Acta Scientific PAEDIATRICS (ISSN: 2581-883X)

Volume 3 Issue 10 October 2020

Review Article

Free Radical Related Diseases of Prematurity - A Morbid Concern of Neonatal Life

Mir Mohammad Yusuf[1]*, Manzoor Hussain[2] and MAK Azad Chowdury[3]

[1]Assistant Professor, Critical Care Department, Bangladesh Institute of Child Health (BICH), Dhaka Shishu (Children) Hospital, Bangladesh

[2]Professor and Head, Department of Pediatric Medicine and Cardiology, Bangladesh Institute of Child Health (BICH), Dhaka Shishu (Children) Hospital, Bangladesh

[3]Professor and Head, Department of Neonatology, Bangladesh Institute of Child Health (BICH), Dhaka Shishu (Children) Hospital, Bangladesh

***Corresponding Author:** Mir Mohammad Yusuf, Assistant Professor, Critical Care Department, Bangladesh Institute of Child Health (BICH), Dhaka Shishu (Children) Hospital, Bangladesh.

Received: July 13, 2020

Published: September 16, 2020

Abstract

Globally one out of every ten babies is born premature (before 37 completed weeks of pregnancy). Infact, it is an universal problem. As problems with prematurity are multifocal from functional immaturity of the organs to vulnerable low temperature as well as exposure to notorious free radical injury. Free radicals generation is an unavoidable consequence of life. During the perinatal period, overproduction of free radicals and insufficiency of antioxidant leading to oxidative stress, a deleterious process. This imbalance with predisposing conditions as hypoxia, ischemia, hypoxia-ischemia-reperfusion injury or inflammation and high levels of non-protein bound iron have been thought to be factor of the so-called "free radical related diseases of prematurity" including Retinopathy of Prematurity (ROP), Bronchopulmonary Dysplasia (BPD), Necrotizing Enterocolitis (NEC), Intraventricular Hemorrahge (IVH), Renal damage, Oxidative hemolysis. The effects of antioxidant therapy remains controversial. So, very much careful individualized control of oxygenation, blood flow perfusion with adequate intake of nutrients that have antioxidant function and rational steps to management of infection seems to be the best attempt to prevent free radical aggression of prematurity. In this review, we provide an update focused on the factors influencing these diseases.

Keywords: Prematurity; Free Radicals; Oxidative Stress

Introduction

Free radicals are unchanged molecule with unpaired valency electrons in an atomic orbit. Typically, highly reactive, short lived and capable of independent existence. Free radicals trying to capture electron from other molecules to become stable and creating a chain of cascade reaction that stops when the free radical pairs up with an electron. Once started, this process can produce a cascade mechanism and disrupt the living cells by damaging biologically relevant molecules e.g. Proteins, DNA, Carbohydrate and Lipids leading to cell damage and hemostatic disruption. The most important oxygen containing free radicals are superoxide anions (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), peroxide (O_2^{0-2}), hydroperoxyl radical (HO_2^0), peroxynitrite ($ONOO^-$) etc. [1]. The present review intends to make a journey into the free radical chemical biology and to update the current knowledge about the role of OS in this pathogenesis of such neonatal diseases of prematurity- a morbid concern of neonatal life.

Free radicals and oxidative stress

Free radicals can be considered a double edged sword. Small amounts of free radicals are generated continuously in the living

body which are necessary in the function of a number of cellular signaling pathways and the induction of a mitogenic response e g. cell growth as well as physiological roles in cellular responses to noxia as in defense against infectious agents.

While the overproduction of Free Radicals and the insufficiency of antioxidant mechanism resulting oxidative stress (OS), a deleterious process and important mediator of damage to cell structures and tissues. It occurs at birth in all newborns as a consequence of the hyperoxic challenge after the transition from the hypoxic intrauterine environment to extrauterine life. During the perinatal period, the impairment of oxidative balance with predisposing conditions as hypoxia, ischemia, hypoxia-reperfusion injury or inflammation and high levels of non-protein bound iron (Figure 1) have been thought to be increased oxidative level may trigger a deleterious state of OS in neonates specially in preterm infants [1] in which lack of adequate antioxidant production for prematurity and the inability to induce antioxidant defenses during the hyperoxic challenge at birth [2] leading to activate the underlying mechanisms that resulting the onset of so-called "oxidative stress related disease in newborn" including Retinopathy of Prematurity, Bronchopulmonary Dysplasia, Necrotizing Enterocolitis, Intraventricular Hemorrahge, Renal damage, Oxidative hemolysis [3].

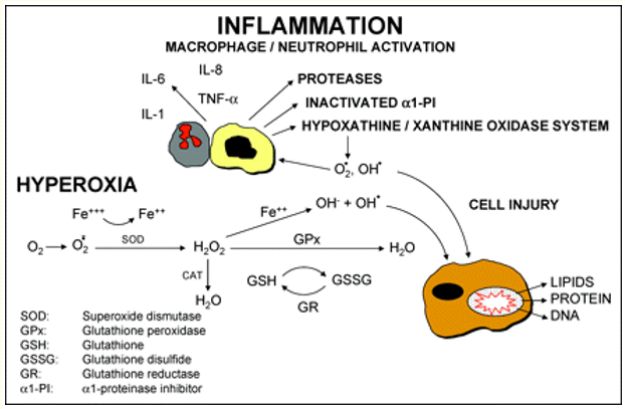

Figure 1: Inflammation/Hyperoxia mechanisms of oxidative aggression and cell damage.

Free radical diseases of prematurity

Premature infants are not developmentally prepared for the extra-uterine life in an oxygen rich environment and exhibit an unique sensitivity to oxidative stress leading so-called free radical diseases of prematurity (Figure 2).

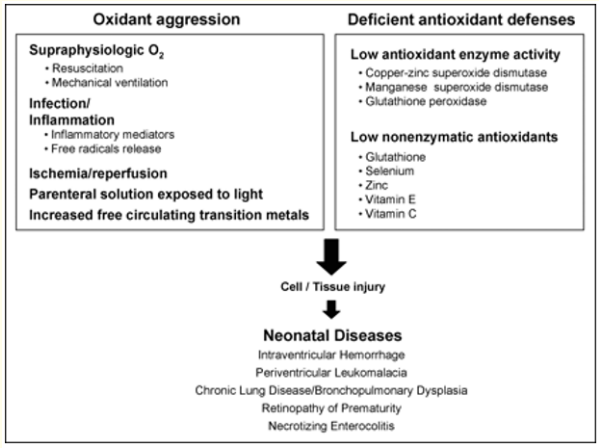

Figure 2: Factors that increase susceptibility of preterm infants to free radical related diseases.

Retinopathy of prematurity (ROP) and free radical aggression

Retinopathy of prematurity is the major cause of visual impairment and blindness in premature neonates worldwide [4]. ROP is a vasoproliferative disorder of immature retina, represents a spectrum of pathologic disturbance in normal course of retinal vascularization [5]. Several factors contribute to ROP, most of which have some aspects related to free radical aggression. Insufficient vasoconstrictor agent synthesis and increased vasodilator production from the endothelium of choroidal vessels expose the retinal vasculature to hypoxia at birth, with subsequent vasoconstriction and vaso-obliteration. Hyperoxia induced retinal vasoconstriction occurs via endothelial cell apoptosis, probably induced by peroxynitrite radicals [5]. The preterm low birth weight infant has an incomplete vascularized retina. With supplemental oxygen therapy, preterm infants have been associated with this disease. Protective effects have been shown by giving the potent antioxidant D-penicillamine and vitamin E [6].

Oxidative stress and bronchopulmonary dysplasia (BPD)

Bronchopulmonary Dysplasia is chronic lung disease (CLD) of the neonate, one of the adverse respiratory outcome having distressing definitive factors that influencing the morbidity as well as mortality of very low birth weight baby [7]. Etiology is unknown but several factors may be responsible for pathogenesis like barotraumas (in mechanical ventilated neonate), surfactant deficit, oxygen toxicity, inflammation, infections, inadequate nutrition which play key role for the development of CLD (e.g. BPD). The generation

of FR is one common pathway shared by these insults [8,9]. Overall incidence increases significantly with declining gestational age [10]. As immature lungs have less fibrotic component and a more delayed alveolar development- while exposure to prolong period of high level inspired oxygen resulting to development of BPD through free radical effect on endothelial and epithelial cell barriers that induce pulmonary edema. Also trigger inflammatory mediators, increase cytokine concentration leading to OS- resulting lung damage further exacerbate by infection (antenatal or postnatal) or by lung stretching [11]. In mechanically ventilated neonates, elevated concentration of TNF-α, interleukin and phagocyte number in tracheal secretions [12]. The lung phagocytic cells mediate their antimicrobial functions through release of lysozymes, peroxidases and proteases, but in addition, Reactive Oxygen Species (ROS) and NO were released. Activated neutrophils and pulmonary type II cells are also important inducers of the Fenton reaction, which lead to a greater ROS generation [13].

Oxidative stress and NEC (Necrotizing enterocolitis)

NEC is a syndrome of intestinal ischemic necrosis, the most common gastrointestinal emergency in preterm infants leading in significant morbidity and mortality [14]. NEC has a multifactoral etiology including low gestational age, low birth weight, low apgar scores, hyaline membrane disease, umbilical vessel catheterization and intestinal ischemia. Other risk factors are the prolonged antibiotic exposure, the genetic polymorphism in vascular endothelial growth factor, IL-10, IL-12 [15]. Among them, a common synergistic effect of OS was described through free radical production during ischemia/reperfusion injury, reintroducing O_2 in the tissues and react with hypoxanthine and xanthine oxidase to produce the superoxide anions, hydrogen peroxide and hydroxyl radical [16]. A strong association is present between intrauterine OS events and further risk of developing NEC [17]. Ozdenir., *et al*. [18] reported a significant increase of intestinal Malondialdehyde (MDA) in preterm infants with NEC. All-trans retinoic acid treatment reduced the intestinal MDA elevation, suggesting an active lipid peroxidation in NEC disease. Consistent with these results, administration of antioxidant drugs has been shown to reduce intestinal mucosal damaged by ischemia or inflammation [19].

Free radical injury and IVH (Intraventricular hemorraghe), PVL (Periventricular leucomalacia)

Intraventricular Hemorraghe in very preterm infants is a common disease associated with long term consequences [20]. The hemorrhage typically involves the periventricular germinal matrix (GM). Pathogenesis of GMH-IVH is multifactorial. An inherent fragility of the GM vasculature predisposes to hemorrhage, and fluctuation in the cerebral blood flow induces rupture of blood vessels (Figure 2). Platelet or coagulation disorders might accentuate or perpetuate the hemorrhage [21]. Recently, more detailed analyses have demonstrated the role of OS in this context [22]. During hypoxia, FR production increases, enhancing all the pathways implicated in microvascular damage and dysfunction. H_2O_2 and nitric oxide radicals (NO^-) are able to activate the soluble enzyme guanylate cyclase, which catalyses the formation of the cyclic "second messenger" guanosine monophosphate (cGMP). cGMP modulates the function of protein kinases, ion channels and other important targets, leading to altered dilatation of arterioles, enhanced fluid filtration, leukocyte plugging in capillaries, and release of inflammatory mediators and platelet activation [23]. The oxidative events that trigger the initiation of bleeding into the germinal matrix promote a cascade leading to disruption of tight junctions, to the increased blood-brain barrier permeability and to microglial activation within the developing periventricular white matter. These events are mediated by cytokines (IL-1ß and TNF) and NO. Finally, reactive microglia release ROS, which in turn not only contribute to endothelial damage but also alter hemostasis and increase anaerobic metabolism [24] leading to necrosis of the cerebral white matter specially to adjacent external angles of lateral ventricles resulting periventricular leukomalacia [25].

Resuscitation of an asphyxiated neonate

An acute increase in oxygenation during transition from an intrauterine hypoxic to extra-uterine normoxic environment leads to production of the ROS. Asphyxiated newborns have been resuscitated using 100% oxygen. However, scientific support for this action never has been established, and concern have been raised regarding the potentially adverse effects of exposure to excessive oxygen on breathing physiology, cerebral circulation, and tissue damage [26].

Renal damage and free radical

The kidney, a primary target organ is often severely damaged after asphyxia. Medullary thick ascending limb cells and the pars recta of the proximal tubule are specially sensitive to injury after hypoxia or ischemia. FR- mediated lipid peroxidation has been implicated as a mechanism of tissue injury during ischemia. Lipid

peroxidation products affect renal function directly by causing renal vasoconstriction or decreasing the glomerular capillary ultrafiltration coefficient and thus the glomerular filtration rate [27].

Patent ductus arteriosus (PDA)

PDA is seen more frequently (20% to 60%) in preterm infants, particularly those born at < 30 weeks' gestation [28]. Hemodynamically significant PDA may cause hypoperfusion of organs [29]. Hypoperfusion, ischemia, and chronic hypoxia lead to production of oxygen radicals [30].

Oxidative hemolysis

Red blood cells (RBC) have a wide array of antioxidant enzymes defending against attacks by FRs. Superoxide dismutase (SOD), catalase (CT), and glutathione peroxidase (GPX) represent great antioxidant resources of these cells against stressors associated with prematurity [31]. Otherwise, after exposure of RBCs to OS, increased susceptibility of red blood cells to the oxidative damage [32].

Antioxidant defense - to oxidative stress

Neonates, particularly those born prematurely, have an incomplete detox response to free radicals. To passive oxidative stress-related damage in newborns, many therapeutic strategies to promote antioxidant status in newborns have been proposed. Supplementation with enzymatic and/or nonenzymatic antioxidants have been experimented with, but the results were mixed [33]. It was reported that an antioxidant supply can prevent oxidant stress-related disease, support the immune system of neonates, reduce stillbirths and enhance neonatal vitality [34]. Antioxidant defense mechanisms include:

- **Enzymatic endogenous antioxidant:** Normally are found in the body. A complex interaction between reducing and oxidizing molecules that defenses the cellular milieu necessary for maintaining cellular, placental, fetal and postnatal growth [35]. Such enzymes have low activity in preterm infants and cannot balance excessive ROS production. The most important antioxidant enzymes are Superoxide dismutase (SODs), found in almost all aerobic cells and in extracellular fluids [36]. SODs are a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide. In humans, three forms of superoxide dismutase are present. SOD1 is located in the cytoplasm, SOD2 in the mitochondria, SOD3 is extracellular. SOD1 is a dimer and SD3 is tetramer both contain copper and zinc, while SOD2 is tetramer but contain manganese in its reactive [37]. Surech., *et al*. [38] concluded that intratracheal administration of recombinant human copper zinc SOD caused an improvement in the antioxidant activity of enzymes in premature infants. Glutathione peroxidase (GPx) in mitochondria and catalase (CAT) in peroxisomes catalyze the reaction of H_2O_2 to molecular oxygen and water. These enzymes, together with vitamin E, play an important role in the peroxidation of polyunsaturated free fatty acids from cell membranes [39].

- **Non-enzymatic antioxidants:** Nutrients such as selenium, copper and zinc may have antioxidant functions as components of antioxidant enzymes. Vitamins E, Vitamin C, ceruloplasmin, transferin, glutathione (GSH), bilirubin and uric acid are considered to have antioxidant properties [39]. Potentially, neonatologists could administer these nutrients in adequate quantities to preterm infants in whom quantities are deficient because fetal uptake occurs in the final 3 months of pregnancy. However, antioxidant action to combat oxidative stress and prevent diseases of the preterm infant in an open field with studies returning contradictory results, meta-analyses with few trails and few encouraging results.

Ascorbic acid (Vitamin C) - is a monosaccharide antioxidant found in both animals and plants. As it cannot be synthesized in humans and must be obtained from the diet, as a vitamin [40]. Ascorbic acid is a reducing agent, can reduce and thereby neutralize ROS such as hydrogen peroxide [41]. Plasma vitamin C concentrations in preterm infants decline rapidly after birth, and supplementation could be helpful in protecting lipids from peroxidation and lungs from inflammation [39].

Tocopherols (Vitamin E) - Vitamin E is fat soluble vitamin and antioxidant properties [42] by blocking natural peroxidation of polyunsaturated fatty acids (PUFAs) found in the lipid layers of cellular membranes, substituting oxygen in the reaction. Approximately 90% of Vitamin E is located in adipose tissue, their vitamin E reserve is lower. Vitamin E supplementation reduced the risk of severe ROP and blindness, but only with doses greater than 3.5 mg/dl [43].

Ceruloplasmin/Transferin/Iron

ROS production is enhanced by the presence of free iron. Under normal circumstances, the iron-binding activity of transferrin

and ferroxidase activity of ceruloplasmin that catalyzes oxidation from ferrous to ferric iron results in these substances behaving as antioxidants and preventing free iron aggression [3]. Plasma concentrations of ceruloplasmin and transferrin are low in preterm infants, and ceruloplasmin synthesis increases in the 6 to 12 weeks after birth. Reduced transferrin and ceruloplasmin concentrations have been observed in asphyxiated preterm infants prior to the development of periventricular-intraventricular brain hemorrhage [44]. Investigators have expressed concern that large intakes of enteral or parenteral iron may overwhelm the iron binding capacity of preterm infant serum, resulting in cell membrane oxidative stress. Iron overload remains of concern in sick preterm infants receiving many blood transfusions. It is important to know an individual preterm infant's iron status at birth to determine subsequent iron requirements.

Bilirubin

In vitro bilirubin has been demonstrated as a potent antioxidant scavenger of peroxyl radicals, with a similar action to vitamin E [39]. In the oxidative process, bilirubin reverts to its precursor biliverdin, a nontoxic product. A direct relationship between the total antioxidant status of newborn plasma and bilirubin concentrations was found in both preterm and term infants [45]. Exchange transfusion by lowering the serum bilirubin concentration, decreases plasma antioxidant capacity.

Melatonin

Melatonin and its metabolites are strong antioxidants and they have important functions to prevent mutilation of crucial molecules by free radicals. It was demonstrated that melatonin reduces all aspects of the ensuing damage in the ischemia and subsequent reperfusion model of the heart, kidney, liver, intestine and brain in cases of excessive of ROS [33]. In 2004, Gitto., *et al*. showed that melatonin treatment can reduce the severity of RDS in preterm newborns by reducing inflammation [46]. Additionally, it has been shown that melatonin can be used in the treatment of hypoxic-ischemic encephalopathy in newborns [47].

Uric acid

Uric acid accounts for roughly half the antioxidant ability of the plasma. In fact, uric acid may have substituted for ascorbate in human evolution [48]. However, like ascorbate, uric acid can also mediate for the production of active oxygen species.

Conclusion

Although free radical injury is well recognized in the pathogenesis of free radical related prematurity diseases, the effects of antioxidant therapy remains controversial. So, very much careful individualized control of oxygenation, blood flow perfusion with adequate intake of nutrients that have antioxidant function and rational steps to management of infection seems to be the best attempt to prevent free radical aggression of prematurity.

Bibliography

1. Weinberger B., *et al*. "Oxygen toxicity in premature infants". *Toxicology and Applied Pharmacology* 181 (2002): 60-67.

2. Shoji H and Koletzko B. "Oxidative stress and antioxidant protection in the perinatal period". *Current Opinion in Clinical Nutrition and Metabolic Care* 10 (2007): 324-328.

3. O'Donovan DJ and Fernandes JC. "Free radicals and diseases in premature infants". *Antioxide Redox Signal* 6 (2004): 169-176.

4. Bas AY., *et al*. "Incidence, risk factors and severity of retinopathy of prematurity in Turkey (TR-ROP study): a prospective, multicentre study in 69 neonatal intensive care units". *British Journal of Ophthalmology* 102.12 (2018): 1711-1716.

5. Wheatley CM., *et al*. "Retinopathy of prematurity: recent advances in our understanding". *British Journal of Ophthalmology* 86 (2002): 697-701.

6. Raju TNK., *et al*. "Vitamin E prophylaxis to reduce retinopathy of prematurity: a reappraisal of published trials". *The Journal of Pediatrics* 131.6 (1997): 844-850.

7. Banks BA., *et al*. "Plasma 3-nitrotyrosine is elevated in premature infants who developed bronchopulmonary dysplasia". *Pediatrics* 101 (1998): 870-874.

8. Attar MA and Donn SM. "Mechanisms of ventilated lung injury in premature infants". *Seminars in Neonatology* 7 (2002): 353-360.

9. Banchan E., *et al*. "Bronchopulmonary dysplasia: changes in pathogenesis, epidemiology at definition". *Seminars in Neonatology* 8 (2003): 63-71.

10. Jensen EA and Schmidt B. "Epidemiology of bronchopulmonary dysplasia". *Birth Defects Research Part A. Clinical and Molecular Teratology* 100.3 (2014): 145-157.

11. Perrone S., *et al*. "Oxidative stress and bronchopulmonary dysplasia". *Journal of Clinical Neonatology* 1.3 (2012): 109-114.

12. Groneck P and Speer CP. "Interleukin-8 in pulmonary effluent fluid of preterm infants". *The Journal of Pediatrics* 123.5 (1993): 839-840.

13. Margraf IR., *et al*. "Morphometric analysis of the lung in bronchopulmonary dysplasia". *The American Review of Respiratory Disease* 143.2 (1991): 391-400.

14. Caplan MS and Fanaroff A. "Necrotizing: a historical perspective". *Seminars in Perinatology* 41.1 (2017): 2-6.

15. Alexander VN., *et al*. "Antibiotic exposure in the newborn intensive care unit and the risk of necrotizing enterocolitis". *The Journal of Pediatrics* 159.3 (2011): 392-397.

16. Perrone S., *et al*. "The role of oxidative stress on necrotizing enterocolitis in very low birth weight infants". *Current Pediatric Reviews* 10.3 (2014): 202-207.

17. Perrone S., *et al*. "May oxidative stress biomarkers in cord blood predict the occurrence of necrotizing enterocolitis in preterm infants?". *The Journal of Maternal-Fetal and Neonatal Medicine* 25.1 (2012): 128-131.

18. Ozdemir R., *et al*. "All-trans-retinoic acid attenuates intestinal injury in neonatal necrotizing enterocolitis". *Neonatology* 104.1 (2013): 22-27.

19. Darlow BA., *et al*. "Vitamin A supplementation to prevent mortality and short- and longterm morbidity in very low birth weight infants". *Cochrane Database of Systematic Reviews* 8 (2016): CD000501.

20. Volpe JJ. "Perinatal brain injury: from pathogenesis to neuroprotection". *Mental Retardation and Developmental Disabilities Research Reviews* 7.1 (2001): 56-64.

21. Ballabh P. "Pathogenesis and prevention of intraventricular hemorrhage". *Clinics in Perinatology* 41.1 (2014): 47-67.

22. Perrone S., *et al*. "Neonatal brain hemorrhage: the role of nonprotein-bound iron". *Journal of Pediatric Biochemistry* 6.2 (2016): 88-91.

23. del Zoppo GJ and Mabuchi T. "Cerebral microvessel responses to focal ischemia". *Journal of Cerebral Blood Flow and Metabolism* 23.8 (2003): 879-894.

24. Ulfig N., *et al*. "Brain macrophages and microglia in human fetal hydrocephalus". *Brain and Development* 26.5 (2004): 307-315.

25. Yonezawa M., *et al*. "Cystine deprivation induces oligodendroglia death: rescue by free radical scavengers and by a diffusible glial factor". *Journal of Neurochemistry* 67 (1996): 566-573.

26. International Liaison Committee on Resuscitation. "Neonatal resuscitation". *Resuscitation* 67 (2005): 293-303

27. Bomzon A., *et al*. "Bile acids, oxidative stress, and renal function in biliary obstruction". *Seminars in Nephrology* 17.6 (1997): 549-562.

28. Fred F and Ferri MD. "Ferri's Clinical Advisor". Philadelphia, Pa, USA: Elsevier (2016).

29. Hamrick SEG and Hansmann G. "Patent ductus arteriosus of the preterm infant". *Pediatrics* 125.5 (2010): 1020-1030.

30. Jensen SJK. "Oxidative stress and free radicals". *Journal of Molecular Structure: THEOCHEM* 666 (2003): 387-392.

31. Belvisi E., *et al*. "Enzyme activities in erythrocytes of term and preterm newborns". *Journal of Pediatric Biochemistry* 6.2 (2016): 114-118.

32. Ferrali M., *et al*. "Iron release and membrane damage in erythrocytes exposed to oxidizing agents, phenylhydrazine, divicine and isouramil". *Biochemical Journal* 285.1 (1992): 295-01.

33. Marseglia L., *et al*. "Oxidative stress-mediated damage in newborns with necrotizing enterocolitis: a possible role of melatonin". *American Journal of Perinatology* 32.10 (2015): 905-909.

34. Mutinati M., *et al*. "Oxidative stress in neonatology: a review". *Reproduction in Domestic Animals* 49.1 (2014): 7-16.

35. Buonocore G., *et al*. "Oxidative stress in preterm neonates at birth and on the seventh day of life". *Pediatric Research* 52 (2002): 46-49.

36. Johnson F and Giulivi C. "Superoxide dismutases and their impact upon human health". *Molecular Aspects of Medicine* 26 (2005): 340-352.

37. Zelko I., *et al*. "Superoxide dismutase multigene family: A comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression". *Free Radical Biology and Medicine* 33 (2002): 337-349.

38. Suresh GK., *et al*. "Superoxide dismutase for preventing chronic lung disease in mechanically ventilated preterm infants". *Cochrane Database of Systematic Reviews* 1 (2001): CD00196865.

39. Thibeault DW. "The precarious antioxidant defenses of the preterm infant". *American Journal of Perinatology* 4 (2000): 167-180.

40. Smirnoff N. "L-ascorbicacid biosynthesis". *Vitamins and Hormones* 2 61 (2001): 241-266.

41. Padayatty S., *et al*. "Vitamin C as an antioxidant: Evaluation of its role in disease prevention". *Journal of the American College of Nutrition* 22 (2003): 18-35.

42. Herrera E and Barbas C. "Vitamin E: Action, metabolism and perspectives". *Journal of Physiology and Biochemistry* 57 (2001): 43-56.

43. Binivale MA., *et al*. "The role of nutrition in the prevention and management of bronchopulmonary dysplasia". *Seminar in Perinatology* 30 (2006): 200-208.

44. Lackmann GM., *et al*. "Reduced iron-associated antioxidants in premature newborns suffering intracerebral hemorrhage". *Free Radical Biology and Medicine* 20 (1996): 407-409.

45. Wiedemann M., *et al*. "Neonatal blood plasma is less susceptible to oxidation than adult plasma owing to its higher content of bilirubin and lower content of oxidizable fatty acids". *Pediatric Research* 53 (2003): 843-849.

46. Gitto E., *et al*. "Oxidative and inflammatory parameters in respiratory distress syndrome of preterm newborns: beneficial effects of melatonin". *American Journal of Perinatology* 21.4 (2004): 209-216.

47. Robertson NJ., *et al*. "Melatonin augments hypothermic neuroprotection in a perinatal asphyxia model". *Brain* 136.1 (2013): 90-105.

48. Jaeschke H., *et al*. "Mechanisms of hepatotoxicity". *Toxicological Sciences* 65 (2002): 166-176.