Accepted Manuscript

Oxidative stress - Related spontaneous preterm delivery challenges in causality determination, prevention and novel strategies in reduction of the sequelae

Vedran Stefanovic, Sture Andersson, Maximo Vento

PII: S0891-5849(18)32495-X

DOI: https://doi.org/10.1016/j.freeradbiomed.2019.06.008

Reference: FRB 14307

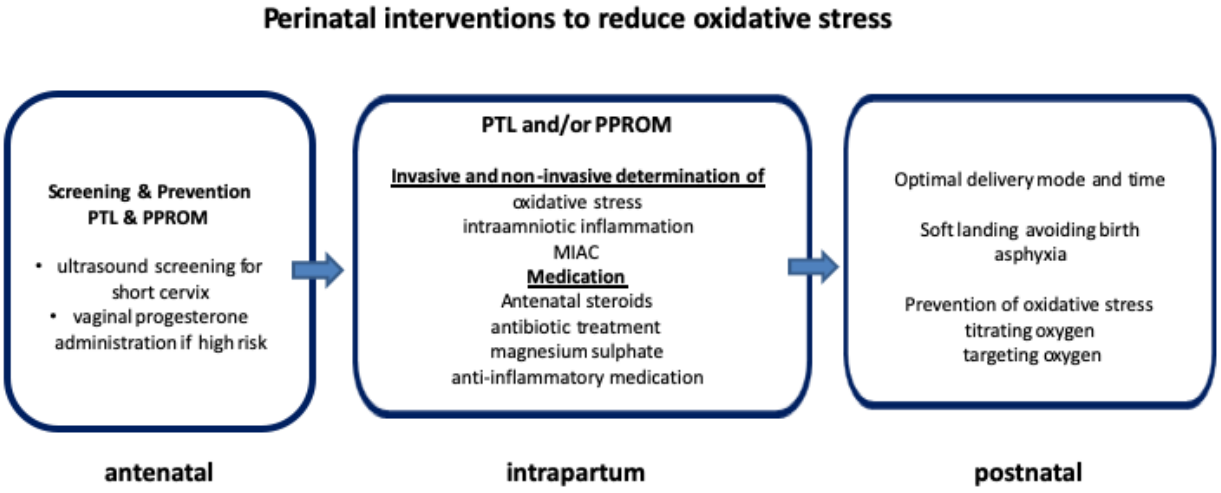
To appear in: *Free Radical Biology and Medicine*

Received Date: 2 December 2018

Revised Date: 5 June 2019

Accepted Date: 6 June 2019

Please cite this article as: V. Stefanovic, S. Andersson, M. Vento, Oxidative stress - Related spontaneous preterm delivery challenges in causality determination, prevention and novel strategies in reduction of the sequelae, *Free Radical Biology and Medicine* (2019), doi: https://doi.org/10.1016/j.freeradbiomed.2019.06.008.



Title:

Oxidative stress - related spontaneous preterm delivery

Challenges in causality determination, prevention and novel strategies in reduction of the sequelae

Authors:

Vedran Stefanovic[1], Sture Andersson[2], Maximo Vento[3,4*]

Affiliations

[1] Department of Obstetrics and Gynecology, Fetomaternal Medical Center, Helsinki University and Helsinki University Hospital, Finland.

[2] Children's Hospital, Pediatric Reserch Center, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

[3]Division of Neonatology; University and Polytechnic Hospital La Fe, Valencia, Spain.

[4]Neonatal Research Group; Health Research Institute La Fe, Valencia, Spain.

***Corresponding Author:**

Maximo Vento MD PhD; Health Research Institute La Fe; Division of Neonatology; University and Polytechnic Hospital La Fe; Avenida Fernando Abril Martorell 106; 46026 Valencia; Spain.

Email address: Maximo.vento@uv.es

Funding

MV acknowledges PI17/0131 grant from the Instituto de Investigación Sanitaria Carlos III (Spanish Ministry of Science, Innovation and Universities) and RETICS funded by the PN 2018-2021 (Spain), ISCIII- Sub-Directorate General for Research Assessment and Promotion and the European Regional Development Fund (FEDER), reference RD16/0022.

VS acknowledges Helsinki University Hospital Research EVO Grants and Ms. Maaria Puupponen (University of Helsinki) for her technical assistance in the preparation of this manuscript.

Key Words

Prematurity, oxidative stress, biomarkers, amniotic fluid, placenta, amniocentesis, intrauterine inflammation, microbial invasion of the amniotic cavity

Conflicts of Interest

Authors declare no having conflicts of interest

Abstract

Spontaneous preterm birth (PTB) is one of the major complications of pregnancy and the main cause of neonatal mortality and morbidity. Despite the efforts devoted to the understanding of this obstetrical syndrome and improved medical care, there has been a tendency for the PTB rate to increase in the last decades globally. The costs of the screening for spontaneous PTB, its management, and treatment of the sequelae represent a major burden to the health service economy of high-income countries. In this scenario, it has been widely acknowledged that oxidative stress (OS) plays an important role in the pathogenicity of human disease in wide range of areas of medicine. There is an emerging evidence that an imbalance between pro-and-antioxidants may be associated with spontaneous PTB. However, there are still many controversies on the mechanisms by which OS are involved in the pathogenesis of prematurity. Moreover, the crucial question whether the OS is the cause or consequence of the disease is yet to be answered.

The purpose of this article is to briefly summarize the current knowledge and controversies on oxidative stress–related spontaneous PTB and to give a critical approach on future perspectives on this topic as a classical example of translational medicine. Placenta-mediated pregnancy adverse outcome associated with OS leading to iatrogenic PTB (e.g. pre-eclampsia, intrauterine growth restriction, gestational diabetes) will not be discussed.

Introduction

Preterm birth, defined as birth before 37 gestational weeks is the leading cause of neonatal morbidity and mortality (1). Data from 184 countries showed that the global average PTB rate in

2010 was 11.1%, ranging from about 5% in northern Europe to 18% in subSaharian Africa (2). During the past few decades, the perinatal outcomes of preterm neonates have improved significantly; however, rates of spontaneous PTB have essentially remained constant despite some promissing interventions in its prevention (3). In high-income countries, the majority of PTBs occur spontaneously, either with intact membranes or preterm premature rupture of membranes (PPROM) (4). The costs of the screening for PTB, its management and treatment of the sequelae represent a major burden to the health service economies (1,2).

Although PTB is a complex condition resulting from multiple etiologic factors, it is well accepted and documented that both infection and inflammation represent highly significant risk factors for PTB (3). However, despite the clinical and public health significance of PTB and recent research achievements, the correlation between infectious agents, molecular and immunological pathways and triggering factors for spontaneous PTB remain poorly understood reflecting the complexity of this obstetrical syndrome. It has been widely acknowledged that oxidative stress (OS) may play an important role in the pathogenesis of human disease and constitutes a major topic in all areas of medicine (5). In this regard, there is emerging evidence that an imbalance between oxidants and antioxidants may be associated with PTB (6). Table 1 lists recent interventional studies aiming to reduce the incidence/severity of oxidative stress in preterm birth.

Oxidative stress, antioxidants and oxidative damage biomarkers

Oxidative stress

Oxygen is the final acceptor of electrons generated during cellular metabolic activity which imply mainly the oxidases'activity (xanthine oxido-reductase; NADPH oxidases), nitric oxide synthase (NOS), and the mitochondrial oxidative phosphorylation. Under physiologic conditions, di-oxygen undergoes a tetravalent reduction and combined with 2 protons will produce water, a neutral element. However, under pathologic conditions oxygen may be incompletely reduced to form reactive oxygen species (ROS). Monovalent reduction, is the most common incomplete reduction

and results in the generation of anion superoxide (•O2-). Further reduction with 2 or 3 electrons leads to the formation of hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH), respectively the latter being highly reactive. In addition, nitric oxide (NO•) may combine with ROS especially anion superoxide to conform peroxynitrite ($ONOO^-$) a reactive nitrogen species (RNS). ROS and RNS may behave as free radicals which are highly reactive species with an extremely short half-life and with great affinity for electrons of nearby molecules. Free radicals react with proteins, DNA, RNA, glucids or free fatty acids causing alteration of both their structure and function, begetting them as free radicals and triggering chain reactions (7,8,9). H_2O_2 is a ROS but not a free radical and acts as a cell-signaling molecule that regulates the redox-mediated oxidation of cysteine residues within proteins and plays, therefore an important role in intracellular antioxidant defense and the redox regulation code (10).

Oxidative stress is broadly referred to as an imbalance between the generation of ROS and RNS and their clearance by the antioxidant defense system in favor of the former (8). ROS are generated in different intracellular compartments, such as the plasma membrane, the peroxisomes, the endoplasmic reticulum, and in the cytosol. The main endogenous source of ROS is the mitochondrial electron transport chain during respiration, being the rate of ROS production proportional to the rate of mitochondrial respiration. In addition, free circulating transition metals (Fenton chemistry) and enzymes that catalyze ROS-generating chemical reactions, such as peroxidases, NADPH oxidase, xanthine oxidase, lipoxygenases, myeloperoxidase, nitric oxide synthase, and cyclooxygenases, are also relevant sources of ROS. However, there are circumstances such as infection, radiation, or inflammation in which other subcellular structures such as peroxisomes, Golgi apparatus, or endoplasmic reticulum become sources of ROS that outweigh the mitochondria (8).

Antioxidant defenses

The redox balance of the different cellular compartments is tightly regulated by the enzymatic and non-enzymatic antioxidant defense systems. Antioxidants can directly reduce free radical and attenuate their reactivity, modify their chemical structure to neutral species, quench damage caused by free radical chain reactions or sequester transition metals to avoid Fenton chemistry (11). According to their characteristics there are low molecular weight antioxidants such as glutathione (GSH), vitamins C and E, or circulating bilirubin or uric acid among others. These compounds are electron donors that reduce free radicals attenuating their reactivity. In addition, there are enzymatic antioxidants that neutralize free radicals. The most relevant are the group of superoxides dismutases, catalase, glutathione redox cycle enzymes, DT diaphorase, and the peroxiredoxin. Finally, high molecular non-enzymatic proteins (thioredoxin, glutaredoxin, and metalothioneins) that have different antioxidant properties such as sequestering transition metals to avoid Fenton chemistry, or act as electron donors, or physically quench chain reactions (12). In a pro-oxidant situation, non-enzymatic antioxidants constitute the first barrier of defense directly reducing or quenching free radicals. Simultaneously, there is an activation of a coordinate array of antioxidant defenses including nuclear factor (erythroid -derived)-related factors (NrFs) family of transcription factors. Among these, NrF2 is the mos relevant and is involved in a wide range of signal transduction pathways that deal with oxidative stimulation (13). Under stressful conditions, NrF2 translocates to the nucleus where it activates the antioxidant responsive element (ARE) gene expression. As a consequence, an array of antioxidant enzymes will be expressed that will neutralize free radicals and balance the oxidant status that leads to the promoting cell survival (14). NrF2 target genes have multiple biological functions such as being responsible for detoxification (*NQ01, GSTs, AKRs)* and the activation of specific antioxidant enzymes such as glutathione peroxidase, glutamate-cysteine ligase, glutamate-cysteine ligase modifier subunit, glutathione reductase, superoxide dismutases family, thioredoxin and peroxiredoxins (15). Under physiologic

conditions, antioxidant response maintains low concentrations of ROS and RNS which act as regulatory mediators in signaling processes. However, at moderate or high concentrations, they act as free radicals altering cellular function and the redox status indispensable for cell reproduction, growth, and differentiation (16).

Oxidative stress and damage biomarkers

The extremely short half life of free radicals doesn't allow to directly measure their concentration in the clinical setting. Therefore, different analytical strategies have been employed to assess the oxidant status. Hence, extracellular (plasma, serum, urine, amniotic fluid, cerebral spinal fluid) or intracellular (erythrocytes, leukocytes) compartments oxidant status has been performed determining the reduced to oxidized quotient of molecules containing sulfur-disulfide bonds such as reduced to oxidized glutathione (GSH/GSSG), cysteine-cystine (Cys-H/Cys-SS) or reduced to oxidized thioredoxin (Trx-SH/Trx-SS) or the activity of enzymatic antioxidants such as SOD, CAT or GPX (12,17). Recently, our lab has developed a new method based on surface enhanced Raman spectroscopy (SERS) to easily analyze GSH.We have performed analytical monitoring with high reliability by using a silver colloid that enhances the GSH signal and allows for the accurate measurement of microvolumes (20 mcl) of blood. This novel analytical tool has been validated and is extremely suitable, using portable optical sensor device to perform point-of-care (POC) testing of OS levels in newborns (18).

Increasingly, the use of highly precise analytical platforms such as high-performance liquid or gas chromatography coupled to mass spectrometry has allowed to determine an array of byproducts resulting from the oxidative damage to specific tissue components such as protein, lipids, carbohydrates or nucleic acids measured in different biofluids are used as surrogates for oxidative stress (17).

Free radicals provoke oxidative modifications of the chemical structure of proteins such as oxidation of sulfur residues to sulfide, hydroxylation of aromatic groups, nitration or chlorination of

tyrosine residues, or conversion to carbonyl derivatives. Among the most widely employed biomarkers are related with the oxidation of Phenyalanine (Phe). Hydroxyl radicals convert Phe to ortho-tyrosine (o-Tyr) or meta-tyrosine (m-Tyr), peroxynitrite leads the formation of 3-nitrotyrosine ($3NO_2$-Tyr) and hypochlorous acid to 3-chlorotyrosine (3Cl-Tyr), the latter ones relative to protein nitration and inflammation (19).

Intrauterine infection, inflammation and oxidative stress in preterm birth etiology

Intrauterine infection and inflammation are the leading etiological factors in the pathogenesis of spontaneous PTB (20) and risk factors for brain damage in the neonate, especially in those born very prematurely (21). Pro-inflammatory stimuli, such as lipopolysaccharide (LPS) and other bacterial, viral or fungal byproducts often recognized by the toll-like receptor (TLR) pathways, or intercellular signaling mediators often recognized as members of the TNF-α superfamily or immunoglobulin domain-containing receptors are strong inducers of NK-kB activity in many cell types including placenta. NFkB is responsible for activating its target genes involved in multiple cellular processes, including inflammation, survival, proliferation, differentiation, apoptosis or cell cycle arrest. Once activated, NF-kB translocates into the nucleus to activate the expression of an ample array of genes that constitute the inflammatory response (22, 23). Hence, genes encoding inflammatory mediators that control cell activation and chemotaxis, such as the proinflammatory cytokines TNF-alpha, IL-1 and IL-12, chemokines such as monocyte chemotactic protein (MCP)-1, interferon (IFN)-inducible protein (IP)-10 and RANTES. In addition, large numbers of NF-kB dependent genes contribute to the innate immune response, anti-microbial peptide beta-defensin-2 or C-reactive protein (22, 23).

Intrauterine infection and inflammation cause an increased production of proinflammatory cytokines in the fetal brain that triggers the activation of microglia with the consequent release of free radicals that inevitably cause injury to oligodendrocytes and neurons in the most critical stage of fetal brain development (24, 25).

In experimental studies in different mammal species, the antioxidant enzyme (AO) levels are not sufficiently efficient to overcome fetal to neonatal transition until late in gestation (26). Hence, during the final 10–15% of gestation, there is an exponential increase of 150–200% activity in the AO enzymes present in lung tissue. (27). Consequently, preterm infants with an immature AO enzyme system will be predisposed to oxidative stress-associated lung damage and respiratory insufficiency (28). Notably, the use of antenatal steroids increases the AO enzyme activity, especially in preterm females improving their ability to satisfactorily transit into the extra uterine world (29).

Little is known about OS in the human fetus and most of the published data are based on results obtained in animal models. However, in type 1 diabetes or insulin-treated gestational diabetes the association of chronic fetal hypoxia with OS has been recently studied using high performance liquid chromatography coupled to quadrupole mass spectrometry (HPLC-MS/MS) after validating a specific method for the determination of markers of oxidative damage to proteins (meta-tyrosine/phenylalanine ratio) and DNA (8-oxo-dihydroguanosine/2-dihydoguanosine ratio), and nitrosative stress (3-NO-tyrosine) in amniotic fluid. Results clearly established a correlation between amniotic fluid markers of oxidative and nitrosative stress and erythropoietin an indirect marker of fetal hypoxia (30). Moreover, fetus with hypoxic events in utero and poor postnatal adaptation evidenced by low Apgar score and metabolic acidosis exhibited higher concentrations of lipid peroxidation biomarkers in cord blood, specifically 8-iso-15 (R)-PGF2α and total isoprostanes than normal controls (31).

Buhimschi et al demonstrated that term labor triggers a compensatory up-regulation of nonenzymatic antioxidant reserve in the fetal red blood cell and plasma compartments which serves as a protective mechanism to the relative postnatal hyperoxia (32). Conversely, the decreased nonenzymatic antioxidant reserve after preterm labor and delivery would enhance the vulnerability to free radical damage of the preterm neonate. Authors speculate that the two compartments of

nonenzymatic antioxidant reserve have distinct defensive roles in the perinatal period. Oxidative stress is linked to both infection and inflammation and may contribute to the perinatal brain damage in premature infants (33). Mass spectrometry methods to study intraamniotic fluid infection (IAA) have been recently validated following the stringent FDA requirements (34, 35). These methods have allowed to approach with high accuracy and reliability the influence of oxidative stress and inflammation in the clinical setting (34, 35). Spontaneous PTB may occur with intact membranes or after premature preterm rupture of membranes (PPROM) with different latency. Athough it has been proposed that the etiology of PTB with intact membranes differs from that with PPROM (36, 37, 38), it has been demonstrated by amniocentesis that IAI and microbial invasion of the amniotic cavity share the similar pattern in both entities (39, 40). The common finding of these studies is that the proportion of samples demonstrating severe inflammation and/or microbial invasion of the amniotic cavity is much higher in pregnancies with lower gestational age, especially before 25 weeks. Furthermore, not only the presence, but the magnitude of IAI was much higer in amniotic fluid samples from lower gestational ages than later on. These findings may ultimately question the results of meta-analyses on antibiotic use in women with preterm labour with intact membranes. Meta–analysis of 11 included trials showed a reduction in maternal infection with the use of prophylactic antibiotics but failed to demonstrate benefit or harm for any of the pre–specified neonatal outcomes (41). Additionally, there was a suggestion of harm with almost significant increase in neonatal mortality in the antibiotic group. These trials included 7428 women, but amniocentesis was not performed in any of them. By combining histological diagnosis of chorioamnionitis with quantitative brain MRI, Anblagan et al have recently shown that inflammation in utero contributes to altered microstructure in major white matter tracts of preterm infants at term equivalent age (42). Chorioamnionitis is a well-known risk factor for fetal and neonatal infection as up to 17% of preterm newborns whose mother has chorioamnionitis develop EOS in both PTL and PPROM (43).

Placental histopathology may have a role in risk stratification for trials of immune-modulatory therapies designed to improve outcome (43). However, it has been demonstrated that twenty-four percent of patients with clinical chorioamnionitis in preterm gestations have no evidence of either culture-proven intraamniotic infection or IAI, and false-positive diagnosis of clinical chorioamnionitis in preterm gestation may lead to unwarranted preterm delivery. (44).

Amniocentesis and placental investigations-evidence for inflammation, infection and oxidative stress-related preterm birth.

Amniocentesis is a safe and feasible procedure that should be performed in selected cases of patients with threatened PTB either with intact membranes or PPROM (45,46,47,48). The information about the status of intrauterine environment (regardless of determination the microbial invasion of the amniotic cavity (MIAC), inflammation and/or oxidative stress) obtained by amniocentesis outweigh its potential risks and costs. These results may properly identify women that would not benefit of certain obstetrical procedures (emergency cerclage, tocolysis) and those that would deliver within a very short interval after amniocentesis (49, 50). This would allow better timing of antenatal steroids for lung maturation, magnesium sulfate for neuroprotection, and optimal antibiotic administration in case of MIAC. Also, the results of amniocentesis would help to restrain from unnecessary and potentially harming procedures as mentioned above. Although, taking into consideration that IAI is present in approximately 14% of women with PTB (with or without PPROM), not all women would need this procedure; however, the non-invasive stratification of the at-risk patients has not been successful so far (51). Although it seems that antibiotic administration rarely eradicates the IAI in women with PPROM. There is an evidence of a sub-group of patients with documented inflammation of the amniotic cavity which demonstrated a decrease in the intensity of the inflammatory process after antibiotic administration (52). Currently, preterm labor is unfortunately too often considered as irreversible in the setting of an infection. This nihilistic view should be challenged due to several reasons: infection-induced preterm labor in the

non-human primate model was significantly delayed by anti-inflammatory and antibiotic administration, and recent data suggest that rapid identification of intra-amniotic infection and inflammation is possible (52,53,54).

Amniotic fluid and placental oxidative stress markers in pregnancies with preterm labor with intact membranes and/or PPROM.

Isoprostanes (F(2)-IP) are produced by ROS attack on polyunsaturated fatty acids and are sensitive and specific biomarkers of lipid-peroxidation in vivo in adults and neonates (9,12,55,56). Dutta et al., have studied concentration of F2-Isoprostane as biomarkers of OS in amniotic fluid samples from pregnancies with PPROM and PTB with intact membranes, demonstrating a 3-fold increase in F2-Isoprostanes in PPROM suggesting that OS-associated damage is more pronounced in PPROM than in PTB (57). In the same line of research, Kwiatkowski et al hypothesized that isoprostanes would be good markers of premature rupture of membranes. For this purpose, they determined 8-iPF2(2α)-III isoprostanes and showed that levels of this specific isoprostane were significantly higher in term infants with intact membranes as compared to preterm or term infants with PPROM. Hence, levels of iPF2(2α)-III in maternal plasma and amniotic fluid can be considered reliable markers of pregnancy at risk of preterm rupture of membranes (58). Studies by Longini et al., have also shown that women with PPROM had higher level of F2-isoprostane in the amniotic fluid at the time of genetic amniocentesis between 15-18 gestational weeks than those with term delivery (59). Biomarkers derived from oxidative damage to DNA such as 8-hydroxy-2 deoxy-dihydroguanosin are also increased in pregnancies with chorioamnionitis (60). Polycyclic aromatic hydrocarbons (PAH) alter the antioxidant defense system and as a consequence provoke an induction of early delivery. In a study performed by Agarwal et al., placenta tissue samples were obtained from healthy pregnant women and levels of PAHs quantified. Oxidative stress was assessed by determining malondialdehyde and reduced glutathione in placental tissue. Increased benzo(a)pyrene and MDA and decreasing concentrations of GSH were found in women with preterm delivery as

compared to term controls. In addition, higher and lower molecular weight PAHs showed significant correlation with the tendency towards depletion of GSH showing that oxidative stress and a pro-oxidant redox status caused by PAH may contribute to preterm delivery (61).

Perrone et al., reported that placentas with histological lesions compatible with chorioamnionitis and inflammation in preterm pregnancies were associated with higher cord blood levels of F2-isoprostanes (62).

A body of evidence has revealed that spontaneous preterm labour with or without PPROM may be triggered by premature placental ageing caused by OS-induced damage, premature senescence of the fetal membranes and telomere reduction (surrogate for OS) (63,64,65). Soydinc et al demonstrated that in non-invasively obtained vaginal washing fluid, levels of markers of total oxidative stress were significantly higher in women with PPROM than in those with intact membranes (66). Furthermore, higher level of oxidative stress and lower level of anti-oxidant capacity markers were observed in women with PPROM with consequent histological chorioamnionitis as compared with PPROM but without chorioamnionitis. Özalkaya et al demonstrated that umbilical cord pro-oxidant TOS level was higher in preterm infants without fetal inflammatory response syndrome (FIRS) and with PPROM compared to those without FIRS and PPROM (67).

Interventions to reduce oxidative stress in preterm birth

1.Antenatal steroids

Antenatal steroid (AS) administration has been a part of routine protocol of the PTB management between 24 and 34 weeks of gestation for many decades. It has been shown indisputably that the effectiveness AS administration to the mother within 7 days before delivery significantly reduces not only the incidence and severity of respiratory distress syndrome, but also other prematurity related problems such as bronchopulmonary dysplasia, patent ductus arteriosus, intracranial

hemorrhage, retinopathy of prematurity and necrotizing enterocolitis. Free radical production and oxidative stress are associated with all of these neonatal conditions. Vento et al., showed that the maximal effect of AS was achieved when administered 2–4 days before delivery (29). Moreover, this study also showed an association between antenatal steroids and activities of antioxidant enzymes and glutathione cycle enzymes in cord blood. Accordingly, extremely preterm infants who received antenatal steroids exhibited decreased levels of biomarkers of oxidative damage to proteins (O-tyrosine) and DNA (8-oxodG) (29). Unfortunately, there is an evidence of an inappropriate timing of the AS administration. Sanya et al., demonstrated that over two-thirds of the women presenting suspicion of PTB delivery after 34 weeks of gestation received AS unnecessarily (68, 51). Furthermore, in large cohorts of women who delivered before 34 weeks of gestation, AS was administered within 7 days of delivery only in approximately 40% of cases (69, 52). Despite these inconveniences, epidemiological studies confirm that more than 80% of preterm infants born in industrialized countries receive at least one dose of AS before being born (70).

2. Magnesium sulfate fetal neuroprotection

Summarizing the results of five randomized control trials, a meta-analysis published in 2009 demonstrated that magnesium sulphate given to mothers shortly before delivery reduced the risk of cerebral palsy by 32% and improved gross motor function in preterm infants born before 32 weeks of gestation without side effects for the mother or long-term consequences for the newborn (71). Based on high quality evidence of benefit, MgSO4 is currently recommended worldwide for women at risk of preterm birth before 32 weeks of gestation for fetal neuroprotection. The number of women needed to be treated to benefit one baby by avoiding cerebral palsy is 63. There is still a lack of understanding of the MgS04 neuroprotective mechanisms. Magnesium is an important cation that regulates cellular calcium influx regulating voltage-gated Ca^{++} channels and as a consequence cerebral vascular tone thus protecting fetal brain (72). Moreover, under stressful situations blocking massive entrance of Ca^{++} reduces the activation of the Krebs' cycle and the

liberation of highly energized electrons that lead to a burst of oxygen free radicals in the mitochondria (72). Additionally, it has been demonstrated in animal models that MgSO4 decreases the production of free radicals during hypoxic-ischemic reperfusion (73). Cerebral vasodilatation and reduction in inflammatory cytokine production have been reported (74,75). This theory is supported in a randomized controlled trial that included 72 women and showed increased levels of brain-derived neurotrophic factor (BDNF) in cord blood from pregnancies with MgSO4 neuroprotection administered before 34 weeks of gestation compared with placebo (76). There is evidence that BDNF is protective against neonatal hypoxic-ischaemic brain injury in vivo (77). Thordstein et al demonstrated that a combination of oxygen radical scavengers and magnesium administered in the phase of resuscitation mitigates perinatal postasphyxial brain damage in the rat (78).

3.Anti-inflammatory medication

Free radicals are generated in large quantities during the inflammatory response shifting the fetomaternal redox balance to a pro-oxidative state compromising the fetus. Selective inactivation of free radicals with N-acetylcysteine (NAC), both an antioxidant and L-cysteine donor for the synthesis of reduced glutathione (GSH), seems an effective way of improving fetal outcome in preterm deliveries associated with inflammation. In experimental studies, NAC administration to pregnant rats significantly reduced oxidized glutathione (GSSG) in isolated hepatocytes that occurred in the fetal-neonatal transition. The GSH/GSSG ratio in liver of NAC-treated newborns was 411 +/- 216 and in liver of controls it was 283 +/- 176. Thus, the oxidative stress in the fetal-to-neonatal transition was significantly reduced by oral NAC administration to pregnant rats (79). Buhimschi et al., demonstrated in a mice model of preterm labor and fetal damage that maternal inflammation in C57Bl/6 mice caused fetal oxidative stress and death, and was associated with maternal and fetal GSH depletion (79). Moreover, they also showed also that oxidative stress caused damage to the fetus independently of prematurity and that restoration of maternal and fetal

oxidative balance by NAC protected the fetus and reduced the rate of preterm birth (80). Buhimshi et al., in another study collected fetal membranes from 7 patients undergoing elective cesarean delivery at term and demonstrated that NAC dramatically inhibited amniochorionic matrix metalloproteinase activity in addition to inhibiting intrinsic superoxide generation within the tissue (81). In a randomized, double-blind, placebo-controlled trial including 280 women that had previously have given birth prematurely, showed that oral NAC reduced the recurrence of preterm birth in patients with bacterial vaginosis (82). The safety and possible pharmacodynamics side effects of NAC were investigated in a randomized, controlled pilot trial that included 22 pregnant women at ≥24 weeks gestation within 4 hours of clinical diagnosis of chorioamnionitis and their infants concluding that antenatal and postnatal NAC was safe, preserved cerebrovascular regulation, and increased an anti-inflammatory neuroprotective protein (83).

4. Antibiotics and intra-amniotic inflammation

Molecular microbiology techniques have evidenced the presence of a great diversity of different microbes in the amniotic fluid in pregnancies with PTB with intact membranes or PPROM. Morimoto et al., demonstrated that broad-range PCR-based approach is more useful for identifying women with IAI as well as women with polymicrobial infection than the culture-based approach is (84). They also found a correlation between the abundance of 16S rDNA and the severity of IAI (84). It is generally accepted that most IAIs result from a chronic bacterial infection and that the overt symptoms and neonatal outcome depend not only on the bacterial load but also in the presence, duration, and magnitude of the inflammation secondary to the infection (85). Azithromycin was introduced as an empirical antibiotic treatment for all women with PPROM in Helsinki University Hospital based on our research and the current literature that describes the bacteria specia detected from amniotic fluid in PPROM women (86). Azithromycin covers, among other bacteria, genital mycoplasmas, ureaplasmas and Fusobacterium nucleatum. This antibiotic exhibits potent anti-inflammatory effects mediated by the inhibition of IL-1β-mediated

inflammation produced by the inflammasome and simultaneous regulation of the NF-kB activity in response to oxidative stress and lipopolysaccharides rendering extreme useful in the control of chronic lung inflammation (87). The possible application of azithromycin for inflammatory processes such as chorioamnionitis in the perinatal period deserves further studies.

5. Progesterone

Experimental studies demonstrated that progesterone receptor membrane component I (PGRMCI) can be regulated by progestins, inflammatory cytokines, and oxidative stress in the fetal membranes and also that PGRMCI acts as a protective element for maintaining fetal membrane integrity by inhibiting oxidative stress-induced chorion cell ageing (88,89). Recent clinical evidence has demonstrated the benefit of administering progesterone and its analogues as prophylaxis for spontaneous PTB in high-risk patients. The mechanism by which vaginal P4 decreases the risk for PTB appears to be related to the pathophysiology of cervical remodeling during the prelude to parturition. Systematic review and meta-analysis of individual patient data of randomized controlled trials comparing vaginal progesterone with placebo/no treatment in women with a singleton gestation and a midtrimester sonographic cervical length $\leq$25 mm showed that vaginal progesterone decreases the risk of preterm birth and improves perinatal outcomes without any demonstrable deleterious effects on childhood neurodevelopment (90).As an example, the number needed to treat to prevent one preterm birth $\leq$ 32 weeks of gestation is six (90).

Conclusions

Spontaneous preterm birth is one of the major complications of pregnancy and the main cause of neonatal mortality and morbidity (1). In survivors, this risk persists through the adulthood causing both medical and social disability and is increased with decreasing gestational age at birth (91). There is accumulated evidence that correlates OS to PTB regardless of the fetal membrane status. Whether OS is a trigger for PTB or its consequence yet remains to be elucidated. Hence, the standardization and clinical evaluation of different markers of OS in should be integrated in the

management protocols of PTB. Strategies to prevent PTB-related OS should include preventive antioxidant administration to women with a high-risk for PTB (including progesterone treatment for women with high-risk for PTB), administration of appropriate antibiotics with proven microbial invasion of the amniotic cavity, and the introduction of anti-inflammatory agents for patients with proven inflammation aiming to reduce oxidative stress and prolong the latency period between PTB clinical signs and after PPROM. Appropriate administration of antenatal steroids and magnesium sulphate for fetal neuroprotection should be a standard of care in every obstetrical unit. All these therapies have minimal or no side-effects to fetus, newborn and pregnant women if administered according to the guidelines. However, these strategies show evidence of short-term benefit but lack large-scale follow-up data of long-term childhood outcomes (92). We expect that future research on preterm birth interventions should include long-term follow-up of the children to address the optimal strategies for the prevention and reducing the sequelae.

References

(1) Harrison MS, Goldenberg RL. Global burden of prematurity. Semin. Fetal Neonatal Med. 21:74-79, 2016. doi:10.1016/j.siny.2015.12.007

(2) Tielsch JM. Global incidence of preterm birth. Nestle Nutr. Inst. Workshop Ser. 81:9-15; 2015. doi:10.1159/000365798

(3) Keelan JA, Newnham JP. Recent advances in the prevention of preterm birth. F1000Research 6 (F1000 Faculty Rev):1139; 2017. Last updated: July 18, 2017.

(4) Rubens CE, Sadovsky Y, Muglia L, Gravett MG, Lackritz E, Gravett C. Prevention of preterm birth: harnessing science to address the global epidemic. Sci Transl Med. 6:262sr5, 2014. doi:10.1126/scitranslmed.3009871

(5) Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V et al. Oxidative stress: harms and benefits for human health. Oxid Med Cell Longev. 2017;2017:8416763. doi.org/10.1155/2017/8416763

(6) Moore TA, Ahmad IM, Zimmerman MC. Oxidative stress and preterm birth: an integrative review. Biol Res Nurs 2018;20:497-512. doi: 10.1177/1099800418791028

(7) Kalyanaraman B. Teaching the basis of redox biology to medical and graduate students: oxidants, antioxidants and disease mechanisms. Redox Biol. 2013;15:244-257. doi:10.1016/j.redox.2013.01.014.

(8) Sies H, Berndt C, Jones DP. Oxidative Stress. Annu Rev Biochem. 2017;86:715-748. doi: 10.1146/annurev-biochem-061516-045037.

(9) Torres-Cuevas I, Parra-Llorca A, Sánchez-Illana A, Nuñez-Ramiro A, Kuligowski J, Cháfer-Pericas C et al. Oxygen and oxidative stress in the perinatal period. Redox Biol 2017;12:674-681. Doi:10-1016/j-redox.2017.03.011.

(10) Sies H. Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. Redox Biol. 2017;11:613-619.

(11) Menon R. Oxidative stress damage as detrimental factor in preterm birth pathology. Front Immunol. 2014;5:567. Doi: 10.3389/fimmu.2014.00567.

(12) Vento M, Hummler H, Dawson JA, Escobar J., Kuligowski J. Use of oxygen in the resuscitation of neonates. In: Dennery P.A., Buonocore G., Saugstad O.D., editors. Perinatal and Prenatal Disorders. 1st edition. Springer Science+Business Media; New York: 2014. pp. 213–244.

(13) Suzuki T, Yamamoto M. Molecular basis of the Keap1-Nrf2 system. Free Radic Biol Med. 2015;88(Pt B):93-100. Doi:10.1016/j.freeradbiomed.2015.06.006

(14) Kobayashi M, Yamamoto, M. Nrf2–keap1 regulation of cellular defense mechanisms against electrophiles and reactive oxygen species. Adv Enzyme Regul. 2006;46:113–140.

(15) Ooi BK, Goh BH, Yap WH. Oxidative stress in cardiovascular diseases: Involvement of NrF2 antioxidant redox signaling in macrophage foam cells formation. Int J Mol Sci 2017;18:2336. Doi: 10.3390/ijms18112336.

(16) Schieber, M, Chandel, NS. ROS Function in Redox Signaling and Oxidative Stress. Curr. Biol. 2014;24:R453–R462. Doi: 10.1016/j.cub.2014.03.034.

(17) Millan I, Piñero-Ramos JD, Lara I, Parra-Llorca A, Torres-Cuevas I, Vento M. Oxidative stress in the newborn period: useful biomarkers in the clinical setting. Antioxidants (Basel). 2018;7.pii:E193. Doi: 10.3390/antiox7120193.

(18) Sánchez-Illana A, Mayr F, Cuesta-García D, Piñeiro-Ramos JD, Cantarero A, Guardia M et al. On-Capillary Surface-enhanced Raman spectroscopy: Determination of glutathione in whole blood microsamples. Anal Chem. 2018;90:9093-9100. Doi: 10.1021/acs.analchem.8b01492.

(19) Torres-Cuevas I, Kuligowski J, Cárcel M, Cháfer-Pericás C, Asensi M, Solberg R et al. Protein-bound tyrosine oxidation, nitration and chlorination by-products assessed by ultraperformance liquid chromatography coupled to tandem mass spectrometry. Anal Chim Acta. 2016, 913, 104–110. Doi:10.1016/aca.2016.01.054.

(20) Kemp, MW. Preterm birth, intrauterine infection, and fetal inflammation. Front. Immunol. 5:74; 2014. doi:10.3389/fimmu.2014.00574

(21) Salmaso N, Jablonska B, Scafidi J, Vaccarino FM, GalloV. Neurobiology of premature brain injury. Nat Neurosci. 17:341–346; 2014. doi:10.1038/nn.3604

(22) Hoffmann A, Baltimore D. Circuitry of nuclear factor kappa B signaling. Immunol Rev. 2006;2010:171-86.

(23) Khanjani S, Kandola MK, Lindstrom TM, Sooranna SR, Melchionda M, Lee YS et al. NF-kB regulates a cassette of immune/inflammatory genes in human pregnant myometrium at term. J Cell Mol Med 2011;15:809-824. Doi:10.1111/j.1582-4934.2010.01069

(24) Tataranno ML, Perrone S, Buonocore G. Plasma biomarkers of oxidative stress in neonatal brain injury. Clin Perinatol. 42:529-539; 2015. doi:10.1016/j.clp.2015.04.011

(25) Burd I, Balakrishnan B, Kannan S. Models of fetal brain injury, intrauterine inflammation, and preterm birth. Am J Reprod Immunol. 67:287-294; 2012. doi:10.1111/j.1600-0897.2012.01110.x

(26) Frank L, Groseclose, EE. Preparation for birth into an O2-rich environment: the antioxidant enzymes in the developing rabbit lung. Pediatr Res 1984;18:240-244. doi:10.1203/00006450-198403000-00004

(27) Frank L, Sosenko, IR. Prenatal development of lung antioxidant enzymes in four species. J Pediatr. 1987;110:106-110; 1987. doi.org/10.1016/S0022-3476(87)80300-1

(28) Torres-Cuevas I, Cernada M, Nuñez A, Escobar J, Kuligowski J, Chafer-Pericas C et al. Oxygen supplementation to stabilize preterm infants in the fetal to neonatal transition: no satisfactory answer. Front Pediatr. 2016;4:29. doi:10.3389/fped.2016.00029. eCollection 2016.

(29) Vento M, Aguar M, Escobar J, Arduini A, Escrig R, Brugada M et al. Antenatal steroids and antioxidant enzyme activity in preterm infants: influence of gender and timing. Antioxid Redox Signal. 2009;11:2945-2955. doi:10.1089/ars.2009.2671

(30) Escobar J, Teramo K, Stefanovic V, Andersson S, Asensi MA, Arduini A et al. Amniotic fluid oxidative and nitrosative stress biomarkers correlate with fetal chronic hypoxia in diabetic pregnancies. Neonatology 2013;103:193-198; doi:10.1159/000345194

(31) Cháfer-Pericás C, Cernada M, Rahkonen L, Stefanovic V, Andersson S, Vento M. Preliminary case control study to establish the correlation between novel peroxidation biomarkers in cord serum and the severity of hypoxic ischemica encephalopathy. Free Radic Biol Med 2016;97:244-249. Doi: 10.1016/j.freeradbiomed.2016.06.006).

(32) Buhimschi IA, Buhimschi CS, Pupkin M, Weiner CP. Beneficial impact of term labor: nonenzymatic antioxidant reserve in the human fetus. Am. J. Obstet. Gynecol. 2003;189:181-188. doi.org/10.1067/mob.2003.357

(33) Burd I, Balakrishnan B, Kannan S. Models of fetal brain injury, intrauterine inflammation, and preterm birth. Am J Reprod Immunol. 2012;67:287-294. doi:10.1111/j.1600-0897.2012.01110.x

(34) Cháfer-Pericás C, Stefanovic V, Sánchez-Illana Á, Escobar J, Cernada M, Cubells E et al. Novel biomarkers in amniotic fluid for early assessment of intraamniotic infection. Free Radic Biol Med. 2015;89:734-740; 2015. doi:10.1016/j.freeradbiomed.2015.09.014

(35) Cháfer-Pericás C, Stefanovic V, Sánchez-Illana Á, Escobar J, Cernada M, Cubells E et al. Mass spectrometric detection of biomarkers for early assessment of intraamniotic fluid infection. Data Brief. 2015;20:1026-1030. doi:10.1016/j.dib.2015.11.020.

(36) Voltolini C, Torricelli M, Conti N, Vellucci FL, Severi FM, Petraglia F. Understanding spontaneous preterm birth: from underlying mechanisms to predictive and preventive interventions. Reprod Sci. 2013;20:1274-1292. doi:10.1177/1933719113477496

(37) Park CW, Park JS, Moon KC, Jun JK, Yoon BH. Preterm labor and preterm premature rupture of membranes have a different pattern in the involved compartments of acute histologoic chorioamnionitis and/or funisitis: Patho-physiologic implication related to different clinical manifestations. Pathol Int. 2016;66:325-332. doi:10.1111/pin.12412

(38) Dutta EH, Behnia F, Boldogh I, Saade GR, Taylor, BD, Kacerovský M et al. Oxidative stress damage-associated molecular signaling pathways differentiate spontaneous preterm birth and preterm premature rupture of the membranes. Mol Hum Reprod. 2016;22:143-157. doi:10.1093/molehr/gav074

(39) Romero R, Miranda J, Chaiworapongsa T, Korzeniewski SJ, Chaemsaithong P, Gotsch F et al. Prevalence and clinical significance of sterile intra-amniotic inflammation in

patients with preterm labor and intact membranes. Am J Reprod Immunol. 2014;72:458-474. doi:10.1111/aji.12296

(40) Romero R, Miranda J, Chaemsaithong P, Chaiworapongsa T, Kusanovic JP, Dong Z et al. Sterile and microbial-associated intra-amniotic inflammation in preterm prelabor rupture of membranes. J Matern Fetal Neonatal Med. 2015;28:1394-1409. doi: 10.3109/14767058.2014.958463.

(41) Combs CA, Gravett M, Garite TJ, Hickok DE, Lapidus J, Porreco R et al. Amniotic fluid infection, inflammation, and colonization in preterm labor with intact membranes. Am. J Obstet Gynecol. 2014;210:125.e1-125.e15. doi: 10.1016/j.ajog.2013.11.032

(42) Flenady V, Hawley G, Stock OM, Kenyon S, Badawi N. Prophylactic antibiotics for inhibiting preterm labour with intact membranes. Cochrane Database Syst Rev. 2013:5;(12):CD000246. doi: 10.1002/14651858.CD000246.pub2.

(43) Anblagan D, Pataky R, Evans MJ, Telford EJ, Serag A, Sparrow S et al. Association between preterm brain injury and exposure to chorioamnionitis during fetal life. Sci Rep. 2016;6:37932. doi:10.1038/srep37932

(44) Voltolini C, Torricelli M, Conti N, Vellucci FL, Severi FM, Petraglia F. Understanding spontaneous preterm birth: from underlying mechanisms to predictive and preventive interventions. Reprod Sci. 2013;20:1274-1292. doi:10.1177/1933719113477496

(45) Oh KJ, Kim SM, Hong JS, Maymon E, Erez O et al. Twenty-four percent of patients with clinical chorioamnionitis in preterm gestations have no evidence of either culture-proven intraamniotic infection or intraamniotic inflammation. Am J Obstet Gynecol. 2017;216:604.e1-604.e11. doi:10.1016/j.ajog.2017.02.035

(46) Musilova I, Bestvina T, Stranik J, Stepan M, Jacobsson B, Kacerovsky M. Transabdominal amniocentesis is a feasible and safe procedure in preterm prelabor rupture of membranes. Fetal Diagn. Ther. 42:257-261; 2017. doi:10.1159/000457951

(47) Lacerte M, Bujold E, Audibert F, Mayrand MH. Amniocentesis for PPROM management: a feasibility study. J Obstet Gynaecol Can. 2008;30:659-664. doi:10.1016/S1701-2163(16)32913-9

(48) Cobo T, Kacerovsky M, Jacobsson B. Noninvasive sampling of the intrauterine environment in women with preterm labor and intact membranes. Fetal Diagn Ther. 2018;43:241-249. doi:10.1159/000480232

(49) Oh KJ, Kim SM, Hong JS, Maymon E, Erez O, Panaitescu B et al. Twenty-four percent of patients with clinical chorioamnionitis in preterm gestations have no evidence of either culture-proven intraamniotic infection or intraamniotic inflammation. Am J Obstet Gynecol. 2017;216:604.e1-604.e11. doi:10.1016/j.ajog.2017.02.035

(50) Musilova I, Bestvina T, Stranik J, Stepan M, Jacobsson B, Kacerovsky M. Transabdominal amniocentesis is a feasible and safe procedure in preterm prelabor rupture of membranes. Fetal Diagn Ther. 2017;42:257-261. doi:10.1159/000457951

(51) Lacerte M, Bujold E, Audibert F, Mayrand MH. Amniocentesis for PPROM management: a feasibility study. J Obstet Gynaecol Can. 2008;30:659-664. doi:10.1016/S1701-2163(16)32913-9

(52) Gomez R, Romero R, Nien JK, Medina L, Carstens M, Kim YM et al. Antibiotic administration to patients with preterm premature rupture of membranes does not eradicate intra-amniotic infection. J. Matern. Fetal Neonatal Med. 2007;20:167-173. doi:org/10.1080/14767050601135485

(53) Gravett M, Sadowsky D, Witkin S, Novy M. Immunomodulators plus antibiotics to prevent preterm delivery in experimental intra-amniotic infection (IAI). Am J Obstet Gynecol. 2003;189:S56. doi.org/10.1016/j.ajog.2003.10.002

(54) Gravett MG, Adams KM, Sadowsky DW, Grosvenor AR, Witkin SS, Axthelm MK, Novy MJ. Immunomodulators plus antibiotics delay preterm delivery after experimental

intra-amniotic infection in a nonhuman primate model. Am J Obstet Gynecol. 2007;197:518.e1–518.e8.

(55) Belik J, González-Luis GE, Pérez-Vizcaíno F, Villamor E. Isoprostanes in fetal and neonatal health and disease. Free Radic Biol Med 2010;48:177-88. doi: 10.1016/j.freeradbiomed.2009.10.043

(56) Milne GL, Musiek ES, Morrow JD. F2-isoprostanes as markers of oxidative stress in vivo: an overview. Biomarkers 2005;10(Suppl 1):S10-S23. doi:10.1080/13547500500216546

(57) Dutta EH, Behnia F, Boldogh I, Saade GR, Taylor BD, Kacerovský M et al. Oxidative stress damage-associated molecular signaling pathways differentiate spontaneous preterm birth and preterm premature rupture of the membranes. Mol Hum Reprod. 2016;22:143-157. doi: 10.1093/molehr/gav074

(58) Kwiatkowski S, Torbé A, Dołegowska B, Błogowski W, Czajka R, Chlubek D et al. Isoprostanes 8-iPF2alpha-III: risk markers of premature rupture of fetal membranes? Biomarkers 2009;14:406-413. doi:10.1080/13547500903045583

(59) Longini M, Perrone S, Vezzosi P, Marzocchi B, Kenanidis A, Centini G et al. Association between oxidative stress in pregnancy and preterm premature rupture of membranes. Clin. Biochem. 2007;40:793-797.

(60) Cháfer-Pericás C, Stefanovic V, Sánchez-Illana Á, Escobar J, Cernada M, Cubells E et al. Novel biomarkers in amniotic fluid for early assessment of intraamniotic infection. Free Radic Biol Med 2015;89:734-740. doi:10.1016/j.freeradbiomed.2015.09.014

(61) Agarwal P, Singh L, Anand M, Taneja A. Association between placental polycyclic aromatic hydrocarbons (PAHS), oxidative stress, and preterm delivery: a case control study. Arch Environ Contam Toxicol. 2018;74:218-227. doi: 10.1007/s00244-017-0455-0

(62) Perrone S, Tataranno ML, Negro S, Longini M, Toti MS, Alagna MG et al. Placental histological examination and the relationship with oxidative stress in preterm infants. Placenta 2016;46:72-78. doi:10.1016/j.placenta.2016.08.084

(63) Menon R, Boldogh I, Hawkins HK, Woodson M, Polettini J, Syed TA et al. Histological evidence of oxidative stress and premature senescence in preterm premature rupture of the human fetal membranes recapitulated in vitro. Am J Pathol. 2014;184:1740-1751. doi:10.1016/j.ajpath.2014.02.011

(64) Menon R, Yu J, Basanta-Henry P, Brou L, Berga SL, Fortunato SJ et al. Short fetal leukocyte telomere length and preterm prelabor rupture of the membranes. PLoS One. 2012;7:e31136. doi:10.1371/journal.pone.0031136

(65) Polettini J, Dutta EH, Behnia F, Saade GR, Torloni MR, Menon R. Aging of intrauterine tissues in spontaneous preterm birth and preterm premature rupture of the membranes: a systematic review of the literature. Placenta 2015;36:969-973. doi:10.1016/j.placenta.2015.05.003

(66) Soydinc HE, Sak ME, Evliyaoglu O, Evsen MS, Turgut A, Özler A et al. Prolidase, matrix metalloproteinases 1 and 13 activity, oxidative-antioxidative status as a marker of preterm premature rupture of membranes and chorioamnionitis in maternal vaginal washing fluids. Int J Med Sci. 2013;10:1344-1351. doi:10.7150/ijms.4802. eCollection 2013.

(67) Özalkaya E, Karatekin G, Topçuoğlu S, Karatepe HÖ, Hafızoğlu T, Baran P et al. Neonatology oxidative status in preterm infants with premature preterm rupture of membranes and fetal inflammatory response syndrome. Pediatr Neonatol. 2017;58:437-441. doi:10.1016/j.pedneo.2016.08.008

(68) Sanya R, Al Naggar E, Gasim M, Ahmed BI. Use or overuse of antenatal corticosteroids for suspected preterm birth. J Matern Fetal Neonatal Med. 2014;27:1454-1456. doi:10.3109/14767058.2013.860523

(69) Adams TM, Kinzler WL, Chavez MR, Vintzileos AM. The timing of administration of antenatal corticosteroids in women with indicated preterm birth. Am J Obstet Gynecol. 2015;212:645.e1-4. doi:10.1016/j.ajog.2014.11.021

(70) Roberts D, Brown J, Medley N, Dalziel SR. Antenatal corticosteroids for accelerating fetal lung maturation for vomwn at risk of preterm delivery. Cochrane Database Syst Rev. 2017;3:CD004454. doi: 10.1002/14651858.CD004454.pub3.

(71) Doyle LW, Crowther CA, Middleton P, Marret, S, Rouse D. Magnesium sulphate for women at risk of preterm birth for neuroprotection of the fetus. Cochrane Database Syst Rev. 2009;1:CD004661. doi:10.1002/14651858.CD004661.pub3

(72) Bara M, Guiet-Bara A. Magnesium regulation of Ca2+ channels in smooth muscle and endothelial cells of human allantochorial placental vessels. Magnes Res. 2001;14:11-8.

(73) Shogi T, Miyamoto A, Ishiguro S, Nishio A. Enhanced release of IL-1beta and TNF-alpha following endotoxin challenge from rat alveolar macrophages cultured in low-mg(2+) medium. Magnes Res. 2003;16:111-119.

(74) Fellman V, Raivio KO. Reperfusion injury as the mechanism of brain damage after perinatal asphyxia. Pediatr Res. 1997;41:599-606.

(75) Sugimoto J, Romani AM, Valentin-Torres AM, Luciano AA, Ramirez Kitchen CM, Funderburg N et al. Magnesium decreases inflammatory cytokine production: a novel innate immunomodulatory mechanism. J. Immunol. 2012;188(12):6338-46. 10.4049/jimmunol.1101765. doi:10.4049/jimmunol.1101765

(76) Bachnas MA, Mose JC, Effendi JS, Andonotopo W. Influence of antenatal magnesium sulfate application on cord blood levels of brain-derived neurotrophic factor in premature infants. J Perinat Med. 2014;42:129-134. doi:10.1515/jpm-2013-0137

(77) Han BH, Holtzman DM. BDNF protects the neonatal brain from hypoxic-ischemic injury in vivo via the ERK pathway. J Neurosci. 2000;20:5775-5781. doi:https://doi.org/10.1523/JNEUROSCI.20-15-05775.2000

(78) Thordstein M, Bågenholm R, Thiringer K, Kjellmer I. Scavengers of free oxygen radicals in combination with magnesium ameliorate perinatal hypoxic-ischemic brain damage in the rat. Pediatr Res. 1993;34:23-26. doi:10.1203/00006450-199307000-00006

(79) Sastre J, Asensi M, Rodrigo F, Pallardó FV, Vento M, Viña J. Antioxidant administration to the mother prevents oxidative stress associated with birth in the neonatal rat. Life Sci 1994;54:2055-9.

(80) Buhimschi IA, Buhimschi CS, Weiner CP. Protective effect of N-acetylcysteine against fetal death and preterm labor induced by maternal inflammation. Am J Obstet Gynecol. 2003;188:203-208. doi.org/10.1067/mob.2003.112

(81) Buhimschi IA, Kramer WB, Buhimschi CS, Thompson LP, Weiner CP. Reduction-oxidation (redox) state regulation of matrix metalloproteinase activity in human fetal membranes. Am J Obstet Gynecol 2000;182:458-464. doi.org/10.1016/S0002-9378(00)70239-0

(82) Shahin AY, Hassanin IM, Ismail AM, Kruessel JS, Hirchenhain J. Effect of oral N-acetyl cysteine on recurrent preterm labor following treatment for bacterial vaginosis. Int J Gynaeco Obstet. 2009;104:44-48. doi:10.1016/j.ijgo.2008.08.026

(83) Jenkins DD, Wiest DB, Mulvihill DM, Hlavacek AM, Majstoravich SJ, Brown TR et al. Fetal and neonatal effects of N-Acetylcysteine when used for neuroprotection in maternal chorioamnionitis. J Pediatr. 2016;168:67–76.e6. doi: 10.1016/j.jpeds.2015.09.076

(84) Morimoto S, Usui H, Kobayashi T, Katou E, Goto S, Tanaka H et al. Bacterial-culture-negative subclinical intra-amniotic infection can be detected by bacterial 16S

ribosomal-DNA-amplifying polymerase chain reaction. Jpn J Infect Dis. 2018;71:274-280. doi:10.7883/yoken.JJID.2017.468

(85) Ericson JE, Laughon MM. Chorioamnionitis: implications for the neonate. Clin Perinatol 2015;42:155-65. doi:10.1016/j.clp.2014.10.011.

(86) Myntti T, Rahkonen L, Pätäri-Sampo A, Tikkanen M, Sorsa T, Juhila J et al. Comparison of amniotic fluid matrix metalloproteinase-8 and cathelicidin in the diagnosis of intra-amniotic infection. J Perinatol. 2016;36:1049-1054. doi:10.1038/jp.2016.147

(87) Tchirikov M, Schlabritz-Loutsevitch N, Maher J, Buchmann J, Naberezhnev Y, Winarno AS et al. Mid-trimester preterm premature rupture of membranes (PPROM): etiology, diagnosis, classification, international recommendations of treatment options and outcome. J Perinat Med. 2018;46:465-488. doi:10.1515/jpm-2017-0027

(88) Meng Y, Murtha AP, Feng L. Progesterone, Inflammatory Cytokine (TNF-α), and Oxidative Stress (H2O2) Regulate Progesterone Receptor Membrane Component 1 Expression in Fetal Membrane Cells. Reprod Sci. 2016;23:1168-78. doi: 10.1177/1933719116630412.

(89) Feng L, Allen TK, Marinello WP, Murtha AP. Roles of Progesterone Receptor Membrane Component 1 in Oxidative Stress-Induced Aging in Chorion Cells. Reprod Sci. 2019;26:394-403. doi: 10.1177/1933719118776790.

(90) Romero R, Conde-Agudelo A, El-Refaie W, Rode L, Brizot ML, Cetingoz E, Serra V, Da Fonseca E, Abdelhafez MS, Tabor A, Perales A, Hassan SS, Nicolaides KH. Vaginal progesterone decreases preterm birth and neonatal morbidity and mortality in women with a twin gestation and a short cervix: an updated meta-analysis of individual patient data. Ultrasound Obstet Gynecol. 2017;49:303-314. doi: 10.1002/uog.17397.

(91) Moster D, Lie RT, Markestad T. Long-term medical and social consequences of preterm birth. N Engl J Med. 2008; 359:262-73. doi: 10.1056/NEJMoa0706475.

(92) Murray SR, Stock SJ, Norman JE. Long-term childhood outcomes after interventions for prevention and management of preterm birth. Semin Perinatol. 2017; 41: 519-27. doi: 10.1053/j.semperi.2017.08.011.

Graphical abstract. Scheme representing the simplified list of interventions to avoid or attenuate oxidative stress in preterm delivery. PTL= preterm delivery; PPROM= preterm premature rupture of membranes. MIAC= microbial invasion amniotic cavity.

Table 1. Published studies describing intervention strategies to prevent preterm birth and its sequelae by decreasing oxidative-stress and inflammation

Intervention/issue studied	Type of the study	Sample and methods	Main findings	Conclusion	References
Antenatal steroids for lung maturation	Prospective observational-cohort clinical study	Extremely low-gestational-age neonates (>28 weeks of gestation) receiving antenatal steroids (CORT, n= 37) or not (NOCORT, n=20)	There is an association between antenatal steroids and activities of antioxidant enzymes and glutathione cycle enzymes in cord blood. In addition, reduced oxidative stress, and decreased oxidation of proteins and DNA was also demonstrated	Antenatal administration of steroids reduces oxidative stress and decreases the intensity of prematurity sequelae (oxygen supplementation, mechanical ventilation, and conditions such as bronchopulmonary dysplasia, intra-periventricular hemorrhage, or retinopathy) related to free-radical production and oxidative stress	Vento et al (2009) Reference #29
Magnesium sulfate (MgS04) for fetal neuroprotection	Randomized controlled clinical trial	72 pregnant women who were divided into three groups: group I (preterm pregnancy with MgSO4), group II (preterm pregnancy without MgSO4), and group III (full-term pregnancy as control group)	The cord blood brain-derived neurotrophic factor (BDNF) levels in premature infants with antenatal MgSO4 was significantly higher than in premature infants without antenatal MgSO4 and was comparable to full-term infants	The application of antenatal MgSO4 in preterm delivery increased cord blood BDNF levels, which downregulation increases sensitivity to oxidative damage under stressful circumstances	Bachnas et al (2014) Reference #76
N-Acetyl-Cysteine (NAC) for prevention of recurrent preterm birth in adjunct to progesterone	A randomized, double-blind, placebo-controlled trial	280 women between 16 and 18 weeks of pregnancy who had 1 previous preterm birth and had just been successfully treated for bacterial vaginosis with metronidazole for 1 week. The women were randomized to receive 0.6 g of NAC per day plus 17-hydroxyprogesterone caproate (17-OHPC) or placebo plus 17-OHPC until 36 completed weeks of pregnancy or active labor.	Reaching 36 weeks of pregnancy was more frequent and gestational age at delivery was significantly higher in the NAC than in the placebo group (37.4 weeks ± 0.4 weeks vs 34.1 weeks ± 1.2 weeks)	Oral NAC (known to be powerful anti-oxidant) was found to reduce the recurrence of preterm birth in patients with bacterial vaginosis	Shahin et al (2009) Reference #82

Antenatal and postnatal NAC treatment of newborns exposed to chorioamnionitis	Randomized, controlled, double-blinded trial	22 pregnant women (24 infants) >24 weeks of gestation presenting within 4 hours of diagnosis of clinical chorioamnionitis were randomized to NAC or saline treatment. Antenatal NAC or saline was given intravenously every 6 hours until delivery. Postnatally, NAC or saline was given every 12 hours for 5 doses	Cerebrovascular coupling was disrupted in infants with chorioamnionitis treated with saline but preserved in infants treated with NAC, suggesting improved vascular regulation in the presence of neuroinflammation. Infants treated with NAC had higher serum anti-inflammatory interleukin-1 receptor antagonist and lower proinflammatory vascular endothelial growth factor over time vs controls.	In this cohort of newborns exposed to chorioamnionitis, antenatal and postnatal NAC was safe, preserved cerebrovascular regulation, and increased an anti-inflammatory neuroprotective protein	Jenkinks et al (2016) Reference #83
Oxidative stress influence on Progesterone receptor membrane component I (PGRMCI) in fetal membranes	Experimental study	Human fetal membranes collected following planned uncomplicated cesarean delivery at term without rupture of membranes or labor. The objective was to evaluate progesterone receptor membrane component I (PGRMCI) expression in cells of the fetal membranes after exposure to progestin (17P, P4, and medroxyprogesterone acetate [MPA]), a proinflammatory cytokine TNF-a, and oxidative stress (H2O2).	Progestin, TNF-a, and oxidative stress act as regulatory factors of PGRMCI in a cell-specific manner in fetal membranes.	PGRMCI can be regulated by progestins, inflammatory cytokines, and oxidative stress in the fetal membranes. This study demonstrated that inflammation and oxidative stress are inseparable in fetal membrane pathology	Meng et al (2016) Reference #86
Oxidative stress influence on fetal membrane senescence	Experimental study	Human fetal membranes collected following planned	Hydrogen peroxide significantly induced cell senescence and p38 MAPK phosphorylation, and it significantly decreased SIRT3 expression in	This study demonstrated that oxidative stress-induced cell aging is one of the mechanisms of PPROM and PGRMCI acts as a protective	Feng et al (2019) Reference #89

		uncomplicated cesarean delivery at term without rupture of membranes or labor. Study aimed to investigate the effects of oxidative stress (represented by hydrogen peroxide [H2O2] on fetal membrane and chorion cell senescence, p38 mitogen-activated protein kinase (MAPK) phosphorylation, and sirtuin (SIRT3) and examine the role of progesterone receptor membrane component I (PGRMCI) in these effects	full-thickness fetal membrane explants and chorion cells. These effects were enhanced by PGRMCI knockdown	element for maintaining fetal membrane integrity by inhibiting oxidative stress-induced chorion cell ageing	
Vaginal progesterone on the prevention of preterm birth	Systematic review and meta-analysis of individual patient data of randomized controlled trials comparing vaginal progesterone with placebo/no treatment in women with a singleton gestation and a midtrimester sonographic cervical length ≤25 mm	974 women (498 assigned to vaginal progesterone, 476 assigned to placebo) with a cervical length ≤25 mm participating in five high-quality trials	Vaginal progesterone significantly decreased the risk of preterm birth <36, <35, <34, <32, <30 and <28 weeks of gestation, spontaneous preterm birth <33 and <34 weeks of gestation, respiratory distress syndrome, composite neonatal morbidity and mortality, birthweight <1500 and <2500 g, and admission to the neonatal intensive care uni	Vaginal progesterone decreases the risk of preterm birth and improves perinatal outcomes in singleton gestations with a midtrimester sonographic short cervix, without any demonstrable deleterious effects on childhood neurodevelopment	Romero et al (2018) Reference #90

HIGHLIGHTS

- Experimental and clinical evidence correlates oxidative stress to preterm delivery regardless of the fetal membrane status.
- However, it is not known if oxidative stress is a trigger for preterm birth of the consequence of infection/inflammation.
- New biomarkers of oxidative stress and oxidative damage biomarkers using mass spectrometry and metabolomics have recently put forward and validated.
- Hence, the standardization and clinical evaluation of different markers should be integrated in the management protocols of preterm birth.
- Strategies to prevent preterm birth-related oxidative stress should include:
    - preventive antioxidant administration to women with a high-risk for preterm birth including
    - administration of appropriate antibiotics with proven microbial invasion of the amniotic cavity
    - introduction of anti-inflammatory agents for patients with proven inflammation.
    - Appropriate administration of antenatal steroids and magnesium sulfate for fetal neuroprotection should be a standard of care in every obstetrical unit.