

Newer Approaches for Persistent Pulmonary Hypertension in Neonates: Mechanism and Treatment

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Abstract

Persistent pulmonary hypertension of the newborn (PPHN) is a clinical syndrome of failed circulatory adaptation at birth, seen in about 2/1000 live born infants. While it is more commonly encountered in term and near term babies, it can be recognized in some premature babies with respiratory distress or bronchopulmonary dysplasia. Most commonly, PPHN is secondary to delayed or impaired relaxation of the pulmonary vasculature associated with diverse neonatal pulmonary pathologies such as meconium aspiration syndrome, congenital diaphragmatic hernia and respiratory distress syndrome.

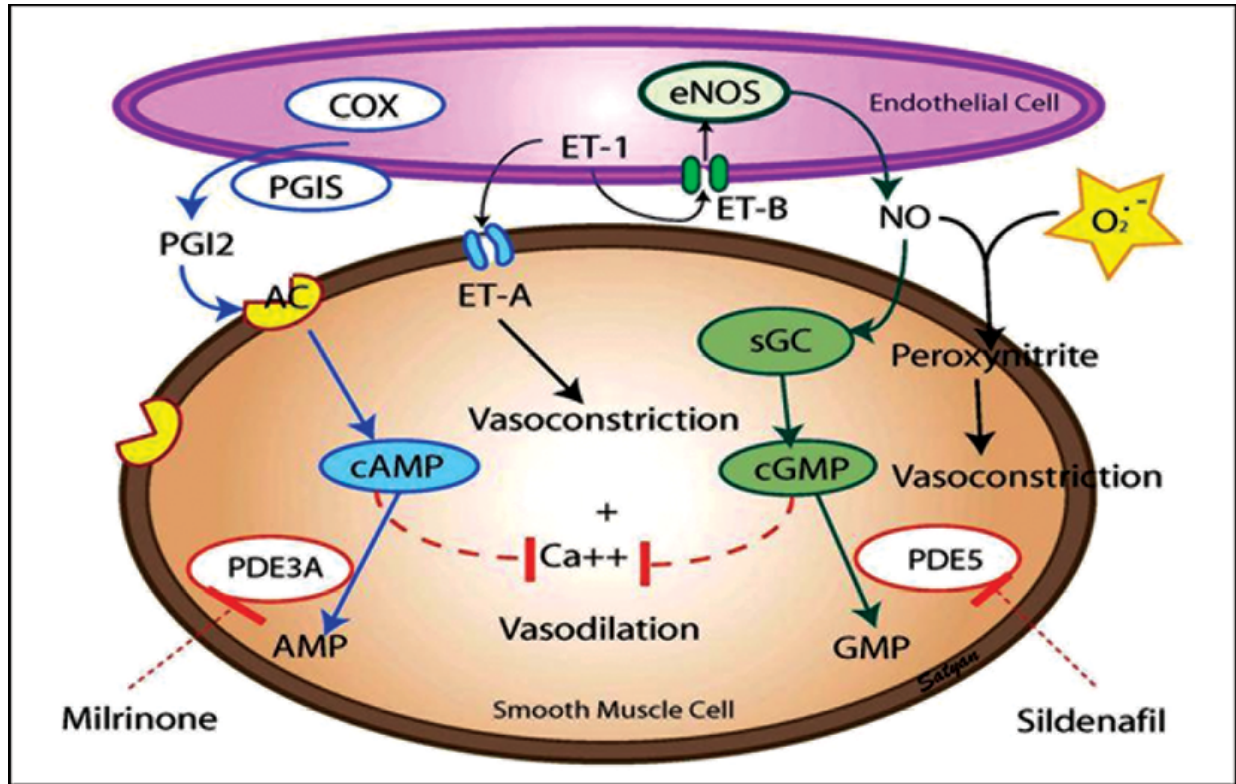
Keywords: PPHN; Neonate; Systemic vascular resistance(SVR); Pulmonary vascular resistance(PVR); ECMO; Inhaled no; Sildenafil; Milrinone.

INTRODUCTION

Persistent pulmonary hypertension of the newborn (PPHN) is a clinical syndrome characterized by sustained elevation of pulmonary vascular resistance (PVR) and is often associated with normal or low systemic vascular resistance (SVR). This leads to extrapulmonary shunting from right to left across persistent fetal channels (patent ductus arteriosus, PDA and patent foramen ovale, PFO) leading to labile hypoxemia. This was previously referred to as persistent fetal circulation (PFC) and is often secondary to an failed pulmonary transition at birth.

FETAL CIRCULATION

Circulation in the fetus is characterized by high PVR and low SVR. The placenta is the site of gas exchange. Numerous factors contribute to the high pulmonary vascular tone in utero, such as mechanical factors (compression of the small pulmonary arterioles by the fluid filled alveoli and a lack of rhythmic distension), the presence of low-resting alveolar and arteriolar oxygen tensions, and a relative lack of vasodilators.¹ Low oxygen tension and elevated levels of vasoconstrictor mediators such as endothelin-1 (ET-1) and thromboxane play a crucial role in maintaining elevated fetal PVR.¹ Serotonin increases fetal PVR^{2,3} and the use of serotonin re-uptake inhibitors (SSRI) during pregnancy has been associated with increased incidence of PPHN.⁴



Transition at Birth

A series of circulatory events take place at birth to ensure a smooth transition from fetal to extra uterine life. Clamping of the umbilical cord removes low resistance placental circulation, increasing systemic arterial pressure (fig. 2). Simultaneously, various mechanisms operate to rapidly reduce pulmonary arterial pressure and increase pulmonary blood flow. Of these, the most important stimuli appear to be ventilation of the lungs and an increase in oxygen tension. With initiation of respiration, the fluid filled fetal lungs are distended with air.¹ There is improved oxygenation of the pulmonary vascular bed, further decreasing PVR.⁵ There is an eight fold increase in pulmonary blood flow, which raises left atrial pressure, closing the foramen ovale. As PVR drops lower than SVR, there is a reversal of flow across the ductus arteriosus. The increase in arterial oxygen saturation leads to closure of the ductus arteriosus and ductus venosus within the first few hours after birth. In the final phase of neonatal pulmonary vascular transition, further decline in PVR is accompanied by rapid structural remodelling of the entire pulmonary bed, from the main pulmonary arteries to the capillaries.⁶

Vascular endothelium releases several vasoactive products that play a primary role in pulmonary

transition at birth. Pulmonary endothelial NO production increases markedly at the time of birth. Oxygen is believed to be an important catalyst for this increased NO production, although the precise mechanism is not clear. It increases oxidative phosphorylation and the release of red blood cell ATP which is a pulmonary vasodilator during fetal life and a potential stimulus for endothelial NO production.^{7,8} In intrapulmonary arteries isolated from near term fetal sheep, both basal and stimulated NO release increase with escalating oxygen tension.⁹ The shear stress resulting from increased pulmonary blood flow and increased oxygenation also induce endothelial nitric oxide synthase (eNOS) expression, thus contributing to NO-mediated pulmonary vasodilation after birth.¹ Nitric oxide exerts its action through sGC and cGMP (fig. 1). Bloch et al report that expression of sGC is higher in late gestation and newborn rats than in adult rats¹⁰, which may explain the better response to NO in neonates. There is a similar developmental regulation of cGMP specific phosphodiesterase 5 (PDE5) expression and activity. Expression of PDE5 in the lungs increases during gestation and in the immediate newborn period.^{11,12}

The arachidonic acid prostacyclin pathway also plays an important role in the transition at birth. The cyclooxygenase enzyme acts on arachidonic

acid to produce prostaglandin endoperoxides. Prostaglandins activate adenylate cyclase to increase cAMP concentrations in vascular smooth muscle cells. Inhibition of prostacyclin production by non-steroidal anti-inflammatory drugs (NSAIDs) during late pregnancy has been associated with PPHN although this association has been recently called into question.¹³ Atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) dilate fetal pulmonary vasculature by increasing cGMP through particulate guanylate cyclase (pGC)¹⁴ and may play a role in pulmonary vascular transition at birth.

Etiology and Pathophysiology of PPHN

Failure of the pulmonary circulation to undergo the normal transition after birth leads to PPHN, which is characterized by an elevated PVR/SVR ratio resulting from either vasoconstriction, structural remodelling of the pulmonary vasculature, intravascular obstruction or lung hypoplasia. There is right to left shunting of blood across the foramen ovale and ductus arteriosus, resulting in hypoxemia and labile oxygen saturations (fig. 3).

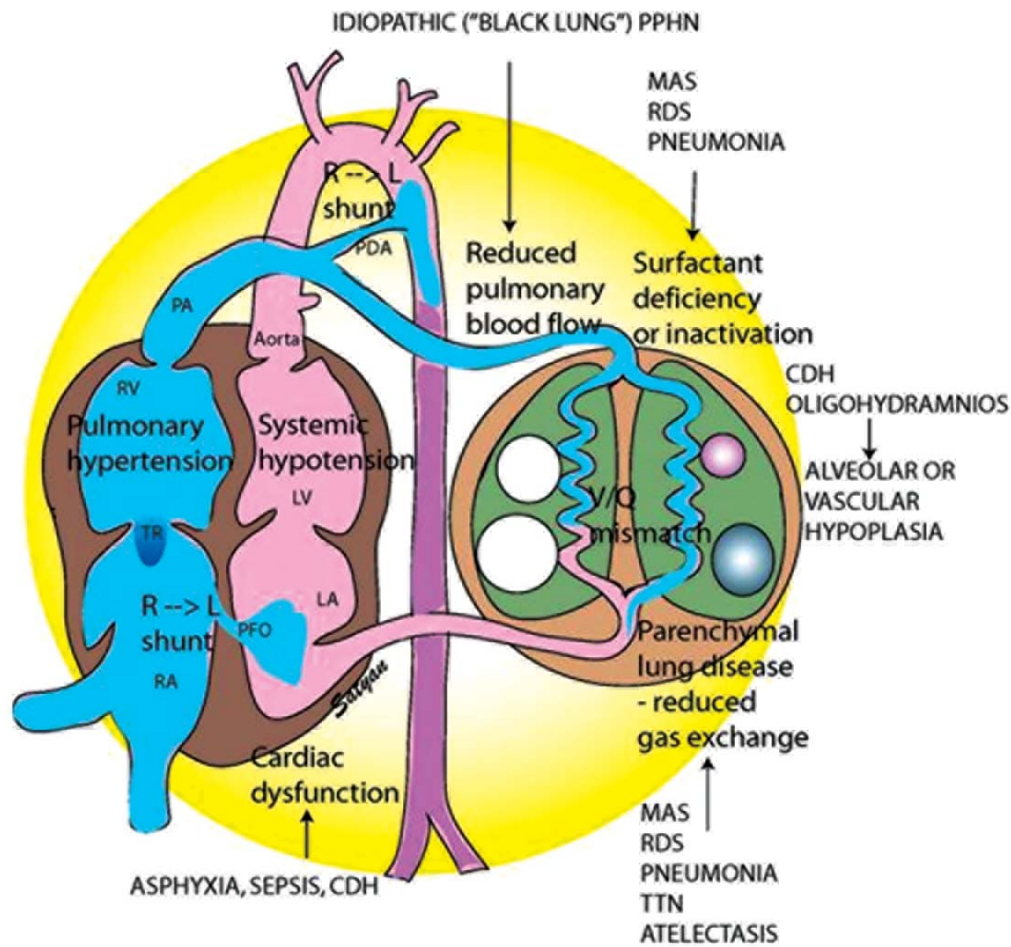


Fig. 3: Various Etiological factors causing PPHN and hemodynamic changes in PPHN

PPHN may be idiopathic (10%) or secondary to certain neonatal pulmonary diseases which cause delayed relaxation of pulmonary vascular bed. Common pulmonary conditions like congenital diaphragmatic hernia (CDH), respiratory distress syndrome (RDS), pneumonia, meconium aspiration syndrome (MAS), transient tachypnea of the newborn (TTN) might be associated with

PPHN. Some of the uncommon causes of severe and intractable PPHN include alveolar capillary dysplasia¹⁵, hyaline membrane disease because of mutations in surfactant protein B (SP-B) gene¹⁶ and respiratory failure because of ATP binding cassette protein member A3 (ABCA3) deficiency.¹⁷ PPHN was significantly associated with genetic variants in corticotropin releasing hormone (cRh) receptor

1, CRHR1 and cRh-binding protein, CRHBP.¹⁸

Meconium Aspiration Syndrome

Meconium aspiration syndrome (MAS) is the leading cause of PPHN, although its incidence has reduced in recent years due to a reduced number of post-term deliveries. Meconium staining of amniotic fluid (MSAF) is seen in 5%-24% of normal pregnancies, however only 5% of infants born with MSAF develop MAS. Meconium can partially or completely block the airway and also inactivate surfactant.¹⁹ This results in decreasing V/Q ratios and rising intrapulmonary right to left shunt. Other segments of the lungs may be over ventilated relative to perfusion, causing rise in physiologic dead space and hypoxemia.

Asphyxia

Multiple mechanisms impart hypoxemic respiratory failure and PPHN in asphyxiated newborns including fetal hypoxemia, ischemia, meconium aspiration, right and left ventricular dysfunction, coagulation defects, hyperoxic resuscitation and effects of mechanical ventilation.²⁰ Acidosis and hypoxia gives rise to PVR. In hypothermia trials for asphyxia, approximately 20% of asphyxiated infants in the control group and 25% in the hypothermia group were diagnosed with PPHN.²¹

Pulmonary Hypertension in Premature Infants

Although PPHN is traditionally considered a disease of term and near term infants, it is increasingly being diagnosed in preterm infants.²² Some preterm infants present with PPHN in the first few days of life.²³ Preterm infants with bronchopulmonary dysplasia (BPD) may present with severe pulmonary hypertension later in the hospital course or after discharge from the Neonatal Intensive Care Unit (NICU). Preterm infants with fetal growth restriction are at high risk for developing BPD with pulmonary hypertension.²⁴ Pulmonary vascular disease contributes to poor outcomes in BPD.²⁵

Idiopathic or "Black Lung" Pphn

PPHN can occur in the absence of any parenchymal disease or lung hypoplasia due to abnormal muscularization of pulmonary arterioles. There is severe hypoxemia with pulmonary vasoconstriction. Conditions such as polycythemia and hyperviscosity may also increase PVR and

contribute to PPHN in the absence of parenchymal lung disease. In-utero closure of the ductus arteriosus due to antenatal exposure to non-steroidal anti-inflammatory drugs such as aspirin and ibuprofen has been associated with PPHN.²⁶ This alliance was brought into question in a recent study.¹³ Antenatal ligation of the ductus Aarteriosus in fetal lambs creates a model of severe PPHN with clinical and pathological features of "black-lung" PPHN.²⁷

Pathogenesis of PPHN

There is proof suggesting that an alteration of the NO pathway contributes to PPHN. Activity and expression of eNOS and sGC in lungs is decreased²⁸⁻³⁰ in fetal lamb model of PPHN. In these lambs, the vascular response to NO itself is diminished,³⁰ where as the response to cGMP is normal. Thus the declined responsiveness appears to result from decreased vascular smooth muscle sensitivity to NO at the level of sGC. Decreased expression of eNOS³¹ and decrease levels of NO metabolites in urine³² have also been noted in neonates with PPHN.

Antenatal and Perinatal Risk Factors

Risk factors for PPHN are meconium stained amniotic fluid, perinatal acidosis and asphyxia, maternal risk factors like prolonged rupture of membranes, maternal fever, or positive group B Streptococcal carrier status. PPHN should be considered as causing factor of hypoxic respiratory failure in term or near term infants. In addition to the known risk factors, it has recently been postulated that perinatal environment, including exposure to nicotine and some medications, maternal obesity and diabetes, epigenetics, painful stimuli and birth by cesarean section may also affect the maladaptation of the lung circulation at birth³³

Management in the Delivery Room

Early recognition of PPHN and correction of factors that prevent reduce in PVR are vital to successful management of late preterm or term neonate with hypoxemic respiratory defect. One of the peculiar features of PPHN is labile hypoxemia. These neonates exhibit frequent desaturation episodes and wide swings in SpO₂ and arterial PO₂ without changes in ventilator settings. A single, loud S₂ and a systolic murmur of tricuspid regurgitation are mostly auscultated.

Neonates with PPHN are often cyanotic

secondary to significant right to left extrapulmonary shunting in the presence of high PVR. Significant resuscitative efforts may be required in the labour room.

Diagnosis

In a term or near term neonate with respiratory distress, an initial evaluation would include a chest X-ray and an arterial blood gas. Hypoxemia disproportionate to the severity of parenchymal disease on chest X-ray should suggest idiopathic PPHN. Evidence of the underlying parenchymal disease such as RDS, MAS, etc. may be seen on chest X-ray in secondary PPHN. This diagnosis can be confirmed by measuring preductal (right extremity) and postductal (either lower extremity) arterial oxygenation. A difference in arterial $PO_2 = 20$ mmHg or oxygen saturation = 5-10% should be considered suggestive of PPHN. Echocardiography remains the gold standard diagnostic tool in PPHN.

In near times, B-type natriuretic peptide (BNP) was proposed as a biomarker in PPHN, is specifically to assess efficacy of treatment and to predict rebound PPHN.^{34,35}

Supportive Measures

Once PPHN is diagnosed, supportive measures are necessary in successful management efforts. Necessary measures should be taken to maintain normothermia and correct metabolic abnormalities such as hypoglycemia, hypocalcemia, acidosis and polycythemia. Intravenous nutrition with appropriate glucose infusion rate, adequate calcium and amino acid supplementation with an optimal chloride: acetate ratio must be administered preferably through a central line.

Covering eyes, ears and maintaining a low noise environment is a known practice. Minimal stimulation, along with meticulous use of sedation and analgesia with narcotic analgesics like morphine and fentanyl or benzodiazepines such as midazolam is suggested.

Systemic blood pressure must be maintained at normal values for gestational age. If there is hypotension and/or poor perfusion indicating hypovolemia, volume replacement in the form of 1-2 fluid boluses should be administered. If hypotension persists despite volume replacement, inotropic agents like dopamine, dobutamine, and epinephrine are indicated. These agents are nonselective to systemic circulation and may be associated with pulmonary vasoconstriction and

elevation of PVR at high doses.

Oxygen and Optimal Oxygen Saturations

It has been shown that brief exposure to 100% oxygen in newborn lungs results in rise of contractility of pulmonary arteries³⁶ and decreases response to iNO.^{37,38} along with direct inactivation of NO, reactive oxygen species can decrease eNOS activity, sGC activity and increase PDE5 activity, resulting in decreased cGMP levels and potentiating pulmonary vasoconstriction. In the ovine ductal ligation model of PPHN, maintaining oxygen saturations in the 90-97% range results in less PVR.³⁸ We suggested maintaining preductal oxygen saturations in low to mid 90s during management of neonate with PPHN with PaO_2 levels between 55 and 80 mmHg.

Ventilation

Adequate lung expansion is essential for adequate oxygenation as well as the effective delivery of iNO.³⁹ Conventional and high frequency ventilation (HFV)⁴⁰ may be used to decrease the V/Q mismatch⁴¹ HFV in combination with iNO resulted in the maximum improvement in oxygenation in some newborns who had severe PPHN complicated by diffuse parenchymal lung disease and underinflation.⁴² Infants with RDS and MAS benefit most from a combination of HFV and iNO therapy.^{43,44} "Gentle" ventilation strategies with adequate PEEP, relatively low PIP and some permissive hypercapnia are now being recommended to ensure adequate lung expansion without causing barotrauma.

In the presence of an patent arterial line, severity of PPHN is determined by calculation of oxygenation index (OI).

$$OI = \frac{\text{Mean airway pressure in cm H}_2\text{O} \times FiO_2 \times 100}{PaO_2 \text{ in mmHg.}}$$

Surfactant

Exogenous surfactant therapy increases oxygenation and decreases the need for ECMO when PPHN was secondary to parenchymal lung disease such as RDS/pneumonia/sepsis/MAS.⁴⁵ Over the last decade, the use of surfactant in treating secondary PPHN and respiratory failure has increased and might have contributed to improved effectiveness of iNO with reduced need for ECMO.

Nitric Oxide

In 1999, inhaled nitric oxide (iNO) was approved by the FDA for use in late-preterm and term

neonate with PPHN. It has been the primary management of PPHN. It achieves potent and selective pulmonary vasodilation without falling of systemic vascular tone. In the intravascular space, it binds with hemoglobin to form methemoglobin, which prevents systemic vasodilation (selective effect). iNO decreases V/Q mismatch by entering only ventilated alveoli and redirecting pulmonary blood by dilating adjacent pulmonary arterioles.

Due to rebound vasoconstriction and resultant pulmonary hypertension on abrupt withdrawal, iNO has to be weaned gradually.⁴⁶ Weaning in steps from 20 ppm gradually over a period of time before its stoppage has been shown to prevent the rebound effect.⁴⁷ If there is oxygenation response, inspired oxygen concentration is primarily weaned below 60% and then iNO is weaned at the rate of 5 ppm every 4 hours. Once iNO dose is 5 ppm, stepwise weaning @ 1 ppm 4 hourly is performed at our institution.

Systemic Vasodilators

Phosphodiesterase Inhibitors

The maximum rate of failure to obtain a sustained oxygenation response to iNO therapy has led to the search for other aims to enhance pulmonary vasodilation. Stoppage of cGMP degrading phosphodiesterase (PDE5) by sildenafil and inhibition of the cAMP degrading phosphodiesterase (PDE3) by milrinone are two of the most promising therapies.

Sildenafil

This drug is presently available both in oral and intravenous route in United States and is FDA approved only for adults with pulmonary hypertension

Studies are suggestive of oral sildenafil (dose range 1-3 mg/kg every 6 h) improves oxygenation and reduces mortality, in centers limited by non-availability of iNO.^{48,49} Intravenous sildenafil has shown to be effective in improving oxygenation in patients with PPHN with or without prior exposure to iNO.⁵⁰ Being systemically administered, the risk of side effects like hypotension due to systemic vasodilation is more. This risk may be reduced by slowly administering a loading dose (0.4 mg load over 3 hours), followed by a maintenance dose (0.07 mg/kg/h). Sildenafil may decrease the rebound pulmonary hypertension noted during iNO weaning.

Milrinone

This inotropic vasodilator is commonly used in paediatric and adult intensive care units but is now currently licensed for use in treating PPHN. It inhibits a PDE3 and dilates pulmonary arteries in the fetal lung model of PPHN.⁵¹ Infants with PPHN resistance to iNO therapy have responded to IV milrinone in 3 case series.⁵²⁻⁵⁴ A loading dose (50 mcg/kg) followed by a maintenance dose (0.33 to 1 mcg/kg/h) is mostly used. As with any systemic vasodilator, hypotension is a side effect so blood pressure needs to be closely monitored.

Milrinone might be the pulmonary vasodilator of choice in the presence of PPHN with left ventricular dysfunction⁵⁵

Bosentan

This non-specific endothelin-1 receptor blocker had been used in the treatment of PPHN, mainly in elderly. In the fetal lamb model of pulmonary hypertension, *Ivy et al.* showed that chronic intrauterine ET receptor blockade reduced PAP in-utero, reduced RVH and distal muscularization of small pulmonary arteries, and rises the fall in PVR at delivery.⁵⁶

Steroids in PPHN

Postnatal systemic steroids have been shown to lower the duration of hospital length of stay and oxygen dependence in MAS.⁵⁷ In the fetal lamb model of PPHN, hydrocortisone treatment postnatally has been proven to improve oxygenation, increase cGMP levels and reduce ROS levels.⁵⁸ These data postulates a potential role for hydrocortisone in PPHN. Care must be taken to avoid using steroids in presence of bacterial or viral infection.

Extracorporeal Membrane Oxygenation

ECMO is a contributory measure that essentially gives time for the neonatal heart and lung to recover from the underlying pathology. With improved ventilation techniques, limitation of oxygen toxicity and the use of therapies like HFOV, surfactant, iNO and other vasodilators, ECMO use for neonatal respiratory disorders has reduced.

Newer approaches

Several newer therapies for PPHN are under investigation. These include free radical scavengers like recombinant human superoxide dismutase (SOD) which give better oxygenation in lambs

with PPHN^{59,60} Apocynin, an NADPH oxidase inhibitor, has also proven attenuate ROS mediated vasoconstriction and increase NOS activity in PPHN lambs.⁶¹

Neurodevelopmental Outcomes

PPHN is a disease with significant long term morbidity, irrespective of the treatment modality. These neonate suffer from long term sequelae such as neurodevelopmental, cognitive and hearing abnormalities.⁶²⁻⁶⁴

Thus, it is important to provide long term multidisciplinary follow-up after discharge.

Summary

PPHN is related with high morbidity and mortality. Inhaled nitric oxide with HFV, surfactant and supportive measures including sedation and blood pressure support remain the prime management in PPHN. ECMO is a choice when these measures fail. Oral/IV sildenafil, IV milrinone and inhaled PGI₂ may have a similar effect with iNO and are being used more frequently. However, larger clinical trials are must to establish their treatment effect.

Long term follow-up is essential for these infants due to the high risk of neurodevelopmental and hearing abnormal.

REFERENCES

1. Lakshminrusimha S, Steinhorn RH. Pulmonary vascular biology during neonatal transition. *Clinics in perinatology*. 1999 Sep 1;26(3):601-19.
2. Delaney C, Gien J, Grover TR, Roe G, Abman SH. Pulmonary vascular effects of serotonin and selective serotonin reuptake inhibitors in the late-gestation ovine fetus. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 2011 Dec;301(6):L937-44.
3. Delaney C, Gien J, Roe G, Isenberg N, Kailey J, Abman SH. Serotonin contributes to high pulmonary vascular tone in a sheep model of persistent pulmonary hypertension of the newborn. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 2013 Jun 15;304(12):L894-901.
4. Chambers CD, Hernandez-Diaz S, Van Marter LJ, Werler MM, Louik C, Jones KL, Mitchell AA. Selective serotonin-reuptake inhibitors and risk of persistent pulmonary hypertension of the newborn. *New England Journal of Medicine*. 2006 Feb 9;354(6):579-87.
5. Teitel DF, Iwamoto HS, Rudolph AM. Changes in the pulmonary circulation during birth-related events. *Pediatric Research*. 1990 Apr;27(4):372-8.
6. Haworth SG, Hislop AA. Adaptation of the pulmonary circulation to extra-uterine life in the pig and its relevance to the human infant. *Cardiovascular research*. 1981 Feb 1;15(2):108-19.
7. Konduri GG, Theodorou AA, Mukhopadhyay A, Deshmukh DR. Adenosine triphosphate and adenosine increase the pulmonary blood flow to postnatal levels in fetal lambs. *Pediatric research*. 1992 May;31(5):451-7.
8. Steinhorn RH, Morin 3rd FC, Van Wylen DG, Gugino SF, Giese EC, Russell JA. Endothelium-dependent relaxations to adenosine in juvenile rabbit pulmonary arteries and veins. *American Journal of Physiology-Heart and Circulatory Physiology*. 1994 May 1;266(5):H2001-6.
9. Shaul PW, Farrar MA, Zellers TM. Oxygen modulates endothelium-derived relaxing factor production in fetal pulmonary arteries. *American Journal of Physiology-Heart and Circulatory Physiology*. 1992 Feb 1;262(2):H355-64.
10. Bloch KD, Filippov G, Sanchez LS, Nakane M, De La Monte SM. Pulmonary soluble guanylate cyclase, a nitric oxide receptor, is increased during the perinatal period. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 1997 Mar 1;272(3):L400-6.
11. Hanson KA, Ziegler JW, Rybalkin SD, Miller JW, Abman SH, Clarke WR. Chronic pulmonary hypertension increases fetal lung cGMP phosphodiesterase activity. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 1998 Nov 1;275(5):L931-41.
12. Sanchez LS, De La Monte SM, Filippov G, Jones RC, Zapol WM, Bloch KD. Cyclic-GMP-binding, cyclic-GMP-specific phosphodiesterase (PDE5) gene expression is regulated during rat pulmonary development. *Pediatric research*. 1998 Feb;43(2):163-8.
13. Van Marter LJ, Hernandez-Diaz S, Werler MM, Louik C, Mitchell AA. Nonsteroidal antiinflammatory drugs in late pregnancy and persistent pulmonary hypertension of the newborn. *Pediatrics*. 2013 Jan 1;131(1):79-87.
14. Lakshminrusimha S, D'Angelis CA, Russell JA, Nielsen LC, Gugino SF, Nickerson PA, Steinhorn RH. C-type natriuretic peptide system in fetal ovine pulmonary vasculature. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 2001 Aug 1;281(2):L361-8.
15. Singh SA, Ibrahim T, Clark DJ, Taylor RS, George DH. Persistent pulmonary hypertension of newborn due to congenital capillary alveolar dysplasia. *Pediatric pulmonology*. 2005 Oct;40(4):349-53.
16. Hamvas A, Cole FS, Nogee LM. Genetic disorders of surfactant proteins. *Neonatology*. 2007;91(4):311-7.

17. Shulenin S, Noguee LM, Annilo T, Wert SE, Whitsett JA, Dean M. ABCA3 gene mutations in newborns with fatal surfactant deficiency. *New England Journal of Medicine*. 2004 Mar 25;350(13):1296-303.
18. Byers HM, Dagle JM, Klein JM, Ryckman KK, McDonald EL, Murray JC, Borowski KS. Variations in CRHR1 are associated with persistent pulmonary hypertension of the newborn. *Pediatric research*. 2012 Feb;71(2):162-7.
19. Dargaville PA, South M, McDougall PN. Surfactant and surfactant inhibitors in meconium aspiration syndrome. *The Journal of pediatrics*. 2001 Jan 1;138(1):113-5.
20. Lapointe A, Barrington KJ. Pulmonary hypertension and the asphyxiated newborn. *The Journal of pediatrics*. 2011 Feb 1;158(2):e19-24.
21. Shah PS, Ohlsson A, Perlman M. Hypothermia to treat neonatal hypoxic ischemic encephalopathy: systematic review. *Archives of pediatrics & adolescent medicine*. 2007 Oct 1;161(10):951-8.
22. Van Meurs KP, Wright LL, Ehrenkranz RA, Lemons JA, Ball MB, Poole WK, Perritt R, Higgins RD, Oh W, Hudak ML, Laptook AR. Inhaled nitric oxide for premature infants with severe respiratory failure. *New England Journal of Medicine*. 2005 Jul 7;353(1):13-22.
23. Kumar VH, Hutchison AA, Lakshminrusimha S, Morin FC, Wynn RJ, Ryan RM. Characteristics of pulmonary hypertension in preterm neonates. *Journal of Perinatology*. 2007 Apr;27(4):214-9.
24. Check J, Gotteiner N, Liu X, Su E, Porta N, Steinhorn R, Mestan KK. Fetal growth restriction and pulmonary hypertension in premature infants with bronchopulmonary dysplasia. *Journal of Perinatology*. 2013 Jul;33(7):553-7.
25. Mourani PM, Abman SH. Pulmonary vascular disease in bronchopulmonary dysplasia: pulmonary hypertension and beyond. *Current opinion in pediatrics*. 2013 Jun 1;25(3):329-37.
26. Alano MA, Ngougumna E, Ostrea EM, Konduri GG. Analysis of nonsteroidal antiinflammatory drugs in meconium and its relation to persistent pulmonary hypertension of the newborn. *Pediatrics*. 2001 Mar 1;107(3):519-23.
27. Wild LM, Nickerson PA, Morin FC. Ligating the ductus arteriosus before birth remodels the pulmonary vasculature of the lamb. *Pediatric research*. 1989 Mar;25(3):251-7.
28. Dukarm RC, Morin FC, Russell JA, Steinhorn RH. Pulmonary and systemic effects of the phosphodiesterase inhibitor dipyridamole in newborn lambs with persistent pulmonary hypertension. *Pediatric research*. 1998 Dec;44(6):831-7.
29. Shaul PW, Yuhanna IS, German Z, Chen Z, Steinhorn RH, Morin 3rd FC. Pulmonary endothelial NO synthase gene expression is decreased in fetal lambs with pulmonary hypertension. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 1997 May 1;272(5):L1005-12.
30. Steinhorn RH, Russell JA, Morin 3rd FC. Disruption of cGMP production in pulmonary arteries isolated from fetal lambs with pulmonary hypertension. *American Journal of Physiology-Heart and Circulatory Physiology*. 1995 Apr 1;268(4):H1483-9.
31. Villanueva ME, Zaher FM, Svinarich DM, Konduri GG. Decreased gene expression of endothelial nitric oxide synthase in newborns with persistent pulmonary hypertension. *Pediatric research*. 1998 Sep;44(3):338-43.
32. Pearson DL, Dawling S, Walsh WF, Haines JL, Christman BW, Bazyk A, Scott N, Summar ML. Neonatal pulmonary hypertension: urea-cycle intermediates, nitric oxide production, and carbamoyl-phosphate synthetase function. *New England Journal of Medicine*. 2001 Jun 14;344(24):1832-8.
33. Storme L, Aubry E, Rakza T, Houeijeh A, Debarge V, Tourneux P, Deruelle P, Pennaforte T, French Congenital Diaphragmatic Hernia Study Group. Pathophysiology of persistent pulmonary hypertension of the newborn: impact of the perinatal environment. *Archives of cardiovascular diseases*. 2013 Mar 1;106(3):169-77.
34. Reynolds EW, Ellington JG, Vranicar M, Bada HS. Brain-type natriuretic peptide in the diagnosis and management of persistent pulmonary hypertension of the newborn. *Pediatrics*. 2004 Nov 1;114(5):1297-304.
35. Vijlbrief DC, Benders MJ, Kemperman H, van Bel F, de Vries WB. B-type natriuretic peptide and rebound during treatment for persistent pulmonary hypertension. *The Journal of pediatrics*. 2012 Jan 1;160(1):111-5.
36. Lakshminrusimha S, Russell JA, Steinhorn RH, Ryan RM, Gugino SF, Morin FC, Swartz DD, Kumar VH. Pulmonary arterial contractility in neonatal lambs increases with 100% oxygen resuscitation. *Pediatric research*. 2006 Jan;59(1):137-41.
37. Lakshminrusimha S, Russell JA, Steinhorn RH, Swartz DD, Ryan RM, Gugino SF, Wynn KA, Kumar VH, Mathew B, Kirmani K, Morin FC. Pulmonary hemodynamics in neonatal lambs resuscitated with 21%, 50%, and 100% oxygen. *Pediatric research*. 2007 Sep;62(3):313-8.
38. Lakshminrusimha S, Swartz DD, Gugino SF, Ma CX, Wynn KA, Ryan RM, Russell JA, Steinhorn RH. Oxygen concentration and pulmonary hemodynamics in newborn lambs with pulmonary hypertension. *Pediatric research*. 2009 Nov;66(5):539-44.
39. Pabalan MJ, Nayak SP, Ryan RM, Kumar VH, Lakshminrusimha S. Methemoglobin to cumulative nitric oxide ratio and response

- to inhaled nitric oxide in PPHN. *Journal of Perinatology*. 2009 Oct;29(10):698-701.
40. Carlo WA, Beoglos A, Chatburn RL, Walsh MC, Martin RJ. High-frequency jet ventilation in neonatal pulmonary hypertension. *Am J Dis Child*. 1989;143:233-8.
 41. Engle WA, Yoder MC, Andreoli SP, Darragh RK, Langefeld CD, Hui SL. Controlled prospective randomized comparison of high-frequency jet ventilation and conventional ventilation in neonates with respiratory failure and persistent pulmonary hypertension. *Journal of Perinatology*. 1997;17:3-9.
 42. Kinsella JP, Abman SH. Clinical approaches to the use of high-frequency oscillatory ventilation in neonatal respiratory failure. *Journal of Perinatology*. 1996;16:S52-5.
 43. Kinsella JP, Abman SH. High-frequency oscillatory ventilation augments the response to inhaled nitric oxide in persistent pulmonary hypertension of the newborn: Nitric Oxide Study Group. *Chest*. 1998;114:100S.
 44. Kinsella JP, Truog WE, Walsh WF, Goldberg RN, Bancalari E, Mayock DE, et al. Randomized, multicenter trial of inhaled nitric oxide and high-frequency oscillatory ventilation in severe, persistent pulmonary hypertension of the newborn. *J Pediatr*. 1997;131:55-62.
 45. Lotze A, Mitchell BR, Bulas DI, Zola EM, Shalwitz RA, Gunkel JH. Multicenter study of surfactant (beractant) use in the treatment of term infants with severe respiratory failure. *Survanta in Term Infants Study Group*. *J Pediatr*. 1998;132:40-7.
 46. Aly H, Sahni R, Wung JT. Weaning strategy with inhaled nitric oxide treatment in persistent pulmonary hypertension of the newborn. *Arch Dis Child Fetal Neonatal Ed*. 1997;76:F118-22.
 47. Sokol GM, Fineberg NS, Wright LL, Ehrenkranz RA. Changes in arterial oxygen tension when weaning neonates from inhaled nitric oxide. *Pediatr Pulmonol*. 2001;32:14-9.
 48. Baquero H, Soliz A, Neira F, Venegas ME, Sola A. Oral sildenafil in infants with persistent pulmonary hypertension of the newborn: a pilot randomized blinded study. *Pediatrics*. 2006;117:1077-83.
 49. Vargas-Origel A, Gomez-Rodriguez G, Aldana-Valenzuela C, Vela-Huerta MM, Alarcon-Santos SB, Amador-Licona N. The Use of Sildenafil in Persistent Pulmonary Hypertension of the Newborn. *Am J Perinatol*. 2009...
 50. Steinhorn RH, Kinsella JP, Butrous G, Dilleen M, Oakes M, Wessel DL. Intravenous sildenafil in the treatment of neonates with persistent pulmonary hypertension of the newborn. *J Pediatr*. 2009 In press.
 51. Lakshminrusimha S, Porta NF, Farrow KN, Chen B, Gugino SF, Kumar VH, et al. Milrinone enhances relaxation to prostacyclin and iloprost in pulmonary arteries isolated from lambs with persistent pulmonary hypertension of the newborn. *Pediatric critical care medicine: a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies*. 2009;10:106-12.
 52. McNamara PJ, Shivananda SP, Sahni M, Freeman D, Taddio A. Pharmacology of milrinone in neonates with persistent pulmonary hypertension of the newborn and suboptimal response to inhaled nitric oxide. *Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies*. 2013;14:74-84.
 53. Bassler D, Choong K, McNamara P, Kirpalani H. Neonatal persistent pulmonary hypertension treated with milrinone: four case reports. *Biol Neonate*. 2006;89:1-5.
 54. McNamara PJ, Laique F, Muang-In S, Whyte HE. Milrinone improves oxygenation in neonates with severe persistent pulmonary hypertension of the newborn. *J Crit Care*. 2006;21:217-22.
 55. Lakshminrusimha S, Steinhorn RH. Inodilators in nitric oxide resistant persistent pulmonary hypertension of the newborn. *Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies*. 2013;14:107-9.
 56. Ivy DD, Parker TA, Ziegler JW, Galan HL, Kinsella JP, Tuder RM, et al. Prolonged endothelin A receptor blockade attenuates chronic pulmonary hypertension in the ovine fetus. *J Clin Invest*. 1997;99:1179-86.
 57. Tripathi S, Saili A. The effect of steroids on the clinical course and outcome of neonates with meconium aspiration syndrome. *J Trop Pediatr*. 2007;53:8-12.
 58. Perez M, akshminrusimha S, Wedgwood S, Czech L, Gugino SF, Russell JA, et al. Hydrocortisone Normalizes Oxygenation and cGMP Regulation in Lambs with Persistent Pulmonary Hypertension of the Newborn. *Am J Physiol Lung Cell Mol Physiol*. 2011.
 59. Lakshminrusimha S, Russell JA, Wedgwood S, Gugino SF, Kazzaz JA, Davis JM, et al. Superoxide dismutase improves oxygenation and reduces oxidation in neonatal pulmonary hypertension. *Am J Respir Crit Care Med*. 2006;174:1370-7.
 60. Steinhorn RH, Albert G, Swartz DD, Russell JA, Levine CR, Davis JM. Recombinant human superoxide dismutase enhances the effect of inhaled nitric oxide in persistent pulmonary hypertension. *American Journal of Respiratory & Critical Care Medicine*. 2001;164:834-9.
 61. Wedgwood S, Lakshminrusimha S, Farrow KN,

- Czech L, Gugino SF, Soares F, et al. Apocynin improves oxygenation and increases eNOS in persistent pulmonary hypertension of the newborn. *Am J Physiol Lung Cell Mol Physiol*. 2012;302:L616-26.
62. 62. Konduri GG, Vohr B, Robertson C, Sokol GM, Solimano A, Singer J, et al. 2007;150:235-40. 40 e1.
63. 63. Robertson CM, Tyebkhan JM, Hagler ME, Cheung PY, Peliowski A, Etches PC. Late-onset, progressive sensorineural hearing loss after severe neonatal respiratory failure. *Otol Neurotol*. 2002;23:353-6.
64. 64. Lipkin PH, Davidson D, Spivak L, Straube R, Rhines J, Chang CT. Neurodevelopmental and medical outcomes of persistent pulmonary hypertension in term newborns treated with nitric oxide. *Journal of Pediatrics*. 2002;140:306-10.

