UNIVERSIDADE DE PASSO FUNDO
FACULDADE DE AGRONOMIA E MEDICINA VETERINÁRIA
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOEXPERIMENTAÇÃO

IMPLICAÇÕES DO ESTRESSE OXIDATIVO MATERNO EM RECÉM-NASCIDO DE GESTAÇÃO A TERMO E DE PARTO PREMATURO

DISSERTAÇÃO DE MESTRADO

Simone Medeiros Beder Reis

Passo Fundo, RS, Brasil
2020

IMPLICAÇÕES DO ESTRESSE OXIDATIVO MATERNO EM RECÉM-NASCIDO DE GESTAÇÃO A TERMO E DE PARTO PREMATURO

Simone Medeiros Beder Reis

Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Bioexperimentação, Área de Concentração em Bioexperimentação, da Faculdade de Agronomia e Medicina Veterinária da Universidade de Passo Fundo (UPF), como requisito parcial para a obtenção do grau de **Mestre em Bioexperimentação**

Orientador: Rômulo Pillon Barcelos

Coorientador: Wânia Eloisa Ebert Cechin

Passo Fundo, RS, Brasil
2020

UNIVERSIDADE DE PASSO FUNDO
FACULDADE DE AGRONOMIA E MEDICINA VETERINÁRIA
PROGRAMA DE MESTRADO EM BIOEXPERIMENTAÇÃO

A comissão examinadora, abaixo assinada, aprova a Dissertação de Mestrado

IMPLICAÇÕES DO ESTRESSE OXIDATIVO MATERNO EM RECÉM-NASCIDO DE GESTAÇÃO A TERMO E DE PARTO PREMATURO

Elaborada por
Simone Medeiros Beder Reis

Como requisito parcial para a obtenção do grau de
Mestre em Bioexperimentação

Comissão Examinadora

Rômulo Pillon Barcelos, Dr. UPF
(Orientador/Presidente)

Wânia Eloisa Ebert Cechin, Dr. UPF
(Coorientadora)

Michele Rechia Fighera, Dr. UFSM

Rafael Frandolosso, Dr. UPF

Passo Fundo, RS, Brasil
2020

CIP – Catalogação na Publicação

R375i Reis, Simone Medeiros Beder
Implicações do estresse oxidativo materno em recém-nascido de gestação a termo e de parto prematuro [recurso eletrônico] / Simone Medeiros Beder Reis. – 2020.
360 KB ; PDF.

Orientador: Prof. Dr. Rômulo Pillon Barcelos.
Coorientadora: Profa. Dra.Wânia Eloisa Ebert Cechin.
Dissertação (Mestrado em Bioexperimentação) – Universidade de Passo Fundo, 2020.

1. Stress Oxidativo. 2. Recem-nascidos – Saúde e higiene. 3. Parto (obstetrícia). 4. Prematuros. I. Barcelos, Rômulo Pillon, orientador. II. Cecchin, Wânia Eloisa Ebert, coorientadora. III. Título.

CDU: 616-053.2

Catalogação: Bibliotecária Juliana Langaro Silveira - CRB 10/2427

AGRADECIMENTOS

A elaboração deste trabalho não teria sido possível sem a colaboração, estímulo e empenho de diversas pessoas. Gostaria de expressar toda a minha gratidão e apreço a todos aqueles que contribuíram para que este trabalho se tornasse uma realidade. Agradeço:

Primeiramente a Deus**,** por este presente maravilhoso que é a vida, pelas oportunidades, pelas pessoas colocadas em meu caminho, onde me inspiram, me ajudam, me desafiam e me encorajam a ser cada dia melhor.

Aos meus pais José e Maria José, pelos ensinamentos e todo o amor que em vida me deram, vivem dentro do meu coração e fizeram de mim a pessoa que hoje sou. Honro a memória de vocês.

Ao meu marido, Péricles, pelo amor, companheirismo e apoio incondicional, agradeço a enorme compreensão, generosidade e por estar sempre ao meu lado.

Aos meus queridos filhos, Pedro e Tiago, que amo incondicionalmente, que são a razão da minha vida, agradeço pelo amor e por compreenderem minha dedicação a este trabalho.

Aos meus irmãos Delano e Mônica, mesmo estando a alguns quilômetros de distância, se mantiveram presentes em suas manifestações de apoio e carinho.

Ao meu orientador professor Dr. Rômulo Pillon Barcelos, grata pela confiança depositada, pela sua dedicação e paciência. Seus conhecimentos fizeram grande diferença no resultado final deste trabalho. Obrigada por me manter motivada durante todo o processo.

Aos Professores do Programa de pós-graduação de Bioexperimentação, em especial ao Professor Dr. Luiz Carlos Kreutz e Professor Dr. Rafael Frandolosso, obrigada pelos ensinamentos e momentos partilhadas de ciência e pesquisa.

Às minhas Natália e Joana. Obrigada pela amizade e principalmente pelas palavras de encorajamento e amor. Nati, muito obrigada pela disponibilidade em me ajudar nos testes, você é muito especial.

Ao Drº Alexandre Tognon, ter me ajudado nos dados e estatísticas, seu trabalho é sensacional.

À Professora Dra. Wânia Cechin, obrigada por todas oportunidades, pela confiança e amizade.

A Júlia, obrigada pela disposição, generosidade e ajuda nos meus testes.

À Professora Dra. Luciana Grando por disponibilizar o laboratório e os reagentes para minha pesquisa.

À Angélica, por ter organizado minhas amostras.

Aos meus queridos alunos residentes, obrigada pelo carinho e compreensão pelos momentos de ausência neste último ano.

A todos meus amigos queridos, obrigada pelo apoio, todo carinho e por sempre estarem aí, por cada um ser especial e único, trazer para minha vida algo também especial e único.

Aos Colegas do Mestrado que compartilharam comigo esses momentos de aprendizado.

A Equipe de profissionais da Maternidade do Hospital São Vicente de Paulo, meu muito obrigada pela disponibilidade em ajudar na coleta das amostras.

As secretárias do Programa de pós-graduação de Bioexperimentação, pelos auxílios quando solicitados.

Agradeço a Universidade de Passo Fundo, pela oportunidade de aprendizado e evolução profissional.

Muito Obrigada!

DEDICATÓRIA

Dedico este trabalho ao meu Pai, José Beder Leite (in memorian), um grande mestre, que nos deixou durante a realização deste mestrado, que sempre me incentivou e este momento seria de grande felicidade.

EPÍGRAFE

Enquanto você sonha, você está fazendo o rascunho do seu futuro

Charles Chaplin

ÍNDICE

LISTA DE FIGURAS

2. REVISÃO DE LITERATURA

3. CAPITULO 1

LISTA DE TABELAS

3. CAPÍTULO 1

…

LISTA DE ABREVIATURAS

ADP	adenosina trifosfato
APGAR	Appearance, Pulse, Grimace, Activity, Respiration.
CAT	Catalase
DNA	Ácido Desoxirribonucleico
EDTA	Ácido etilenodiamino tetra-cético
EROS	Espécies Reativas de Oxigênio
g/L	Gramas/litro
H_2O_2	Peróxido de Hidrogênio
IG	Idade Gestacional
Kg	Quilograma
LPO	Lipoperoxidação lipídica
µg	Microgramas
mg	Miligramas
mg/L	Miligrama/litro
RN	Recém-nascido
NO	Óxido Nítrico
SCU	Sangue do cordão umbilical
TBARS	Espécies Reativas do Ácido Tiobarbitúrico
ul	microlitro
ugmol.g^{-1}	micromol/grama
UPF	Universidade de Passo Fundo
8-OHdG	8-hydroxydeoxyguanosine

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Bioexperimentação
Universidade de Passo Fundo

IMPLICAÇÕES DO ESTRESSE OXIDATIVO MATERNO EM RECÉM-NASCIDO DE GESTAÇÃO A TERMO E DE PARTO PREMATURO

Autor: Simone Medeiros Beder Reis
Orientador: Rômulo Pillon Barcelos
Passo Fundo, 02 de dezembro de 2020

A prematuridade é o maior determinante de mau prognóstico infantil em termos de sobrevivência e qualidade de vida. A etiologia do parto prematuro é multifatorial, pesquisas sugerem que um desequilíbrio oxidativo do corpo da mãe sobre o feto levariam a um parto prematuro. O objetivo deste estudo foi avaliar o estresse oxidativo da mãe e suas implicações em recém-nascidos (RN) a termo e em RN de parto prematuro. Fizemos a análise dos marcadores lipoperoxidação lipídica (TBARS), 8-Hydroxydeoxyguanosine (8-OHdG), óxido nítrico e atividade da enzima catalase em sangue materno e sangue de cordão umbilical (SCU) de 39 binômios mães – filhos. Foram divididos em 2 grupos: a termo (n=23) e prematuro (n=18), o qual foi subdividido em prematuro espontâneo (n=8) e prematuro placentário (n=8). Nossos resultados mostraram um aumento de TBARS das mães do termo em relação ao prematuro. Nas mães do subgrupo prematuro encontramos aumento nos níveis de TBARS e óxido nítrico do prematuro placentário. Na análise do SCU dos RN encontramos aumento na dosagem do 8-OHdG e lipoperoxidação lipídica no termo em relação ao prematuro. Concluímos que o estresse oxidativo está evidentemente relacionado ao aumento de TBARS e óxido nítrico em gestantes com patologias placentárias, ainda não é possível estabelecer se o estresse oxidativo é fator causal do parto prematuro. O marcador 8-OHdG em gestantes parece não representar um dano oxidativo, mas a capacidade de reparar um dano oxidativo prévio.

Palavras-Chave: Estado Redox, dano oxidativo, gravidez, prematuridade, TBARS, estudo clínico, 8-OHdG.

ABSTRACT

Master's Tesis

Post-Graduation Program in Bioexperimentation – University of Passo Fundo

IMPLICATIONS OF MATERNAL OXIDATIVE STRESS IN A NEWBORN WITH TERM PREGNANCY AND PREMATURE BIRTH

Author**:** Simone Medeiros Beder Reis
Advisor: Rômulo Pillon Barcelos
Passo Fundo, December 02th 2020

Prematurity is the major cause of poor child prognosis in terms of survival and life quality. The etiology of premature birth is multifactorial. Researchers suggest that an oxidative imbalance of the mother's body over the fetus would lead to premature birth. The aim of this study was to assess the mother's oxidative stress and its implications for full-term newborns and newborns. We dosed the oxidative stress markers for lipoperoxidation (TBARS), 8-Hydroxydeoxyguanosine (8-OHdG), nitric oxide, and catalase activity in maternal blood and umbilical cord blood (SCU) from 39 mother-child binomials. They were divided into 2 groups: term (n = 23) and premature (n = 18), which was subdivided into a spontaneous premature (n = 8) and placental premature (n = 8). Our results showed increased TBARS levels of mothers in the term group compared to the premature. We found increased TBARS levels and nitric oxide of the premature placental for the mothers of the premature subgroups. In the analysis of the SCU from newborns, we found increased 8-OHdG and TBARS levels in the term compared to the premature. We conclude that oxidative stress is related to increased lipid peroxidation and nitric oxide levels in pregnant women with placental pathologies. It is suggested that oxidative stress is the causative factor of premature birth, but it needs confirmation. Besides, the 8-OHdG levels in pregnant women do not represent oxidative damage, but the ability to repair previous oxidative damage, which occur more at non-premature births.

Keyword: Redox status; oxidative damage, pregnancy; prematurity, New-borns, clinical study, 8-OHdG.

1. INTRODUÇÃO

A prematuridade é considerada como o maior determinante de mau prognóstico infantil em termos de sobrevivência e qualidade de vida (1). Além disso, há uma preocupação crescente devido à alta letalidade, complicações associadas à prematuridade e ao alto custo econômico e social, a curto, médio e longo prazo (2). A gravidez é um estado fisiológico associado ao aumento do estresse oxidativo, como resultado de um maior *turn over* metabólico e elevados requerimentos de oxigênio tecidual (3). É um estado de desafio metabólico entre mãe e feto em desenvolvimento, apresentando níveis elevados de estresse oxidativo em comparação com estado não-gestante (4,5).

O equilíbrio do estado oxidante e antioxidante (redox) é um processo que começa antes do nascimento (6), onde fetos, recém-nascidos e principalmente prematuros, são altamente propensos ao insulto de espécies reativas e oxidativas, devido ao desafio hipóxico-hiperóxico, suscetibilidade a infeçções, deficiência de defesa antioxidante e a altos níveis de ferro livre (7).

Os estudos sugerem que um desequilíbrio no estado redox pode estar associado à prematuridade (8). Na pré-eclâmpsia foi observado metabolismo anormal dos lipídios e altas concentrações de peróxido lipídico que podem levar ao estresse oxidativo e à disfunção vascular (9).Desta forma, qualquer situação que coloque em desequilíbrio o organismo da mãe parece ter grande impacto no recém-nascido. Os estudos clínicos delimitam mães com fatores de risco para avaliar o estresse oxidativo no contexto da gestação (3,4,9–11), no entanto, estudos com componentes maternos e neonatais e suas relações são relativamente menores.

Neste contexto, o objetivo deste trabalho é avaliar os marcadores de stress oxidativo na díade mãe-filho de gestações normais e gestações de partos prematuros para verificar a relação do estresse oxidativo materno com prematuridade.

Essa dissertação compreende: introdução; revisão de literatura abordando prematuridade, estresse oxidativo e marcadores do estresse oxidativo. Os resultados obtidos estão apresentados no capítulo 1 em forma de artigo científico intitulado **Implications of maternal oxidative stress in a newborn with term pregnancy and premature birth** o qual foi submetido ao periódico *The JOURNAL of PEDIATRICS* por fim, apresentamos as conclusões do estudo e as considerações finais.

2. REVISÃO DE LITERATURA

2.1. PREMATURIDADE

A prematuridade é definida como neonato que nasce antes das 37 semanas de gestação e é subdividida de acordo com a idade gestacional (IG): prematuro extremo (< 28 semanas de gestação), muito prematuro (28 a 32 semanas) e prematuro moderado a tardio (32 a 37 semanas)(12), sendo o maior determinante de mau prognóstico infantil em termos de sobrevivência e qualidade de vida (1). Além disso, há uma preocupação crescente devido à alta letalidade, complicações associadas à prematuridade e ao alto custo econômico e social, a curto, médio e longo prazo (2).

Os recém-nascidos (RN) prematuros são particularmente vulneráveis a complicações e sequelas devido à alta incidência de patologias associadas como: insuficiência respiratória, dificuldade de alimentação, desregulação térmica e suscetibilidade a infecções (13). Dados apontam que ocorrem cerca de 15 milhões de nascimentos prematuros anualmente no mundo, sendo equivalente a 11% do total de nascimentos, aproximadamente (14). O Brasil está classificado entre os 10 principais países do mundo com o maior número de nascimentos prematuros (15), com estimativa de prevalência de 11,5% (16), sendo que a principal causa de óbito em menores de 5 anos foram complicações de e pós partos prematuros(17).

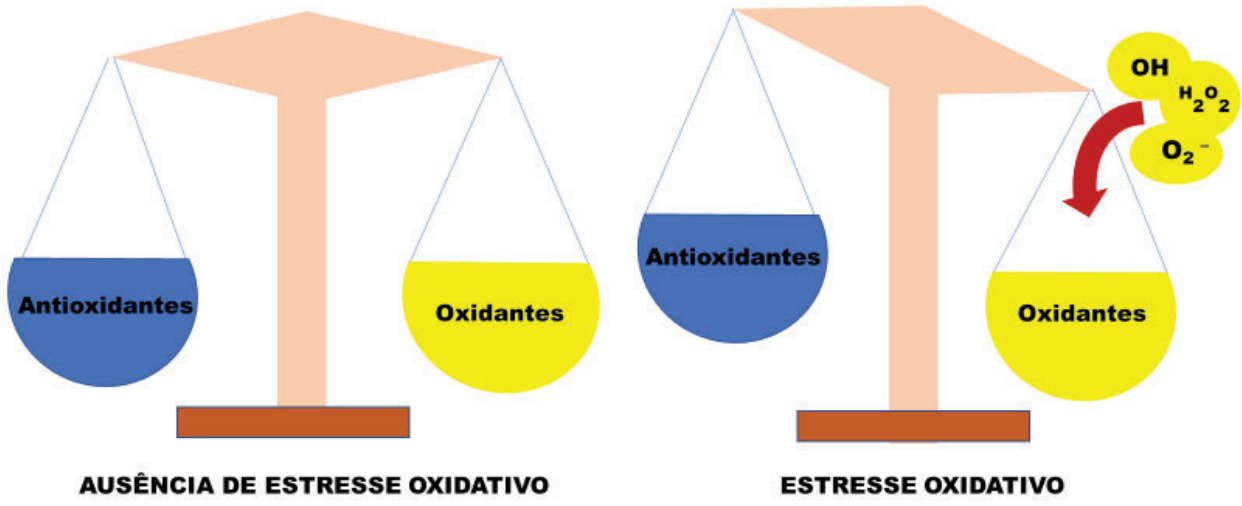
A etiologia do parto prematuro é multifatorial, onde o perfil das mães juntamente com as condições socioeconômicas e sanitárias certamente influenciam (18). Um estudo feito em Pelotas – RS com um total de 4.142 mulheres, os fatores que permaneceram significativamente associados ao parto prematuro foram: cor da pele negra, baixa escolaridade, pobreza, idade materna jovem, primiparidade, prematuro anterior, inadequação do pré-natal e hipertensão (19). Outro estudo de revisão com base populacional feito no Brasil, encontrou os seguintes fatores de risco para a ocorrência de prematuridade: baixo peso materno pré-gestacional e extremos de idade materna, história prévia de natimorto, tabagismo na gravidez, ganho de peso materno insuficiente, hipertensão arterial, infecção do trato geniturinário, cinco ou menos consultas de pré-natal e estresse materno(20). Embora seja uma condição complexa resultante de múltiplos fatores etiológicos, é também bem aceito que tanto a infecção quanto a inflamação representam fatores de risco altamente significativos para o parto prematuro (21). Dados de 20 coortes em 13 países, mostraram que um recém-nascido prematuro tem risco aumentado de 6 a 26 vezes de morte neonatal do que recém-nascidos a termo (22). Assim, as causas da prematuridade e como evita-la é o objetivo de muitas pesquisas.

2.2. ESTRESSE OXIDATIVO

A geração de radicais livres constitui, por excelência, um processo contínuo e fisiológico, cumprindo funções biológicas relevantes. Durante os processos metabólicos, esses radicais atuam como mediadores para a transferência de elétrons nas várias reações bioquímicas. Sua produção, em proporções adequadas, possibilita a geração de adenosina trifosfato (ATP) - (energia). Porém, a produção excessiva pode conduzir a danos oxidativos (23).

O estresse Oxidativo é definido como o desequilíbrio homeostático dentro do ambiente de redução-oxidação (redox) que envolve uma desregulação entre oxidantes e antioxidantes(24). A instalação do processo de estresse oxidativo decorre da existência de um desequilíbrio entre compostos oxidantes e antioxidantes, em favor da geração excessiva de radicais livres ou em detrimento da velocidade de remoção desses (Figura1). Tal processo conduz à oxidação de biomoléculas com consequente perda de suas funções biológicas e/ou desequilíbrio homeostático, cuja manifestação é o dano oxidativo potencial contra células e tecidos(25).As espécies reativas de oxigênio (ROS) são moléculas contendo oxigênio e radicais livres que agem como agentes oxidantes removendo um elétron ou adicionando oxigênio outras moléculas(24).

Figura 1. Processo de desequilíbrio – origem do estresse oxidativo



Fonte: o autor (2020)

2.3. ESTRESSE OXIDATIVO E GRAVIDEZ

A vida humana *in útero* decorre em um ambiente que é relativamente hipóxico em comparação com o *ex- útero*. No entanto, a disponibilidade de oxigênio é fornecida por mecanismos adaptativos que permitem um crescimento e desenvolvimento extraordinários que excedem qualquer outro período da vida. Condições maternas durante a gravidez podem causar hipóxia fetal. A hipóxia crônica é causada por alterações vasculares ou metabólicas da mãe, como pré-eclâmpsia, obesidade ou diabetes. Os fetos hipóxicos apresentam maior risco de desenvolver estresse oxidativo, que pode ser determinante para o seu desenvolvimento *in útero* e pós-natal(26).

O estresse oxidativo tem sido implicado em muitas complicações de saúde durante a gravidez, incluindo trabalho de parto prematuro. Existem lacunas na descrição das vias exatas do trabalho de parto prematuro espontâneo porque vários processos biológicos estão provavelmente envolvidos, incluindo infecção, doença vascular da placenta (27).

A identificação de associações entre medições de estresse oxidativo e resultados perinatais se tornou popular, à medida que cientistas e médicos se esforçam para entender a fisiopatologia que leva a essas complicações associadas à gravidez.

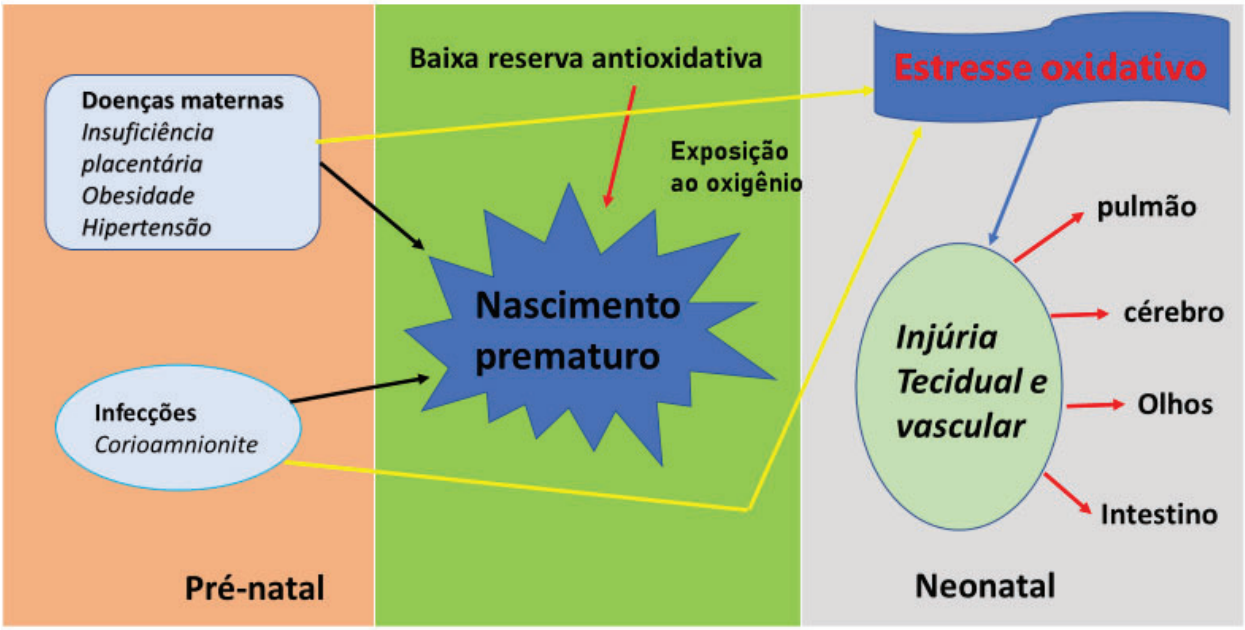
2.4. PREMATURIDADE E ESTRESSE OXIDATIVO

O equilíbrio do estado oxidante e antioxidante (redox) é um processo que começa antes do nascimento (6), onde fetos, recém-nascidos e principalmente prematuros, são altamente propensos ao insulto de espécies reativas e oxidativas, devido ao desafio hipóxico-hiperóxico, suscetibilidade a infeções, deficiência de defesa antioxidante e a altos níveis de ferro livre(7). As complicações da prematuridade, a grande maioria como displasia broncopulmonar, enterocolite necrosante, hemorragia intraventricular, leucomalácia periventricular e retinopatia estão relacionadas ao estresse oxidativo (28), ocorrendo principalmente devido a um descompasso entre a produção de radicais livres e a capacidade antioxidante do recém-nascido prematuro (7).

Saugstad propôs a existência da "Doença dos radicais livres de oxigênio em neonatologia", devido à grande propensão dos neonatos à injúria causada pelas tóxicas espécies reativas de oxigênio (EROS) (29). Sullivan sugeriu que o termo mais correto seria o de "Doença dos radicais livres de oxigênio da prematuridade", devido à grande incidência de tais patologias no prematuro, denotando que algum aspecto da prematuridade aumenta a suscetibilidade aos efeitos tóxicos dos radicais livres (30). Atualmente, após 30 anos de estudo e investigação, a

complexidade do estresse oxidativo, tanto no estado considerado normal, quanto em estados patológicos, é melhor compreendida, especialmente no que se refere aos mecanismos mitocondriais de estresse oxidativo neonatal relativos à hiperóxia (31) (Figura2).

Figura 2. Efeitos do dano oxidativo no nascimento prematuro



Fonte: o autor (2020)

Os estudos sugerem que um desequilíbrio no estado redox pode estar associado à prematuridade (8). Na pré-eclâmpsia foi observado metabolismo anormal dos lipídios e altas concentrações de peróxido lipídico que podem levar ao estresse oxidativo e à disfunção vascular (9). Assim, o estresse oxidativo é o fator mais importante nas complicações durante o segundo e o terceiro trimestre da gravidez(32).No mesmo sentido, as razões mais comuns para nascimentos prematuros incluem pré-eclâmpsia ou eclampsia e restrição de crescimento intrauterino (33); um estudo feito na Índia em mães com pré-eclâmpsia e com mães saudáveis mostrou que o maior índice de estresse oxidativo ocorre nas mães e nos RN do grupo com pré-eclâmpsia (34). Em outro estudo recente, foi analisado todos os nascimentos prematuros entre 1986 e 2010 na Austrália, sendo que nas análises multivariadas os fatores associados à disfunção placentária foram responsáveis por >10% do risco de parto prematuro (35).

Já é amplamente reconhecido que o estresse oxidativo pode desempenhar um papel importante na patogênese da doença humana e constitui um tópico importante em todas as Áreas da medicina (36). No entanto, a comparação de recém-nascidos nascidos a termo, com recém-nascidos prematuros, os sistemas antioxidantes são extremamente deficientes.(37).

2.5. BIOMARCADORES DE ESTRESSE OXIDATIVO

Biomarcador é definido como um indicador de um processo biológico normal ou anormal. A identificação de biomarcadores é essencial para a determinação do estresse oxidativo e, provavelmente, para a descoberta precoce de doenças a ele associadas. Um bom biomarcador deve ter alta sensibilidade e alta especificidade além de sua medição ser reprodutível(27).

Devido há necessidade crescente de ferramentas confiáveis para avaliar, controlar e reduzir o estresse oxidativo em recém-nascidos, os biomarcadores, obtidos a partir da oxidação de biomoléculas, foram desenvolvidos (38), uma vez que a meia meia-vida extremamente curta dos radicais livres não permite medir diretamente sua concentração(23).

Os biomarcadores avaliam a suscetibilidade do hospedeiro ao estresse oxidativo por meio da avaliação de proteínas, lipídios e danos ao DNA. Os métodos analíticos gerais disponíveis atualmente para o estudo dos danos causados pelo estresse oxidativo podem ser divididos em duas categorias: aqueles que detectam a oxidação em lipídios, proteínas e DNA e aqueles que detectam o risco potencial de estresse oxidativo (como o ferro não ligado a proteínas) (27)**.** Neste estudo optamos por usar os biomarcadores que detectam a oxidação dos lipídeos, proteínas e DNA.

a) Peroxidação lipídica: as membranas biológicas, que contêm lipídios poli-insaturados, tornam-se suscetíveis à oxidação na presença de um radical livre. O dano induzido por radicais livres aos lipídios da membrana pode induzir lesões celulares críticas e irreversíveis, iniciando um estado de doença. A lipoperoxidação é um processo pelo qual o ácido graxo poli-insaturado presente nos fosfolipídios das membranas celulares sofre uma reação com o oxigênio, produzindo hidroperóxidos lipídicos. A reação é desencadeada pela liberação de radicais livres reativos. Os hidroperóxidos são os principais produtos moleculares da lipoperoxidação e podem ser medidos no plasma.(27).

b) Catalase: é uma proteína reativa responsável pela decomposição e eliminação de espécies reativas de oxigênio. Catalisa o peróxido de hidrogênio em água e oxigênio. A presença desse proteína pode reduzir o dano oxidativo e o estresse oxidativo celular (39)

c)Óxido nítrico: é um dos principais radicais livres e sua produção excessiva é resultante da falta de sistemas de proteção antioxidante, acarretando o estresse oxidativo. Modelos animais têm mostrado um aumento na produção de NO durante a hipóxia (40)**.** A asfixia perinatal está associada a níveis aumentados de NO. (41)**.** Óxido nítrico desempenha papel na fisiopatologia da hipertensão pulmonar persistente do recém-nascido. As espécies reativas de oxigênio podem ser produzidas pela cadeia de transporte de elétrons da mitocôndria ou a partir de enzimas, como

a óxido nítrico sintase. O óxido nítrico (NO) é um radical livre e avidamente se liga ao ânion superóxido para formar peroxinitrito (42).

d)Dano oxidativo DNA: 8-hydroxydeoxyguanosine (8-OHdG) é um marcador de estresse oxidativo e indica dano ao DNA, uma vez que é liberado quando o reparo do DNA está danificado. Pode ser detectado em tecidos humanos, amostras de sangue e na urina (27)**.** A guanina é a base nitrogenada mais propensa à oxidação, sua interação com o radical hidroxila gera 8-oxo-2'-desoxiguanosina (8-OHdG), biomarcador de dano oxidativo ao DNA mais estudado (40)**.** Em relação aos biomarcadores de oxidação de DNA, 8-hidroxidesoxiguanosina (8-OHdG) tem sido o composto mais estudados O 8-OHdG é utilizado como biomarcador de estresse oxidativo em amostras de sangue e aspirados traqueais (8). É um biomarcador muito útil para avaliar o risco de displasia broncopulmonar em bebês de muito baixo peso(43)**.** Foi considerado biomarcador potencial de retinopatia da prematuridade (44).

3.CAPÍTULO 1

IMPLICATIONS OF MATERNAL OXIDATIVE STRESS IN A NEWBORN WITH TERM PREGNANCY AND PREMATURE BIRTH

Simone Medeiros Beder Reis[ad], Julia Spanhol da Silva[b], Natália Freddo[a], Wânia Eloisa Ebert Cechin[d], Mylena Munaro Bruschi[d], Aline Zuanazzi Pasinato[c], Luciana Grazziotin Rossato Grando[a], Rômulo Pillon Barcelos[abc]*

[a]Post-Graduation Program in Bioexperimentation, University of Passo Fundo (UPF), Passo Fundo, Rio Grande do Sul, Brasil

[b] Post-Graduation Program in Toxicological Biochemestry, Center of Naturaln and Exacts Sciences (CCNE), Federal University of Santa Maria (UFSM), Santa Maria, Rio Grande do Sul, Brazil

[c] Institute of Biological Sciences, University of Passo Fundo (UPF), Passo Fundo, Rio Grande do Sul, Brazil

[d] College of Medicine, University of Passo Fundo (UPF), Passo Fundo, Rio Grande do Sul, Brazil

*Corresponding author: Rômulo Pillon Barcelos

Institute of Biological Sciences, University of Passo Fundo,

Av. Brasil Leste, 285 - São José, Passo Fundo - RS, Brazil. 99052-900.

Phone: (55) 991316601.

E-mail: romulopillon@upf.br

ABSTRACT

Prematurity is the major cause of poor child prognosis in terms of survival and life quality. The etiology of premature birth is multifactorial. Researchers suggest that an oxidative imbalance of the mother's body over the fetus would lead to premature birth. The aim of this study was to assess the mother's oxidative stress and its implications for full-term newborns and newborns. We dosed the oxidative stress markers for lipoperoxidation (TBARS), 8-Hydroxydeoxyguanosine (8-OHdG), nitric oxide, and catalase activity in maternal blood and umbilical cord blood (SCU) from 39 mother-child binomials. They were divided into 2 groups: term (n = 23) and premature (n = 18), which was subdivided into a spontaneous premature (n = 8) and placental premature (n = 8). Our results showed increased TBARS levels of mothers in the term group compared to the premature. We found increased TBARS levels and nitric oxide of the premature placental for the mothers of the premature subgroups. In the analysis of the SCU from newborns, we found increased 8-OHdG and TBARS levels in the term compared to the premature. We conclude that oxidative stress is related to increased lipid peroxidation and nitric oxide levels in pregnant women with placental pathologies. It is suggested that oxidative stress is the causative factor of premature birth, but it needs confirmation. Besides, the 8-OHdG levels in pregnant women do not represent oxidative damage, but the ability to repair previous oxidative damage, which occur more at non-premature births.

Keywords: Redox status; oxidative damage; pregnancy; prematurity; 8-OHdG; TBARS; clinical study.

1. INTRODUCTION

Prematurity is considered the primary determinant of poor child prognosis in survival and life quality (1). Also, there is a growing concern due to high lethality, complications associated with prematurity, and elevated economic and social cost in the short, medium, and long term (2).

Pregnancy is a physiological state associated with increased oxidative stress due to a more significant metabolic turnover and elevated tissue oxygen requirements (3). It is a state of metabolic challenge between a developing mother and fetus, with high oxidative stress levels compared to a non-pregnant state (4,5).

The balance of the oxidative and antioxidant state (redox) is a process that begins before birth (6), where fetuses, newborns, and especially premature, are highly prone to produce reactive and oxidative species due to the hypoxic-hyperoxic challenge, susceptibility to infections, antioxidant defense deficiency and high levels of free iron (7).

The etiology of premature birth is multifactorial, influenced by mothers' profiles, socioeconomic, and health conditions (8). There are several risk factors associated with prematurity. Among them, we can mention low pre-gestational maternal weight, maternal age, previous history of stillbirth, smoking during pregnancy, insufficient maternal weight gain, arterial hypertension, genitourinary tract infection, and maternal stress (9). Literature suggests that an imbalance in the redox state is associated with prematurity (10). In preeclampsia, abnormal lipid metabolism and high concentrations of lipid peroxide are observed, leading to oxidative stress and vascular dysfunction (11).

Thus, any situation that puts the mother's organism out of balance seems to significantly impact the newborn. Clinical studies delimit mothers with risk factors to assess oxidative stress in the context of pregnancy (3,5,11–13). However, studies with maternal and neonatal components and their relationships are relatively less frequent, minor, and sometimes controversial. Considering that early diagnosis of maternal oxidative stress can be of paramount importance for the outcome of pregnancy, this study's objective was to evaluate the markers of oxidative stress in the mother-child dyad of normal pregnancies and premature births pregnancies to verify the relationship of maternal oxidative stress with prematurity.

2. MATERIALS AND METHODS

This section describes the material and methods used in this research.

2.1 Experimental design

It is an observational/cross-sectional clinical trial where mothers and their respective children were randomly selected in the Maternity Sector of Hospital São Vicente de Paulo from January to April 2020. Mothers and their respective children with a gestation of 28 to 36 weeks as a group experimental, called "premature." For the control group, called "term group", mothers and children with full-term pregnancies (over 37 weeks) were selected. Mothers and their respective children in the premature group were divided into subgroups. Mothers and their children who had a spontaneous premature delivery or premature rupture of the membranes were combined into a single group called "spontaneous premature" infants; a second group called "placental premature" included mothers and their respective children whose premature births were determined as a result of preeclampsia (14).

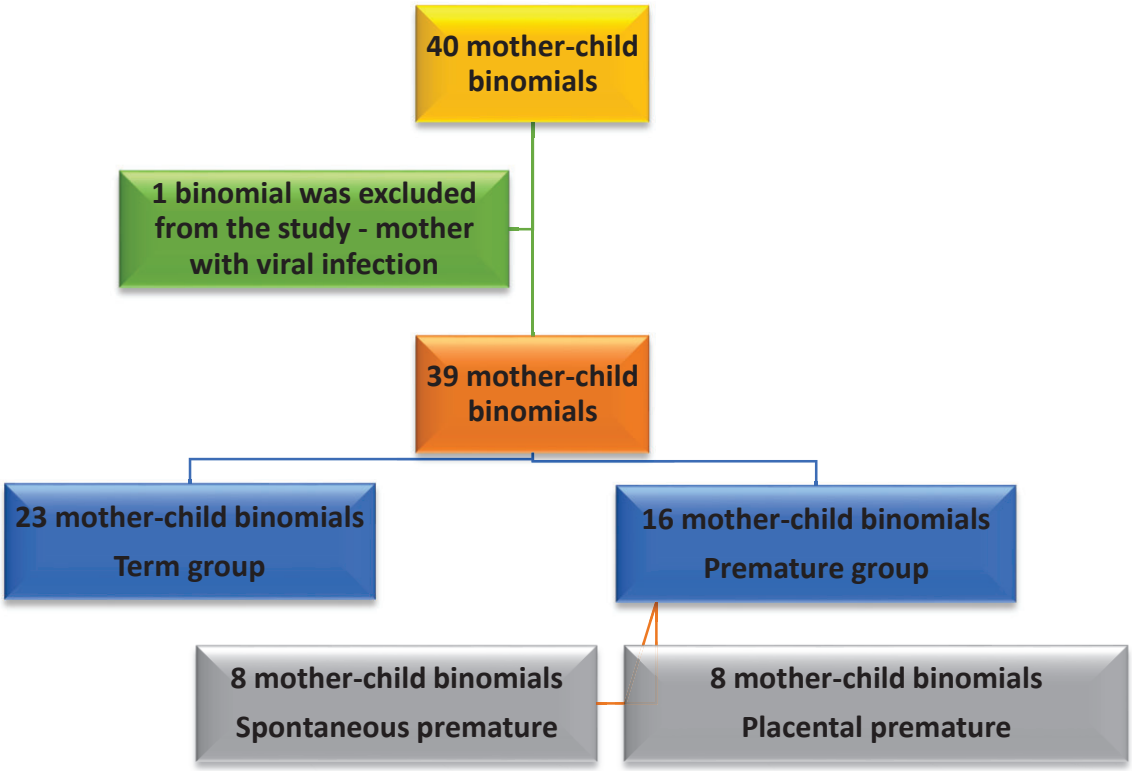


Figure 1: Experimental design

The inclusion criteria were: maternal age > 18 years and gestational age calculated by obstetric ultrasound performed between the 7th and 20th week (15). The exclusion criteria were mothers and their children who had severe or multiple birth malformations (16) or who had the possible congenital viral infections.

Pregnant women on admission to the maternity sector were invited to participate in the study, with the informed consent form's signature. Variables were collected from their medical records that included age, medical and obstetric history, mode of delivery, and smoking habits. A sample (5ml) of venous blood was collected from the mothers, half of the volume was placed in a tube with ethylene diamine tetra acetic acid (EDTA), and the other half was placed in a dry tube with separating gel and centrifuged for 5 min at 4000 rpm. Serum and plasma were separated and stored at -70ºC.

Immediately after birth delivery, the umbilical cord was clamped at two different locals, 25 cm apart, and the umbilical cord blood sample (SCU) was collected from this section, allocated in an EDTA tube and in a dry tube with separating gel, centrifuged for 5 minutes at 4000 rpm. SCU serum and plasma were stored at -70ºC. Newborn variables were collected from their medical records: birth weight and APGAR score (Appearance, Pulse, Grimace, Activity, Respiration.) (17).

2.2 Oxidative stress markers

2.2.1 Lipid peroxidation

Lipid peroxidation was evaluated by the reaction of reactive species to thiobarbituric acid (TBARS). The product of this reaction was quantified by spectrophotometry at 534 nm. The results were expressed with ugmol.g-1 protein (18).

2.2.2 Nitric oxide

The concentration of nitric oxide (NO) was evaluated colorimetrically using the Griess reagent. The product of this reaction was quantified by spectrophotometry at 540 nm. The results were expressed as protein ugmol.g-1 (19).

2.2.3 Protein quantification

The protein was determined using the Coomassie blue - Bradford method and bovine serum albumin was used as a standard. The samples were run in duplicate and the absorbance was measured at 595 nm. The results were expressed in g/L (20).

2.2.4 Catalase

It was determined according to the method of Nelson and Kiesow (1972). The absorbance was recorded for 1 minute (every 15 seconds) at 240nm and the results were given in nmol/mg protein/minute (21).

And in the mothers' plasma and in the umbilical cord blood of their respective children it was dosed:

2.2.5 8-Hydroxydeoxyguanosine (8-OHdG)

For the determination of 8-OHdG the competitive commercial enzyme immunoassay kit: E-EL-0028 (Elabscience) was used according to the manufacturer's instructions. The results were expressed in mg/ml (22).

2.3 Statistical analysis

The data table was built using Microsoft Excel. Statistical analysis was performed using IBM SPSS Statistics version 26 for Windows.

Quantitative variables were expressed as mean ± standard deviation or as median (25th percentile - 75th percentile). Comparisons of oxidative stress markers between premature and full-term pregnancies were performed using analysis of variance with a classification criterion. For variables that violated the assumption of homoscedasticity, assessed by the Levene test, was presented by the Brown-Forsythe robust estimator. The comparisons between gestational history, APGAR in the 1st and 5th minutes and premature and term pregnancies were performed using the U test of Mann-Whitney: Probability values <0.05 were considered statistically significant.

3. RESULTS

Blood and data were collected from the medical records of 40 mother-child binomials, where 1 binomial was excluded from the study because the mother presented cytomegalovirus serology in the postpartum exams. Thus, we included 39 women with their respective children, with 16 mother-child binomials in the premature group and 23 mother-child binomials in the term group. Among the 16 mother-child binomials in the premature group, 8 were classified as spontaneous preterm infants and 8 as placental preterm infants. The characteristics of the participants are summarized in table 1.

Table 1. Characteristics of mothers (n=39) and of newborns (n=39).			
	Premature *(n=16)*	**Term** *(n=23)*	P
Mothers			
Age	29,6 ± 7,2	26,8 ± 6,1	0,210
Pregnancy age (weeks)	33,2 ± 2,4	39,1 ± 1,0	**< 0,001**
Smoker	1 (6,3%)	2 (8,7%)	1,000
Previous pregnancy	2,0 (1,3 – 4,5)	3,0 (1,0– 3,0)	0,832
Previous abortion	0 (0 – 0,8)	0 (0 – 0)	0,437
Previous birth	1,0 (0 – 2,8)	1,0 (0 – 2,0)	0,921
Way of birth			0,411
Vaginal	3 (18,8%)	7 (30,4%)	
Operative	13 (81,2%)	16 (69,6%)	
Newborns			
Birth weight (grams)	1.803,4 ± 640,7	3.184,6 ± 342,8	**< 0,001**
Apgar 1º minute	7,0 (5,3 – 8,0)	9,0 (8,0 – 9,0)	**< 0,001**
Apgar 5º minute	9,0 (8,0 – 10,0)	9,0 (9,0 – 10,0)	0,207
Values express mean ± standard deviation, absolute and relative frequency or median (25th percentile - 75th percentile)			

There was no statistically significant difference in the age and obstetric history of the mothers in the 2 groups. The mean gestational age of the premature group was (33.2 ± 2.4 weeks), and the term group was (39.1 ± 1.0 weeks) with statistical significance between the groups (p <0.001). With regard to smoking, 1 mother in the premature group (6.3%) and 2 mothers in the term group (8.7%) representing 15% of the total sample, with no statistically significant difference between the groups. The operative mode of delivery was the most prevalent, representing 69.6% of the total sample (Table 1), with no statistical significance between the groups (p = 0.411). The characteristics of newborns participants are summarized in table 1.

Premature newborns had significantly lower birth weight compared to full-term newborns (1803.4 ± 640.7g and 3,184.6 ± 342.8g, respectively), p <0.001.

The APGAR score measured in the first minute showed a median of 7 in the premature group and a median of 9 in the control group, with a significant difference between the groups, however in the APGAR measured in the fifth, there was no significant difference.

The markers: lipid lipoperoxidation, 8-OHdG, nitric oxide, proteins, and catalase dosed in mothers in the premature group and the term group are shown in table 2. We found an increase with statistical significance in the concentrations of lipid lipoperoxidation in the term group compared to the premature group. However, there was no statistically significant difference between groups in the other markers studied.

Table 2. Oxidative stress markers of mothers and SCU of newborns.

	Premature *(n=16)*	**Term** *(n=23)*	**P**
Mothers			
Lipid peroxidation, ugmol.g^{-1}	15,6 ± 7,3	21,7 ± 7,1	**0,013**
8-OHdG, ng/mL	126,0 ± 37,8	122,0 ± 21,9	0,705
Nitric oxide, ugmol.g^{-1}	13,8 ± 6,1	16,9 ± 12,5	0,370
Protein, g/L	135,7 ± 42,3	121,6 ± 40,8	0,302
Catalase ugmol.g^{-1}	0,05 ± 0,03	0,05 ± 0,03	0,844
SCU of newborns			
Lipid peroxidation, ugmol.g^{-1}	15,0 ± 5,3	21,2 ± 7,2	**0,006**
8-OHdG, ng/mL	46,4 ± 30,6	73,8 ± 33,2	**0,013**
Nitric oxide, ugmol.g^{-1}	15,0 ± 7,2	16,6 ± 10,3	0,584
Protein, g/L	131,9 ± 37,7	125,3 ± 38,5	0,599
Catalase, ugmol.g^{-1}	0,17 ± 0,21	0,07 ± 0,04	0,094
Values express mean ± standard deviation			

Table 2 shows the results of oxidative stress markers measured in the SCU of newborns (premature and term group). We found statistically significant higher values in the oxidative stress markers of the term group: 8-OHdG (P = 0.013) and lipid lipoperoxidation (P = 0.006). In the other markers, there was no statistically significant difference between the groups.

Table 3 shows the results of the oxidative stress markers of mothers of the subgroups of preterm infants (spontaneous preterm infants and placental preterm infants). The results of lipid lipoperoxidation and nitric oxide measurements were statistically higher in the premature placental subgroup compared to the spontaneous premature subgroup (Figure 2). The other

markers studied were not statistically significant. Finally, table 3 shows the results of the oxidative stress markers of the SCU of newborns in the subgroups of preterm infants (spontaneous preterm infants and placental preterm infants). There was no statistical significance among all studied markers.

Table 3. Oxidative stress markers of mothers and SCU of newborns of the premature subgroup.

	Spontaneous premature *(n=8)*	**Placental premature** *(n=8)*	**P**
Mothers			
Lipid peroxidation, ugmol.g^{-1}	11,4 ± 3,9	19,8 ± 7,7	**0,021**
8-OHdG, ng/mL	118,9 ± 36,6	133,0 ± 40,1	0,861
Nitric oxide, ugmol.g^{-1}	9,9 ± 3,4	17,8 ± 5,6	**0,005**
Protein, g/L	161,8 ± 31,6	109,7 ± 36,1	**0,008**
Catalase, ugmol.g^{-1}	0,04 ± 0,02	0,05 ± 0,04	0,321
SCU of newborns			
Lipid peroxidation, ugmol.g^{-1}	13,9 ± 5,6	16,2 ± 5,0	0,393
8-OHdG, ng/mL	60,1 ± 36,3	32,6 ± 16,1	0,071
Nitric oxide, ugmol.g^{-1}	15,0 ± 7,9	15,0 ± 6,9	0,996
Protein, g/L	145,0 ± 44,3	118,9 ± 26,4	0,175
Catalase, ugmol.g^{-1}	0,17 ± 0,27	0,16 ± 0,15	0,930
Values express mean ± standard deviation			

4. DISCUSSION

In this observational/cross-sectional clinical study, we assessed the level of DNA damage, oxidative stress markers, and antioxidant enzymes in mother-child binomials in a group with premature newborns compared to a group with full-term newborns. We found higher values in the dosage of lipid lipoperoxidation in the mothers of the term group, in the levels of lipid lipoperoxidation and nitric oxide in the mothers of the placental premature subgroup compared to the spontaneous premature subgroup. We found higher lipid lipoperoxidation and 8- OHdG levels in the SCU of newborns in the term group when compared to the premature group.

Lipid lipoperoxidation was significantly higher in mothers with term pregnancies compared to mothers who had premature newborns, corroborating with studies that show that in normal pregnancy, there is an increase in lipoperoxidation products as the pregnancy progresses (23–26). During physiological pregnancy, the development of fetal tissues and organs requires the provision of an adequate amount of nutrients and oxygen. Their reactive forms produced in the body of the mother and fetus affect the replication, differentiation, and maturation of developing cells. Its balanced activity and the maintenance of the balance of oxidative processes are necessary factors for the organism's proper development and functioning (27). There are also numerous anatomical, physiological, and metabolic changes in the mother's body. According to the researchers, they are presumed to support ROS production, especially in the second half of pregnancy. This is mainly due to the increase in primary metabolism and the "consumption" of oxygen, and the use of fatty acids as the primary source of energy for most maternal placental tissues (28).

The last trimester of pregnancy is a special period of increased insulin resistance, fat catabolism, and free fatty acids released. These processes might lead to an increase in the production of free radicals (29). The placenta, filled out with mitochondria, is the primary source of pro-oxygenates, the so-called "factory" of ROS. The superoxide anion radical is the precursor of other more reactive forms of oxygen-derived radicals, such as hydrogen peroxide and hydroxyl radical. Its production increases with pregnancy development, which is mainly associated with an increase in placental mass (30).

Thus, a balanced redox metabolism is necessary from conception for the proper placenta development. The relatively low oxygen pressure in the initial placenta development aims to prevent ROS's excessive production, protecting the embryo, and fetus against free radicals' harmful and teratogenic effects (31). The redox balance processes are essential for appropriate implantation and embryonic development. Simultaneously, uncontrolled production of oxygen radical peroxidation products can lead to embryonic resorption, embryopathy, preeclampsia and placenta degeneration, leading to growth decificts, low birth weight, or even premature birth (6,31,32).

In our study, we confirm this theory. When analyzing only the mothers of the premature group with (placental premature) and without (spontaneous premature) pathologies during pregnancy, we found that the lipid lipoperoxidation significantly higher in the mothers of the premature placental group (mothers with preeclampsia) in comparison with mothers without pathologies (spontaneous premature). In addition, lipid lipoperoxidation was significantly higher in the SCU of full-term newborns compared to premature newborns. As far as we know,

this is the first time that it is reported. One study found augmented TBARS levels in mothers and SCU of full-term newborns immediately after birth (33), in line with our study, in which we found higher lipid lipoperoxidation levels in the mothers and the SCU of newborns in the term group. However, when we analyzed the lipid lipoperoxidation of the SCU of newborns in premature subgroups, there was no significant difference.

Our results corroborate with the Tastekin et al. (34) study. They reported that the lipid lipoperoxidation in mothers' cord blood (32 weeks gestational age) was similar between groups (mothers with preeclampsia and without preeclampsia). However, Zadir et al. (35) obtained different results, in which the TBARS levels were increased in the cord blood of mothers with preeclampsia compared to the control group. With these data, we can demonstrate oxidative stress markers in umbilical cord blood, indicating the fetal exposure of ROS during intrauterine life. However, how newborns react to this exposure is still unclear.

In the same line, we evaluated the 8-OHdG levels, an oxidized nucleoside released after the DNA repair, commonly used as a marker of oxidative stress, as one of the most sensitive biomarkers of oxidative stress, and, therefore, widely used as a biomarker of oxidative damage to DNA (36). In our study, no differences were found in 8-OHdG levels between mothers (preterm and term), as well as between mothers in the preterm subgroups (spontaneous x placental preterm). However, the literature is controversial. Some studies show higher concentrations of 8-OHdG in mothers of premature newborns than mothers of full-term newborns (37,38). Other studies show variable levels of 8-OHdG during pregnancy, with higher levels throughout the pregnancy (26,39). It is worth mentioning that all of these studies analyzed urinary levels of 8-OHdG. However, a recent study in which maternal serum 8-OHdG levels were measured using the ELISA method (40) found results similar to our study, with no statistically significant difference in 8-OHdG levels between groups with preeclampsia. mild, severe preeclampsia and the group of healthy pregnant women. Studies are summarized in table 4.

Table 4. Study with the 8-OHdG marker

Biomarker	Study population	Results	Reference
8-OHdG urinary	487 Mothers of premature newborns and mothers of full-term newborns.	Higher levels of 8-OHdG in mothers of preterm infants.	(37)
8-OHdG urinary	503 healthy pregnant women 24-26 weeks of gestation were followed prospectively until postpartum.	Higher levels of 8-OHdG in women with complicated pregnancies who progressed to premature birth.	(38)

8-OHdG urinary	26 healthy pregnant women at 20, 30 weeks and at delivery.	Variable levels of 8-OHdG during pregnancy, with higher levels later compared to early pregnancy.	(39)
8-OHdG urinary	105 women with uncomplicated pregnancies at various stages of pregnancy and 6 to 8 weeks after delivery and 40 healthy women of reproductive age, but not pregnant, as controls.	8-OHdG levels with significant increase in the third trimester of pregnancy returning to non-pregnant levels in the postpartum period.	(26)
8-OHdG serum	22 pregnant women with severe preeclampsia, 18 pregnant women with mild preeclampsia and 40 healthy pregnant women at 25 and 41 weeks of gestation	8-OHdG levels with no statistically significant difference between groups.	(40)

An alternative hypothesis defends the fact that 8-OHdG tracks not only oxidative damage to DNA but also the successful execution of the repair process (41), so, in this scenario, 8-OHdG is not a stress marker oxidative, but the ability to repair oxidative damage. Our study found a significant difference with higher levels in the SCU of full-term newborns compared to the SCU of premature newborns, corroborating this theory. We suggest that it may indicate that a full-term newborn is more likely to execute the repair process of intrauterine oxidative damage successfully when pregnancy evolves than those who had born prematurely. It was also suggested in another study with women who delivered babies with congenital malformations presented lower levels of 8-OHdG compared to mothers with healthy babies (37).

Besides, when analyzing the subgroups of preterm infants, we found no significant difference in the levels of 8-OHdG in the SCU between the spontaneous preterm and placental preterm groups. Another study evaluating 8-OHdG in the cord blood of pregnant women with newborns (32 to 41 weeks of gestation) also found no significant correlation between the levels of 8-OHdG in the SCU among newborns as having no relation to maternal factors (42), reinforcing the conclusion of the study by Akinci et al. (40), that the marker 8-OHdG does not support the concept of participation in the etiopathogenesis of preeclampsia and consequent premature given birth.

Another interesting marker analyzed was nitric oxide (NO). It is a key molecule involved in various biological functions in the human body, and some studies are investing in establishing the NO role in perinatal medicine. NO is a potent signaling molecule that maintains endothelial integrity, regulating vasodilation, leukocyte adhesion to blood vessels, platelet

aggregation (43), and participates in the regulation of vascular reactivity uteroplacental and fetal-placental circulations (44). We did not find any significant differences in NO levels when comparing mothers in premature groups with mothers in term groups. However, when we evaluated the subgroups (spontaneous preterm and placental preterm), NO levels were higher in the maternal serum of the preterm placental group (mothers with preeclampsia) compared to the spontaneous preterm group (mothers without preeclampsia).

However, there are contradictory reports on NO levels concerning preeclampsia. The increased levels of NO observed in women with preeclampsia in our study are in agreement with the findings of some studies (45–48). One study reported decreased levels of NO in pregnant women with preeclampsia compared to healthy pregnant women (49). On the other hand, studies have found no change in NO levels compared to normal pregnancies (50–52). In particular, a study carried out in Ghana by Adu-Bonsaffoh et al. (45) observed a high level of NO in preeclampsia compared to normotensive pregnancy. The authors attributed the high levels of NO to an unregulated compensatory reaction to restore generalized endothelial damage. As well, AH Shaamash et al. (48) concluded in their study that the increase in ON in pregnancies with preeclampsia is due to a protective compensatory mechanism to maintain blood flow and limit platelet aggregation in maternal-fetal circulations.These studies are summarized in table 5.

Table 5. Studies with the nitric oxide marker

Biomarker	Study population	Results	Reference
Serum NO	75 non-pregnant, 102 healthy pregnant women and 100 pregnant women with preeclampsia	Elevated serum NO levels in women with preeclampsia compared to healthy and non-pregnant pregnant women	(45)
Serum NO	60 pregnant women in four different groups: 10 normotensive pregnant women, 17 pregnant women with preeclampsia, 18 pregnant women with gestational hypertension and 15 pregnant women with chronic hypertension.	Serum nitrite levels were higher in pregnant women with preeclampsia, lower in chronic hypertensive pregnant women and similar in pregnant women with gestational hypertension compared to the control group	(46)
Serum NO	34 healthy pregnant women and 34 pregnant women with preeclampsia	Seriously high serum NO levels in pregnant women with preeclampsia compared to healthy pregnant women	(47)
Serum NO	31 full-term pregnant women with preeclampsia and eclampsia, 32 healthy full-term pregnant women and	Seriously high serum ON levels in pregnant women with preeclampsia compared to healthy pregnant women	(48)

	21 healthy non-pregnant women of the same age.		
Serum NO	64 pregnant women with preeclampsia and 30 healthy pregnant women	Decreased ON levels in pregnant women with preeclampsia compared to healthy pregnant women	(49)
Serum NO	30 pregnant women with preeclampsia and 30 healthy pregnant women	No difference in nitric oxide levels between pregnant women with preeclampsia and healthy pregnant women	(50)
Serum NO	39 normal pregnant women 34 women with preeclampsia	No difference in nitric oxide levels between pregnant women with preeclampsia and healthy pregnant women	(51)
Serum NO	We studied 26 patients with preeclampsia and 27 normotensive pregnancies.	No difference in nitric oxide levels between pregnant women with preeclampsia and healthy pregnant women	(52)

Besides, when analyzing the serum levels of NO in the SCU of the newborns here, we did not find any difference relating to premature vs. terms newborns, and when comparing the spontaneous vs. placental premature subgroups.. AH Shaamash et al. (48) previously found different results in NO analysis in the SCU of their newborns, with Seriously higher serum NO levels in pregnant women with preeclampsia compared to healthy pregnant women.

In conclusion, oxidative stress is related to the increased TBARS and nitric oxide levels in pregnant women with placental pathologies (mothers with preeclampsia). However, it is not yet possible to establish whether oxidative stress is the causative factor of premature birth. Among the markers of oxidative stress studied, 8-OHdG is considered one of the most sensitive. Nevertheless, it does not seem to correspond to oxidative damage in pregnant women but to the ability to repair oxidative damage. Further investigation might uncover the diagnostic value of oxidative stress markers in pregnant women and newborns and, thus, improve pregnancy outcomes and the consequent prognosis of children.

ACKNOWLEDGEMENTS

The work was supported by CAPES/PROEX 23038.005848/2018-31 research grants. JSS, received a fellowship from CAPES.

REFERENCES

1. McCormick MC. The Contribution of Low Birth Weight to Infant Mortality and Childhood Morbidity. N Engl J Med. 1985;312(2):82–90.
2. Walsh EM, Li SX, Black LK, Kuzniewicz M. Incremental Cost of Prematurity by Week of Gestational Age. AJP Rep. 2019;9(1):e76–83.
3. Chiarello DI, Abad C, Rojas D, Toledo F, Vázquez CM, Mate A, et al. Oxidative stress: Normal pregnancy versus preeclampsia. Biochim Biophys Acta - Mol Basis Dis. 2020;1866(2).
4. Little RE, Gladen BC. Levels of lipid peroxides in uncomplicated pregnancy: A review of the literature. Reprod Toxicol. 1999;13(5):347–52.
5. Morris JM, Gopaul NK, Endresen MJR, Knight M, Linton EA, Dhir S, et al. Circulating markers of oxidative stress are raised in normal pregnancy and preeclampsia. BJOG An Int J Obstet Gynaecol. 1998;105(11):1195–9.
6. Marseglia L, D'Angelo G, Manti S, Arrigo T, Barberi I, Reiter RJ, et al. Oxidative stress-mediated aging during the fetal and perinatal periods. Oxid Med Cell Longev. 2014;2014.
7. Ozsurekci Y, Aykac K. Oxidative Stress Related Diseases in Newborns. Oxid Med Cell Longev. 2016;2016.
8. Ramos HÂ de C, Cuman RKN. Fatores de risco para prematuridade: pesquisa documental. Esc Anna Nery. 2009;13(2):297–304.
9. SILVEIRA MF et al. Increase in preterm births in Brazil: review of population-based studies.
10. Moore TA, Ahmad IM, Zimmerman MC. Oxidative Stress and Preterm Birth: An Integrative Review. Biol Res Nurs. 2018;20(5):497–512.
11. Bhat P V., Vinod V, Priyanka AN, Kamath A. Maternal serum lipid levels, oxidative stress and antioxidant activity in preeclampsia patients from Southwest India. Pregnancy Hypertens. 2019;15:130–3.
12. Cambonie G, Comte B, Yzydorczyk C, Ntimbane T, Germain N, Lê NLO, et al. Antenatal antioxidant prevents adult hypertension, vascular dysfunction, and

microvascular rarefaction associated with in utero exposure to a low-protein diet. Am J Physiol - Regul Integr Comp Physiol. 2007;292(3).

13. Chełchowska M, Ambroszkiewicz J, Gajewska J, Mazur J, Lewandowski L, Reśko-Zachara M, et al. Influence of active exposure to Tobacco smoke on nitric oxide status of pregnant women. Int J Environ Res Public Health. 2018;15(12).

14. McElrath TF, Hecht JL, Dammann O, Boggess K, Onderdonk A, Markenson G, et al. Pregnancy disorders that lead to delivery before the 28th week of gestation: An epidemiologic approach to classification. Am J Epidemiol. 2008;168(9):980–9.

15. Pereira APE, Gama SGN da, Domingues RMSM, Leal M do C, Bastos MH, Schilithz AOC. Determinação da idade gestacional com base em informações do estudo Nascer no Brasil. Cad Saude Publica. 2014;30(suppl 1):S59–70.

16. OPAS/OMS. Prevenção e controle de enfermidades genéticas e os defeitos congênitos. 1984;(460).

17. Casey BM, McIntire DD, Leveno KJ. The Continuing Value of the Apgar Score for the Assessment of Newborn Infants. Obstet Gynecol Surv. 2001;56(7):406–7.

18. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351–8.

19. Bracht A I-I EL. Métodos de laboratório em bioquímica. Manole. São Paulo; 2003.

20. Kruger NJ. The Bradford method for protein quantitation. Methods Mol Biol. 1994;32:9–15.

21. Saccol R da SP, da Silveira KL, Manzoni AG, Abdalla FH, de Oliveira JS, Dornelles GL, et al. Antioxidant, hepatoprotective, genoprotective, and cytoprotective effects of quercetin in a murine model of arthritis. J Cell Biochem. 2020;121(4):2792–801.

22. kit Cat # E-EL-0028- Lot X944HDNPDM - Exp. 17/05/2021. -Fornecedor Elabscience (Texas-USA).

23. Mitsuaki M, Ikuo N, Masako H, Yutaka T, Kunio Y. Lipid peroxide levels and lipid content of serum lipoprotein fractions of pregnant subjects with or withoutpreeclampsia. Clin Chim Acta. 1981;115(2):155–61.

24. Patil SB, Kodliwadmath M V., Kodliwadmath SM. Study of oxidative stress and

enzymatic antioxidants in normal pregnancy. Indian J Clin Biochem. 2007;22(1):135–7.

25. Carone D, Loverro G, Greco P, Capuano F, Selvaggi L. Lipid peroxidation products and antioxidant enzymes in red blood cells during normal and diabetic pregnancy. Eur J Obstet Gynecol Reprod Biol. 1993;51(2):103–9.

26. Hung TH, Lo LM, Chiu TH, Li MJ, Yeh YL, Chen SF, et al. A Longitudinal study of oxidative stress and antioxidant status in women with uncomplicated pregnancies throughout gestation. Reprod Sci. 2010;17(4):401–9.

27. Bak A, Roszkowski K. Oxidative stress in pregnant women. Arch Perinat Med. 2013;19(3):150–5.

28. Moore TA, Ahmad IM, Schmid KK, Berger AM, Ruiz RJ, Pickler RH, et al. Oxidative Stress Levels Throughout Pregnancy, at Birth, and in the Neonate. Biol Res Nurs [Internet]. 2019;21(5):485–94.

29. Duhig K, Chappell LC, Shennan AH. Oxidative stress in pregnancy and reproduction. Obstet Med. 2016;9(3):113–6.

30. Sultana Z, Maiti K, Aitken J, Morris J, Dedman L, Smith R. Oxidative stress, placental ageing-related pathologies and adverse pregnancy outcomes. Am J Reprod Immunol. 2017;77(5):1–10.

31. Jauniaux E, Burton GJ. Le rôle du stress oxydant dans les pathologies placentaires de la grossesse. J Gynecol Obstet Biol la Reprod. 2016;45(8):775–85.

32. Bogavac M, Lakic N, Simin N, Nikolic A, Sudji J, Bozin B. Biomarkers of oxidative stress in amniotic fluid and complications in pregnancy. J Matern Neonatal Med. 2012;25(1):104–8.

33. Wilinska M, Borszewska-Kornacka MK, Niemiec T, Jakiel G. Oxidative stress and total antioxidant status in term newborns and their mothers. Ann Agric Environ Med. 2015;22(4):736–40.

34. Tastekin A, Ors R, Demircan B, Saricam Z, Ingec M, Akcay F. Oxidative stress in infants born to preeclamptic mothers. Pediatr Int. 2005;47(6):658–62.

35. Howlader MZH, Parveen S, Tamanna S, Khan TA, Begum F. Oxidative stress and

antioxidant status in neonates born to pre-eclamptic mother. J Trop Pediatr. 2009;55(6):363–7.

36. Scholl TO, Stein TP. Oxidant damage to DNA and pregnancy outcome. J Matern Fetal Med. 2001;10(3):182–5.

37. Stein TP, Scholl TO, Schluter MD, Leskiw MJ, Chen X, Spur BW, et al. Oxidative stress early in pregnancy and pregnancy outcome. Free Radic Res. 2008;42(10):841–8.

38. Hsieh TTA, Chen SF, Lo LM, Li MJ, Yeh YL, Hung TH. The association between maternal oxidative stress at mid-gestation and subsequent pregnancy complications. Reprod Sci. 2012;19(5):505–12.

39. Shoji H, Franke C, Campoy C, Rivero M, Demmelmair H, Koletzko B. Effect of docosahexaenoic acid and eicosapentaenoic acid supplementation on oxidative stress levels during pregnancy. Free Radic Res. 2006;40(4):379–84.

40. Akinci S, Özcan HC, Balat, Uğur MG, Öztürk E, Taysi S, et al. Assessment of 8-hydroxydeoxyguanosine levels in patients with preeclampsia: A prospective study. Clin Exp Obstet Gynecol. 2017;44(2):226–9.

41. Wu LL, Chiou CC, Chang PY, Wu JT. Urinary 8-OHdG: A marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. Clin Chim Acta. 2004;339(1–2):1–9.

42. Ebina S, Chiba T, Ozaki T, Kashiwakura I. Relationship between 8-hydroxydeoxyguanosine levels in placental/umbilical cord blood and maternal/neonatal obstetric factors. Exp Ther Med. 2012;4(3):387–90.

43. Poniedzialek-Czajkowska E, Marciniak B, Kimber-Trojnar Z, Leszczynska-Gorzelak B, Oleszczuk J. Nitric Oxide in Normal and Preeclamptic Pregnancy. Curr Pharm Biotechnol. 2012;12(5):743–9.

44. Sánchez-Aranguren LC, Prada CE, Riaño-Medina CE, Lopez M. Endothelial dysfunction and preeclampsia: Role of oxidative stress. Front Physiol. 2014;5(OCT).

45. Adu-Bonsaffoh K, Ansong Antwi D, Obed SA, Gyan B. Nitric oxide dysregulation in the pathogenesis of preeclampsia among ghanaian women. Integr Blood Press Control. 2015;8:1–6.

46. Bartha JL, Comino-Delgado R, Bedoya FJ, Barahona M, Lubian D, Garcia-Benasach F. Maternal serum nitric oxide levels associated with biochemical and clinical parameters in hypertension in pregnancy. Eur J Obstet Gynecol Reprod Biol. 1999;82(2):201–7.

47. Shobith S, Tirthal R, Srinidhi U, Harshini D, Shilpa S. A Study on the levels of Nitric oxide and Lipid Peroxides in Preeclampsia. Int J Recent Sci Res. 2016;7(5):11012–5.

48. Shaamash AH, Elsnosy ED, Makhlouf AM, Zakhari MM, Ibrahim OA, El-Dien HM. Maternal and fetal serum nitric oxide (NO) concentrations in normal pregnancy, preeclampsia and eclampsia. Int J Gynecol Obstet. 2000;68(3):207–14.

49. Mao D, Che J, Li K, Han S, Yue Q, Zhu L, et al. Association of homocysteine, asymmetric dimethylarginine, and nitric oxide with preeclampsia. Arch Gynecol Obstet. 2010;282(4):371–5.

50. Darkwa EO, Djagbletey R, Essuman R, Sottie D, Dankwah GB, Aryee G. Nitric oxide and preeclampsia: A comparative study in Ghana. Open Access Maced J Med Sci. 2018;6(6):1023–7.

51. Diejomaoh FME, Omu AE, Al-Busiri N, Taher S, Al-Othman S, Fatinikun T, et al. Nitric oxide production is not altered in preeclampsia. Arch Gynecol Obstet. 2004;269(4):237–43.

52. Di Iorio R, Marinoni E, Emiliani S, Villaccio B, Cosmi E V. Nitric oxide in preeclampsia: Lack of evidence for decreased production. Eur J Obstet Gynecol Reprod Biol. 1998;76(1):65–70.

5. CONCLUSÃO

O estresse oxidativo materno relacionado como causa de parto prematuro está sendo muito pesquisado recentemente. Existem muitos estudos na literatura, no entanto os resultados ainda são muito controversos. As principais conclusões deste estudo são:

1. As gestantes com patologias placentárias (mães com pré-eclâmpsia) apresentam estresse oxidativo com níveis aumentados de TBARS e óxido nítrico.
2. O marcador 8-OHdG é considerado um dos marcadores mais sensíveis de estresse oxidativo, no entanto, em gestantes isso não foi comprovado.
3. Não é possível estabelecer se o estresse oxidativo materno é fator causal do parto prematuro.

Futuros estudos devem investigar o valor diagnóstico dos marcadores de estresse oxidativo em gestantes e recém-nascidos e assim, diminuir a incidência de parto prematuro, e consequente prognóstico dos recém-nascidos.

6. CONSIDERAÇÕES FINAIS

Para o desenvolvimento deste estudo objetivamos pesquisar causas de partos prematuros, problema que abrange o mundo, com grande impacto na saúde e economia da população. Sabemos que são muitas as causas maternas relacionadas ao parto prematuro e a intervenção precoce destas gestantes poderia evitar parto prematuro. Tem se estudado muito os efeitos do dano oxidativo nas gestantes com resultados ainda controversos. Essa situação ressalta a importância de recorrer a abordagens baseadas em evidências a fim de esclarecer as principais causas de parto prematuro com diagnóstico e tratamento adequado das gestantes.

7. REFERENCIAS

1. MC M. The contribution of low birth weight to infant mortality and childhood morbidity. N Engl J Med. 1985;10(312):82–90.
2. Walsh EM, Li SX, Black LK, Kuzniewicz M. Incremental Cost of Prematurity by Week of Gestational Age. AJP Rep. 2019;9(1):e76–83.
3. Chiarello DI, Abad C, Rojas D, Toledo F, Vázquez CM, Mate A, et al. Oxidative stress: Normal pregnancy versus preeclampsia. Biochim Biophys Acta - Mol Basis Dis. 2019;(October):1–11.
4. Morris JM, Gopaul NK, Endresen MJR, Knight M, Linton EA, Dhir S, et al. Circulating markers of oxidative stress are raised in normal pregnancy and preeclampsia. BJOG An Int J Obstet Gynaecol. 1998;105(11):1195–9.
5. Little RE, Gladen BC. Levels of lipid peroxides in uncomplicated pregnancy: A review of the literature. Reprod Toxicol. 1999;13(5):347–52.
6. Marseglia L, D'Angelo G, Manti S, Arrigo T, Barberi I, Reiter RJ, et al. Oxidative Stress-Mediated Aging during the Fetal and Perinatal Periods. Oxid Med Cell Longev. 2014;2014:1–8.
7. Ozsurekci Y, Aykac K. Oxidative Stress Related Diseases in Newborns. Oxid Med Cell Longev. 2016;2016:1–9.
8. Moore TA, Ahmad IM, Zimmerman MC. Oxidative Stress and Preterm Birth: An Integrative Review. Biol Res Nurs. 2018;20(5):497–512.
9. Bhat P V., Vinod V, Priyanka AN, Kamath A. Maternal serum lipid levels, oxidative stress and antioxidant activity in preeclampsia patients from Southwest India. Pregnancy Hypertens. 2019;15(July 2018):130–3.
10. Chełchowska M, Ambroszkiewicz J, Gajewska J, Mazur J, Lewandowski L, Reśko-Zachara M, et al. Influence of Active Exposure to Tobacco Smoke on Nitric Oxide Status of Pregnant Women. Int J Environ Res Public Health. 2018;15(12):2719.
11. Cambonie G, Comte B, Yzydorczyk C, Ntimbane T, Germain N, Lê NLO, et al. Antenatal antioxidant prevents adult hypertension, vascular dysfunction, and microvascular rarefaction associated with in utero exposure to a low-protein diet. Am J Physiol Integr Comp Physiol. 2006;292(3):R1236–45.
12. WHO. WORLD HEALTH ORGANIZATION, 2018. Disponível em:<https://www.who.int/news-room/fact->.Acesso em 26 de junho 2019. 2018;
13. Escobar GJ, McCormick MC, Zupancic JAF, Coleman-Phox K, Armstrong MA,

Greene JD, et al. Unstudied infants: Outcomes of moderately premature infants in the neonatal intensive care unit. Arch Dis Child Fetal Neonatal Ed. 2006;91(4):238–44.

14. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: A systematic analysis and implications. Lancet. 2012;379(9832):2162–72.
15. Kinney M V., Lawn JE, Howson CP, Belizan J. 15 million preterm births annually: What has changed this year? Reprod Health. 2012;9(1):2–5.
16. Do Carmo Leal M, Esteves-Pereira AP, Nakamura-Pereira M, Torres JA, Theme-Filha M, Domingues RMSM, et al. Prevalence and risk factors related to preterm birth in Brazil. Reprod Health. 2016;13(Suppl 3).
17. Liu L, Oza S, Hogan D, Chu Y, Perin J, Zhu J, et al. Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet. 2016;388(10063):3027–35.
18. Ramos, HAC; Cuman R. Prematuridade e fatores de risco Prematuridade. Anna Nery Rev Enferm. 2009;13(132):297–304.
19. Silveira MF, Victora CG, Barros AJD, Santos IS, Matijasevich A, Barros FC. Determinants of preterm birth: Pelotas, Rio Grande do Sul State, Brazil, 2004 birth cohort. Cad Saude Publica. 2010;26(1):185–94.
20. Silveira MF, Santos IS, Barros AJD, Matijasevich A, Barros FC, Victora CG. Increase in preterm births in Brazil: review of population-based studies. Rev Saude Publica. 2008;42(5):957–64.
21. Keelan JA, Newnham JP. Recent advances in the prevention of preterm birth. F1000Research. 2017;6(0):1–11.
22. Katz J, Lee ACC, Kozuki N, Lawn JE, Cousens S, Blencowe H, et al. Mortality risk in preterm and small-for-gestational-age infants in low-income and middle-income countries : Lancet. 2013;382(August 3):417–25.
23. Stefanovic V, Andersson S, Vento M. Oxidative stress - Related spontaneous preterm delivery challenges in causality determination, prevention and novel strategies in reduction of the sequelae. Free Radic Biol Med. 2019;142(June):52–60.
24. Sies H. Oxidative stress: A concept in redox biology and medicine. Redox Biol. 2015;4:180–3.
25. Halliwell B, Whiteman M. Measuring reactive species and oxidative damage in vivo

and in cell culture: How should you do it and what do the results mean? Br J Pharmacol. 2004;142(2):231–55.

26. Vento M, Teramo K. Evaluating the fetus at risk for cardiopulmonary compromise. Semin Fetal Neonatal Med. 2013;18(6):324–9.
27. Perrone S, Laschi E, Buonocore G. Biomarkers of oxidative stress in the fetus and in the newborn. Free Radic Biol Med. 2019;142(January):23–31.
28. Perrone S, Santacroce A, Longini M, Proietti F, Bazzini F, Buonocore G. The Free Radical Diseases of Prematurity: From Cellular Mechanisms to Bedside. Oxid Med Cell Longev. 2018;2018:1–14.
29. Saugstad OD. Oxygen radical disease in neonatology. Semin Neonatol. 1998;3(3):229–38.
30. Sullivan JL. Iron,Plasma Antioxidants,and the "Oxygen Radical Disease of Prematurity." Am J Dis Child. 1988;142(12):1341–4.
31. Perez M, Robbins ME, Revhaug C, Saugstad OD. Oxygen radical disease in the newborn, revisited: Oxidative stress and disease in the newborn period. Free Radic Biol Med. 2019;142(December 2018):61–72.
32. Schoots MH, Gordijn SJ, Scherjon SA, van Goor H, Hillebrands JL. Oxidative stress in placental pathology. Placenta. 2018;69:153–61.
33. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. The Lancet. 2008;371(9606):75–84.
34. Bharadwaj S, Bhat VB, Vickneswaran V, Adhisivam B, Zachariah B, Habeebullah S. Oxidative stress in preeclamptic mother–newborn dyads and its correlation with early neonatal outcome–a case control study. J Matern Neonatal Med. 2018;31(12):1548–53.
35. Farrant BM, White SW, Shepherd CCJ. Trends and predictors of extreme preterm birth: Western Australian population-based cohort study. PLoS One. 2019;14(3):1–16.
36. 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.
37. Ofman G, Tipple TE. Antioxidants & bronchopulmonary dysplasia: Beating the system or beating a dead horse? Free Radic Biol Med. 2019;142(February):138–45.
38. Peña-Bautista C, Durand T, Vigor C, Oger C, Galano JM, Cháfer-Pericás C. Non-invasive assessment of oxidative stress in preterm infants. Free Radic Biol Med. 2019;(February):0–1.
39. Zhang H, Wang L, Chu Y. Reactive oxygen species: The signal regulator of B cell. Free Radic Biol Med. 2019;142(November 2018):16–22.

40. Torres-Cuevas I, Corral-Debrinski M, Gressens P. Brain oxidative damage in murine models of neonatal hypoxia/ischemia and reoxygenation. Free Radic Biol Med. 2019;142(May):3–15.
41. Albrecht M, Zitta K, Groenendaal F, van Bel F, Peeters-Scholte C. Neuroprotective strategies following perinatal hypoxia-ischemia: Taking aim at NOS. Free Radic Biol Med. 2019;142(November 2018):123–31.
42. Wedgwood S, Steinhorn RH, Lakshminrusimha S. Optimal oxygenation and role of free radicals in PPHN. Free Radic Biol Med. 2019;142(April):97–106.
43. Mohamed I, Elremaly W, Rouleau T, Lavoie JC. Ascorbylperoxide Contaminating Parenteral Nutrition Is Associated with Bronchopulmonary Dysplasia or Death in Extremely Preterm Infants. J Parenter Enter Nutr. 2017;41(6):1023–9.
44. Ates O, Alp HH, Caner I, Yildirim A, Tastekin A, Kocer I, et al. Oxidative DNA damage in retinopathy of prematurity. Eur J Ophthalmol. 2009;19(1):80–5.
45. 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;89:734–40.