some of these trials have in fact shown harm with these interventions in very immature infants (figure 7).

After birth, supplemental oxygen and hyperoxia are well-known sources of oxidant stress and risk factors for chronic lung disease. However, recent clinical trials have revealed the complexities of oxygen supplementation and oxygen targets for premature infants.

Five large trials from Australia, New Zealand, the United States, Canada, and the United Kingdom randomized extremely preterm infants to management with either 85-89% or 91-95% saturation. [91] The Neonatal Oxygenation Prospective Metaanalysis (NeOProM) Collaboration performed a metaanalysis of the individual patient data for 4965 patients in these trials and found that rates of the primary outcome of death or major disability at 18 to 24 months corrected age were not different between the two groups (53.5% for the lower target vs. 51.6% for the higher target). Rates of bronchopulmonary dysplasia were significantly reduced in the lower vs. higher target saturation group (25% vs. 30%, $P<.001$). However, this pulmonary benefit was offset by an increased risk of death at 18 to 24 months in the lower vs. higher saturation group (19.9% vs. 17.1%; $P = .02$) and severe necrotizing enterocolitis was more frequent in the lower vs. higher saturation group (9.2% vs. 6.9%; $P = .003$). Fetal growth restriction or other risk factors did not alter the risk of morbidity and mortality for either saturation range. This higher target range of 91-95% has now been widely adopted in most neonatal units in the US and Europe. Finding ways to selectively reduce the pulmonary toxicity of oxygen in this population, while retaining the systemic benefits, is an important research and clinical goal for the next decade.

Early PPHN in preterm infants:

Early respiratory failure after preterm birth commonly occurs due to surfactant deficiency and lung immaturity, and supplemental oxygen is required to support life. These infants often demonstrate clinical, echocardiographic and histological evidence of pulmonary hypertension. [93] This problem is compounded by antenatal stressors such as placental insufficiency that appear to increase oxidant stress prior to birth. Mothers with pregnancies subsequently affected by intrauterine growth restriction have been reported to have elevated urinary 8-oxodG in the first and second trimester, indicating that oxidative stress (as evidenced by DNA oxidation) may precede the placental and clinical changes of placental insufficiency. [94] In other prospective cohort studies, infants that developed pulmonary hypertension as a complication of prematurity were more likely to have experienced placental insufficiency and fetal growth restriction prior to birth. [95, 96] Therapies targeted towards addressing hypoxemic respiratory failure and PPHN such as supplemental oxygen and iNO can exacerbate free radical mediated damage. [97]

Oxidative stress, bronchopulmonary dysplasia (BPD) and pulmonary hypertension:

Preterm infants with severe BPD are at high risk of pulmonary hypertension presenting later in their NICU course. [98] There is evidence to suggest that oxidative stress early in the course of these preterm infants is associated with later onset of BPD and pulmonary hypertension. Examination of cord blood metabolomic profiles revealed that markers of oxidative stress were strongly associated with the later onset of PH and chronic lung disease in preterm infants. [99] Oxygenated lipids derived from linoleic acid or α-linolenic acid were uniformly elevated by a variety of mechanisms including soluble epoxide hydrolase activity and levels of 9-HETE, a non-enzymatic oxidation product of arachidonic acid, were elevated more than 2-fold. A recent large meta-analysis of 12 non-overlapping studies reinforces these associations, and found that PH was associated with fetal growth restriction. [100] Collectively, these findings suggest that smaller and more premature infants may experience abnormal pulmonary vascular development long before they are challenged by the pulmonary insults such as mechanical ventilation and sepsis associated with postnatal care (figure 6)

Rodent models have been widely used to identify the mechanisms that give rise to PH and BPD in preterm infants. Neonatal rats and mice are born with developmentally immature lungs at the saccular stage in development, similar to extremely premature infants at <28 weeks gestation. Exposure of rodent pups to hyperoxia (>60% oxygen) leads to pulmonary hypertension, right ventricular hypertrophy, pulmonary vascular remodeling, and alveolar simplification, similar to human infants with PH and BPD. Recent studies indicate that just 24 hours of hyperoxic exposure shortly after birth is sufficient to cause PH and right ventricular hypertrophy that persists to day 14, along with abnormal vascular function due to diminished soluble guanylate cyclase and increased cGMP phosphodiesterase activity. [101]

Other stresses common to the sick preterm infant, such as nutritional deficiency, likely amplify the effect of hyperoxia on the pulmonary vasculature. [102] A novel rodent model causes postnatal growth restriction (PNGR) by increasing the number of pups per dam, which limits milk and fat intake. Combining PNGR with exposure to 75% oxygen for the

first 14 days of life produced a more severe phenotype of PH with decreased pulmonary vessel density, thickened pulmonary arteriolar walls and right ventricular hypertrophy (figure 6). PNGR and hyperoxia, both individually and in combination, also caused decreased expression of key modulators of angiogenesis and vascular tone including VEGF, VEGF receptor 2, HIF1α, HIF2α, eNOS and NOS metabolites. In addition, PNGR was associated with increased activity of soluble epoxide hydrolase (sEH) as evidenced by an increase in the ratio of 14,15-DiHETE (pro-inflammatory oxylipins) to 14,15-EET (anti-inflammatory oxylipins) in the plasma of exposed rat pups. [103] Soluble epoxide hydrolase rapidly converts the more biologically active and anti-inflammatory EpFAs to less active and more pro-inflammatory di-hydroxy fatty acids.

There has been limited progress in the prevention of PH associated with prematurity. As noted above, antioxidant therapy with rhSOD had no effect on prevention of chronic lung disease, although it did reduce some aspects of chronic respiratory morbidity at a year of life. Inhibitors of sEH have been promising developments for treatment of adult diseases of chronic inflammation such as diabetes, metabolic syndrome, atherosclerosis, asthma, COPD, and systemic hypertension. In adult rodents, sEH inhibition attenuated PH and deletion of sEH reduced acute hyperoxic lung injury [104, 105] but this novel approach still needs to be tested in neonatal models of PH. It is also possible that nutritional modifications or supplements could reduce the PH and lung injury associated with postnatal growth restriction. For instance, the offspring of pregnant rats fed ω-3 polyunsaturated fatty acids (PUFA) and exposed to hyperoxia had decreased lung injury typical of bronchopulmonary dysplasia while ω-6 PUFA supplementation had no benefit. [106] Whether these nutritional approaches selectively reduce the pulmonary impact of oxidant stress is an important research question.

Conclusion: Clinical implications of free radicals in PPHN

Supplemental oxygen is a necessary and life-saving therapy for infants who experience a delayed or failed pulmonary vascular transition in the delivery room, or who have neonatal respiratory failure due to PPHN or prematurity. However, supplemental oxygen also increases oxidant stress through production of free radicals by the mitochondrial matrix and enzymatic sources in the cytosol. Longer exposures to hyperoxia also activate PDE5 in the pulmonary vasculature which reduces cGMP generation in response to pulmonary vasodilators such as inhaled NO. Therapies to mitigate the impact of oxidant stress in the lung and pulmonary vasculature are not yet selective enough to the lung and/or to subcellular oxidant signaling. The effects of free radical damage may extend well beyond infancy [97], as suggested by the increased risk of childhood malignancy after neonatal exposure to hyperoxia [107] and iNO. [108] There is an urgent need for basic science, translational, clinical, and epidemiologic studies to solve these important problems.

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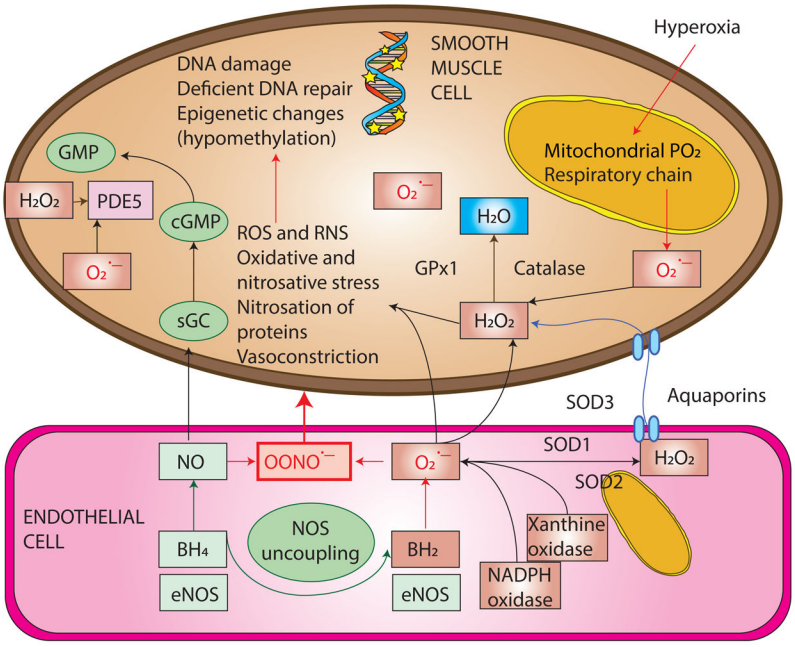

Figure 1. Free radicals - reactive oxygen (ROS) and nitrogen species (RNS) in persistent pulmonary hypertension of the newborn.

Reactive oxygen species such as superoxide anions ($O_2^{\cdot -}$) can be produced by the electron transport chain (ETC) in the mitochondria, due to exposure to hyperoxia or from enzymes such as uncoupled nitric oxide synthase (NOS), NADPH oxidase, and xanthine oxidase or the Fenton reaction. Nitric oxide (NO) is a free radical and avidly binds to superoxide anion to form peroxynitrite ($OONO^{\cdot -}$) at the rate of 6.7/M/s. This rate is considerably faster than the rate of dismutation of superoxide anions by superoxide dismutase (SOD) to form hydrogen peroxide (H_2O_2). Hydrogen peroxide can diffuse across membranes through aquaporins. Hydrogen peroxide is broken down by catalase and glutathione peroxidase (GPx1) to water. NO and oxygen are vasodilators but peroxynitrite is a potent vasoconstrictor. ROS (superoxide anions and hydrogen peroxide) stimulate phosphodiesterase 5 (PDE5) enzyme and breakdown cGMP limiting vasodilator effect of NO. Copyright Satyan Lakshminrusimha.

BH4, tetrahydrobiopterin; SOD1 – superoxide dismutase; SOD3, extracellular superoxide dismutase; eNOS, endothelial nitric oxide synthase; SOD2 or MnSOD, manganese superoxide dismutase; sGC, soluble guanylate cyclase; cGMP – cyclic guanosine monophosphate;

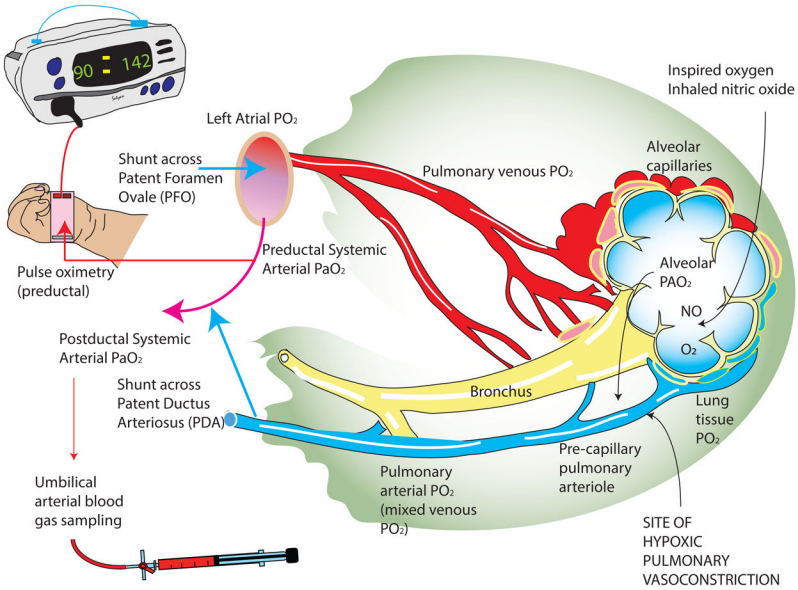

Figure 2. Oxygen tension in different sites of pulmonary circulation and pulmonary vascular resistance (PVR).

The precise site of hypoxic pulmonary vasoconstriction and the sensing mechanisms are not clear but is thought by most to be the precapillary pulmonary arteriole in the lung. The pulmonary arterial smooth muscle cells (PASMC) are exposed to lung tissue PO_2, alveolar PAO_2, and pulmonary arterial (mixed venous) PO_2. It is thought that the rapid diffusion from alveolar PAO_2 is the predominant determinant of oxygen tension in PASMC. In infants with persistent pulmonary hypertension of the newborn (PPHN), PAO_2 can be approximately calculated using preductal PaO_2 values. The presence of a right-to-left shunt at the atrial level or ductal level can reduce PaO_2 levels in PPHN. Heterogeneous lung disease can also interfere with the relationship between alveolar PAO_2 and PVR. Copyright Satyan Lakshminrusimha. Modified from Hemodynamics and Cardiology: Neonatology Questions and Controversies 3rd Edition.

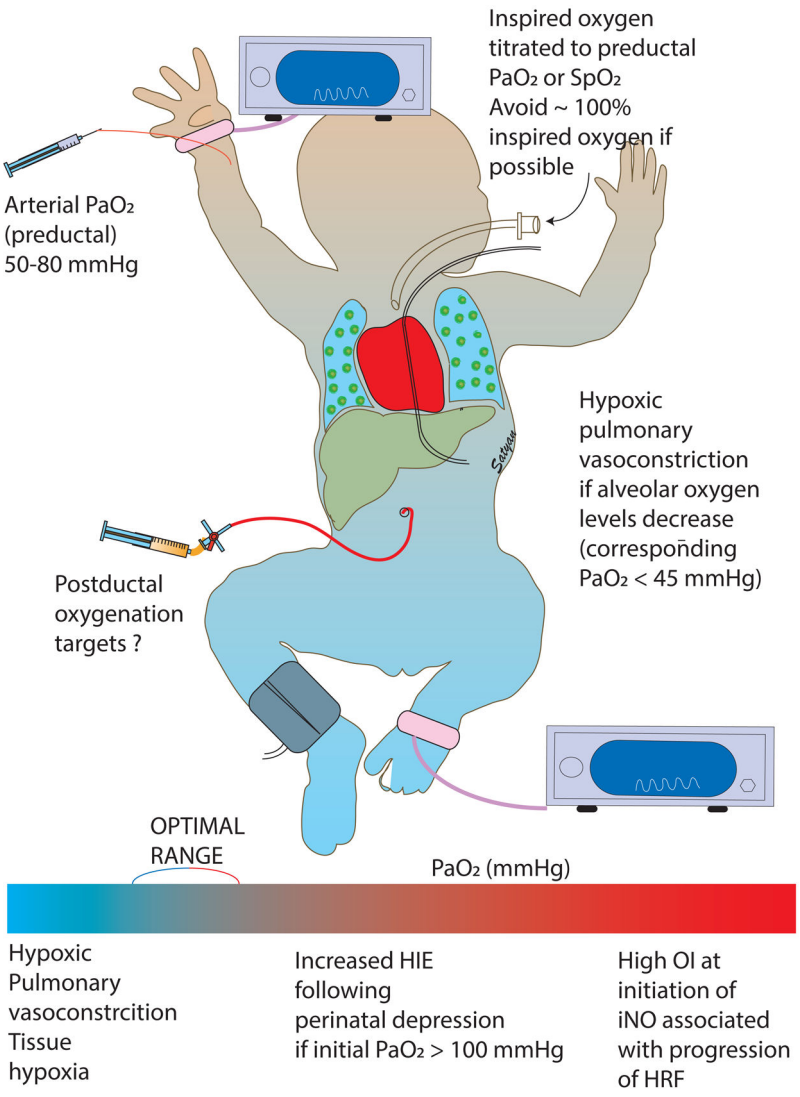

Figure 3. Optimal targets for oxygenation in the management of acute PPHN in term infants.

Based on preclinical data, we recommend a preductal PaO_2 of 50 to 80 mmHg in infants with PPHN. The corresponding SpO_2 targets are approximately 90 to 97%. Oxygen targets below this range are associated with hypoxic pulmonary vasoconstriction. Targets above this range are associated with poor response to inhaled nitric oxide and higher incidence of HIE following perinatal depression.

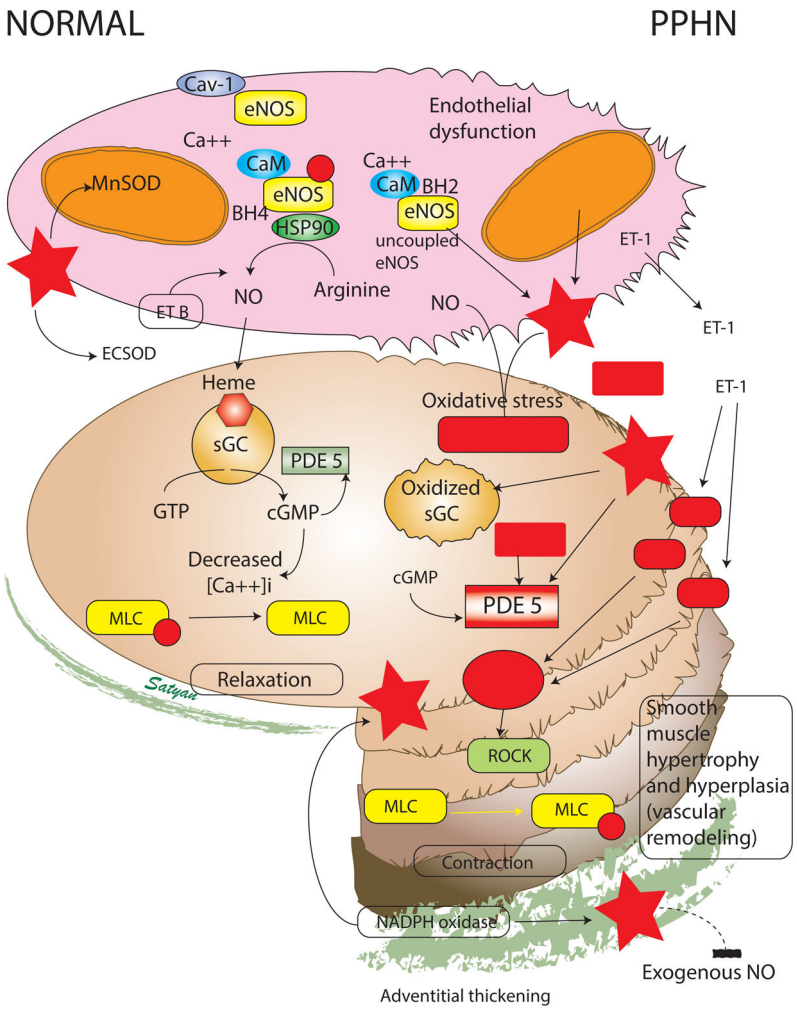

Figure 4. Cellular and biochemical changes in PPHN secondary to oxidative and nitrosative stress.

Pulmonary arteries from human neonates and animal models of PPHN demonstrate thickening of the muscular layer and adventitia. The normal pulmonary arterial endothelium produces nitric oxide (NO) from phosphorylated endothelial nitric oxide synthase (eNOS) coupled to heat shock protein 90 (HSP90) with tetrahydrobiopterin (BH_4) as a cofactor with adequate supply of arginine as substrate. The eNOS protein is bound to caveolin-1 (Cav-1) prior to its release by a calcium-calmodulin (CaM) dependent process. Endothelin acting through endothelin-B receptor (ET_B) on the endothelium stimulates NO production. Manganese superoxide dismutase (MnSOD or SOD-2) is present in the mitochondria and scavenges superoxide anions. Extracellular superoxide dismutase (ecSOD) limits the interaction (and inactivation) of NO with superoxide anions in the endothelial-smooth muscle interface. NO reaches the smooth muscle cell and binds to reduced soluble guanylate cyclase (sGC) which in turn catalyzes the conversion of GTP to cGMP. Cyclic GMP is an important second messenger that reduces the cytosolic concentration of ionic calcium [$Ca^{++}]_i$. Reduced concentration of ionic calcium leads to dephosphorylation of myosin light chains (MLC) resulting in smooth muscle relaxation. In PPHN, endothelial dysfunction leads to uncoupling of eNOS. Low levels of MnSOD and possibly ecSOD increase oxidative

stress and formation of superoxide anions. Superoxide anions inactive nitric oxide resulting in the formation of toxic peroxynitrite. Oxidized sGC cannot be activated by NO to produce cGMP. Superoxide anions stimulate phosphodiesterase 5 (PDE5) activity and enhance breakdown of cGMP. Pulmonary arterial endothelial cells from PPHN pulmonary arteries produce increased levels of endothelin-1 (ET-1), a powerful pulmonary vasoconstrictor. ET-1 acts through ET_A receptor and stimulates Rho-A, Rho-kinase (ROCK) pathway leading to phosphorylation of MLC and smooth muscle contraction. The pulmonary arterial endothelial cells have low levels of ET_B receptors. The net effect is reduced cGMP (vasodilator second messenger) and sensitization of the smooth muscle to ionic calcium. (*Copyright-Lakshminrusimha and Steinhorn*). *Modified from Polin and Fox – Fetal and Neonatal Physiology 5th Edition*

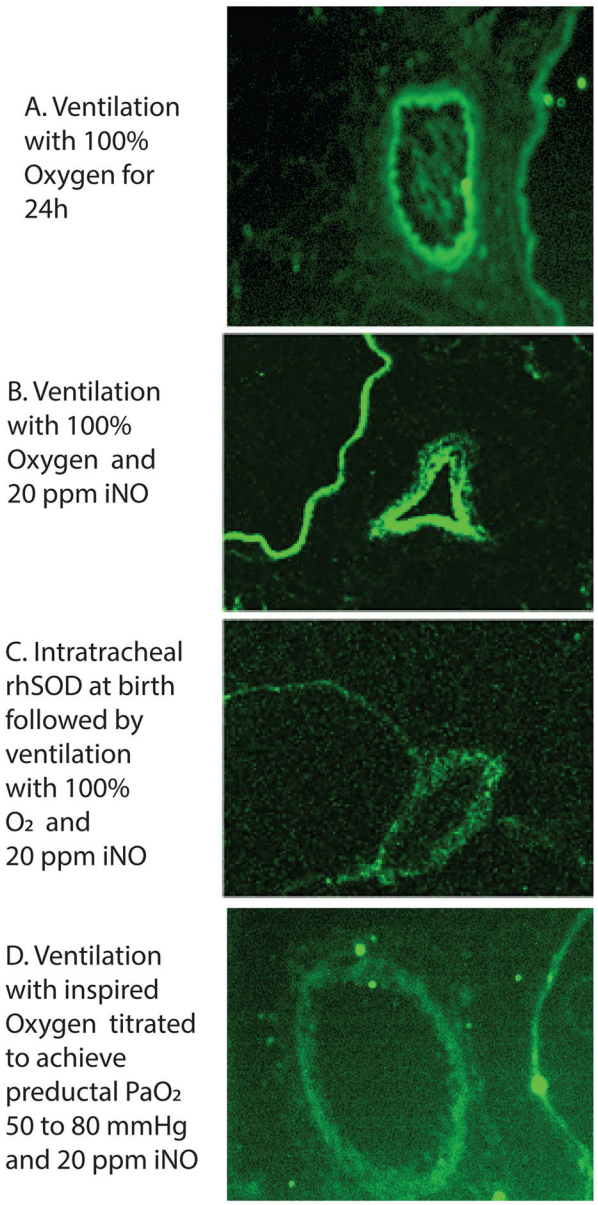

Figure 5. Nitrosative stress and the role of inhaled nitric oxide and inspired oxygen.

3-NT staining of lung sections from lambs with PPHN induced by antenatal ligation of the ductus arteriosus and ventilated for 24 hours.

A. Lambs were ventilated with 100% oxygen for 24 hours irrespective of PaO_2 levels.

B. Lambs were ventilated with 100% oxygen and 20 ppm iNO for 24 hours irrespective of PaO_2 levels.

C. Lambs were ventilated with 100% oxygen and 20 ppm iNO for 24 hours irrespective of PaO_2 levels. These lambs received a dose of intratracheal recombinant human superoxide dismutase (rhSOD) mixed with surfactant at birth.

D. Lambs were ventilated with titrated inspired oxygen to maintain preductal PaO_2 between 50 and 80 mmHg and 20 ppm iNO for 24 hours.

Modified from reference # 44

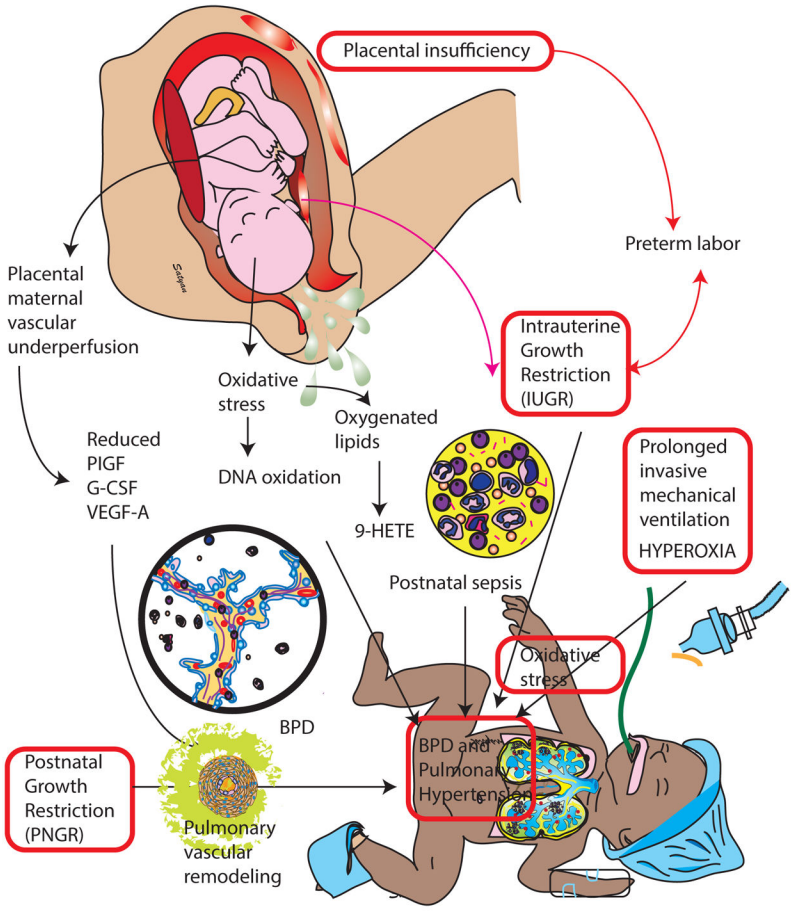

Figure 6.

Role of prenatal and postnatal oxidative stress in the pathogenesis of bronchopulmonary dysplasia (BPD) and pulmonary hypertension (PH). Growth restriction, both prenatal (IUGR) and postnatal (PNGR) contribute to development of PH. Cord blood placental growth factor (PIGF), granulocyte-colony stimulating factor (G-CSF), and vascular endothelial growth factor-A (VEGF-A) Copyright Satyan Lakshminrusimha

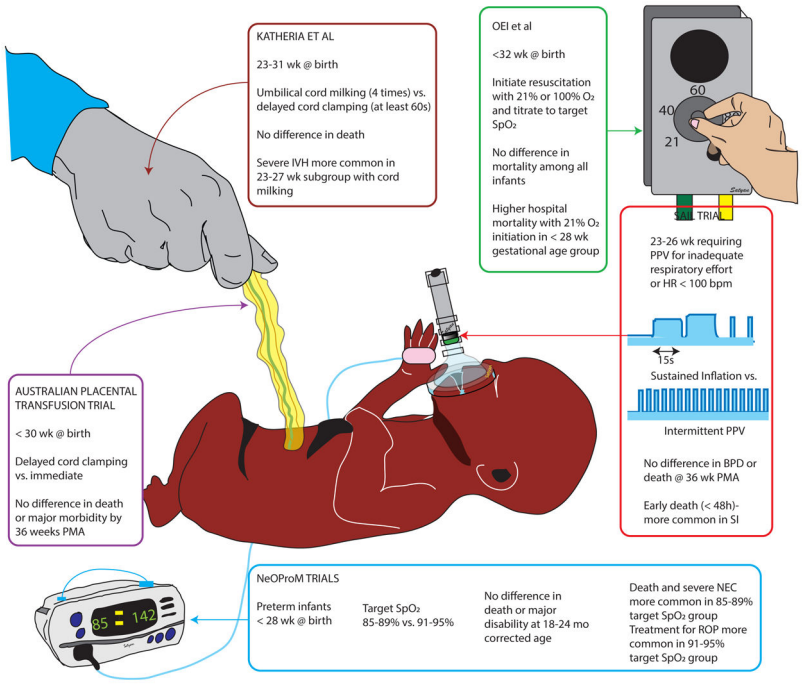

Figure 7.

Recent randomized controlled trials in extremely preterm infants with interventions that could potentially reduce oxidative stress and their outcomes. Some of these trials were stopped early due to negative outcomes in the intervention group. The Australian placental transfusion trial [88], Oei et al [90], Katheria et al [89], NeOProM trials [91] and the SAIL randomized control trial [92] are highlighted in this figure. The patient population studied, intervention, primary outcome and negative findings in subgroups (if any) are shown in boxes. Copyright Satyan Lakshminrusimha