UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE CIÊNCIAS MÉDICAS

Renato Teixeira Souza

UMA ABORDAGEM AMPLA E INTEGRADA PARA INVESTIGAR FATORES ASSOCIADOS AO PARTO PREMATURO, SEUS RESULTADOS PERINATAIS E SUA PREDIÇÃO ATRAVÉS DA METABOLÔMICA

A COMPREHENSIVE INTEGRATIVE APPROACH TO INVESTIGATE FACTORS ASSOCIATED WITH PRETERM BIRTH, RELATED PERINATAL OUTCOMES AND ITS PREDICTION USING METABOLOMIC MARKERS

CAMPINAS

2019

Renato Teixeira Souza

UMA ABORDAGEM AMPLA E INTEGRADA PARA INVESTIGAR FATORES ASSOCIADOS AO PARTO PREMATURO, SEUS RESULTADOS PERINATAIS E SUA PREDIÇÃO ATRAVÉS DA METABOLÔMICA

A COMPREHENSIVE INTEGRATIVE APPROACH TO INVESTIGATE FACTORS ASSOCIATED WITH PRETERM BIRTH, RELATED PERINATAL OUTCOMES AND ITS PREDICTION USING METABOLOMIC MARKERS

Tese apresentada ao Programa de Pós-Graduação em Tocoginecologia da Faculdade de Ciências Médicas da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de doutor em Ciências da Saúde, área de concentração em Saúde Materna e Perinatal.

Thesis presented to the Post-Graduate Program of Obstetrics and Gynaecology from the School of Medical Sciences of the University of Campinas as part of the requirements needed for obtaining the PhD degree on Health Sciences, concentration area of Maternal and Perinatal Health.

ORIENTADOR: JOSÉ GUILHERME CECATTI

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DA TESE DEFENDIDA PELO ALUNO RENATO TEIXEIRA SOUZA, E ORIENTADO PELO PROF. DR. JOSÉ GUILHERME CECATTI.

CAMPINAS

2019

Essa tese foi desenvolvida com projetos de pesquisa e com o auxílio de bolsa de pós-graduação financiados pelas seguintes agências de fomento: CNPq e FAPESP (chamada AP.PPSUS, processo 2009/53245-5); MCTI/CNPq/DECIT e Fundação Bill e Melinda Gates (processo 401636/2013-5). Bolsa de doutorado pela CAPES (demanda social) e PDSE, processo CAPES/PROEX.

Agência(s) de fomento e nº(s) de processo(s): CAPES
ORCID: https://orcid.org/0000-0002-9075-9269

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Ciências Médicas
Ana Paula de Morais e Oliveira - CRB 8/8985

So89c

Souza, Renato Teixeira, 1985-
Uma abordagem ampla e integrada para investigar fatores associados ao parto prematuro, seus resultados perinatais e sua predição através da metabolômica / Renato Teixeira Souza. – Campinas, SP : [s.n.], 2019.

Orientador: José Guilherme Cecatti.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Ciências Médicas.

1. Trabalho de parto prematuro. 2. Ruptura prematura de membranas fetais. 3. Fatores de risco. 4. Programas de rastreamento. 5. Metabolômica. I. Cecatti, José Guilherme, 1957-. II. Universidade Estadual de Campinas. Faculdade de Ciências Médicas. III. Título.

Informações para Biblioteca Digital

Título em outro idioma: A comprehensive integrative approach to investigate factors associated with preterm birth, related perinatal outcomes and its prediction using metabolomic markers
Palavras-chave em inglês:
Premature obstetric labor
Premature rupture of fetal membranes
Risk factors
Screening programs
Metabolomics
Área de concentração: Saúde Materna e Perinatal
Titulação: Doutor em Ciências da Saúde
Banca examinadora:
José Guilherme Cecatti [Orientador]
Mary Angela Parpinelli
João Paulo Dias de Souza
Matias Costa Vieira
Ruth Guinsburg
Data de defesa: 22-02-2019
Programa de Pós-Graduação: Tocoginecologia

BANCA EXAMINADORA DA DEFESA DE DOUTORADO

RENATO TEIXEIRA SOUZA

ORIENTADOR: JOSÉ GUILHERME CECATTI

MEMBROS:

1. PROF. DR. JOSÉ GUILHERME CECATTI

2. PROFA. DRA. MARY ANGELA PARPINELLI

3. PROF. DR. JOÃO PAULO DE SOUZA

4. PROF. DR. MATIAS COSTA VIEIRA

5. PROFA. DRA. RUTH GUINSBURG

Programa de Pós-Graduação em Tocoginecologia da Faculdade de Ciências Médicas da Universidade Estadual de Campinas

A ata de defesa com as respectivas assinaturas dos membros da banca examinadora consta no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretariado Programa do Programa da Unidade.

Data: 22/02/2019

Dedicatória

À minha família...

AGRADECIMENTOS

Aos meus pais, que, com muito amor, me ensinam sobre a vida.

Às minhas irmãs, mulheres por quem tenho profunda admiração.

Aos meus familiares, a quem agradeço pelo carinho de sempre.

Aos amigos, cujos ombros estão sempre por perto.

À Vitória, a quem há pouco encontrei e me apaixonei. Obrigado por poder celebrar a vida ao seu lado.

Ao Dr. Guilherme, um exemplar líder, pesquisador e orientador, com quem aprendo além das fronteiras da ciência.

Às pacientes e seus familiares, por confiarem e contribuírem com esses estudos.

Aos colaboradores dos Estudos EMIP e Preterm SAMBA, sem os quais nada disso seria possível.

Ao CNPq, Decit/MS, FAPESP e Fundação Bill e Melinda Gates pelo apoio financeiro aos projetos envolvidos nessa tese.

RESUMO

Introdução: O parto prematuro é uma das principais causas de morbidade e mortalidade perinatal e neonatal. Identificar quais são as mulheres de maior risco e desenvolver modelos de predição é ainda um grande desafio, potencialmente impactando na eficácia de medidas preventivas. **Objetivo:** Desenvolver uma abordagem ampla aos fatores clínicos e epidemiológicos associados ao parto prematuro, seus preditores metabolômicos e respectivos desfechos perinatais. **Métodos:** Duas análises secundárias de um estudo multicêntrico de corte transversal avaliando a associação do índice de massa corpórea (IMC), o ganho de peso gestacional e fenótipos maternos com a ocorrência de prematuridade e desfechos maternos e perinatais; uma revisão narrativa sobre ciência ômica aplicada na área de saúde materna e perinatal, com enfoque na metabolômica; protocolo de revisão sistemática sobre a performance da metabolômica em predizer parto prematuro espontâneo (PPE) em mulheres assintomáticas; dois artigos abordando o desenvolvimento do método e dos procedimentos técnicos para um estudo multicêntrico prospectivo para investigar parto prematuro; um estudo caso-controle aninhado a uma coorte multicêntrica internacional para identificar preditores clínicos e metabolômicos para PPE; dois artigos originais abordando a incidência, fatores de risco e os desfechos maternos e perinatais associados ao parto prematuro em uma coorte multicêntrica no Brasil com gestantes nulíparas de baixo risco. **Resultados:** Nas análises secundárias do EMIP, observou-se que independente do IMC inicial, quanto maior o ganho de peso materno, maior a probabilidade para todos os subtipos de prematuridade, exceto para PPE em mulheres com IMC normal ou sobrepeso. Foram identificados três clusters de mulheres com parto prematuro, sendo um caracterizado principalmente por mulheres sem nenhuma das condições de risco, o segundo por mulheres com várias condições e o terceiro por mulheres que tiveram pré-eclâmpsia, eclâmpsia, síndrome HELLP e/ou restrição de crescimento fetal. A metabolômica, uma das ciências ômicas, é uma abordagem factível e promissora para investigar a fisiopatologia e identificar biomarcadores de doenças complexas como o PPE. A técnica de metabolômica usando cromatografia gasosa acoplada a espectrômetro de massa identificou mais de 140 metabólitos nas amostras de soro de gestantes nulíparas; três destes foram significativamente associados com

parto prematuro espontâneo nas amostras de Cork, Irlanda. O modelo preditor usando marcadores clínicos e metabolômicos mostrou uma área sob a curva ROC de 0,73 para PPE. Na coorte multicêntrica com mulheres Brasileira e nulíparas, a incidência de parto prematuro espontâneo foi de 6,7% e os fatores significativamente associados à sua ocorrência foram uso de álcool durante a primeira metade da gestação e medida do colo uterino. Os desfechos perinatais adversos foram muito mais frequentes nos casos de parto prematuro, especialmente parto prematuro terapêutico, quando comparados com os partos a termo. **Conclusão:** O ganho de peso gestacional é um fator modificável associado com a probabilidade de parto prematuro. Um número considerável de mulheres não possui nenhuma condição potencialmente associada ao parto prematuro. A ciência Ômica parece ser uma abordagem adequada para a identificação da etiologia e de marcadores para predição de complicações maternas e perinatais, embora ainda necessitem de sucessivas validações e evidência de reprodutibilidade. O desenvolvimento, implementação e coordenação de um estudo multicêntrico para estudar preditores e fatores associados ao parto prematuro requer recursos humanos qualificados, infraestrutura para pesquisa adequada, comprometimento institucional e envolvimento de agências de fomento e desenvolvimento de pesquisa. O modelo preditor para parto prematuro espontâneo em mulheres nulíparas mostra resultados de boa performance, embora requeira futuras validações. A medida do colo demonstra-se um marcador importante e que deve ser melhor explorado, assim como intervenções preventivas para reduzir os desfechos perinatais adversos relacionados a PPE. A resolução baseada em evidência é essencial nos casos de prematuridade terapêutica.

Palavras-chave: trabalho de parto prematuro, ruptura prematura de membranas fetais, fatores de risco, programas de rastreamento, cuidado pré-natal, perinatologia, metabolômica.

ABSTRACT

Introduction: Preterm birth is the leading cause of perinatal and neonatal morbidity and mortality. Identifying women at higher risk and developing prediction models remains a great challenge, potentially affecting preventive interventions. **Objectives:** To develop a comprehensive approach investigate risk factors associated with preterm birth, its metabolomics predictors and respective perinatal outcomes. **Methods:** Two secondary analysis of a multicentre cross-sectional with a nested case-control study addressing the association of maternal body mass index (BMI), gestational weight gain per week and phenotypes with the occurrence of preterm birth and maternal and perinatal outcomes; an integrative review about omics sciences applied to maternal and perinatal health, focusing on metabolomics; a systematic review and respective protocol investigating the performance of metabolomics to predict spontaneous preterm birth (sPTB) in asymptomatic women; two articles describing the methods, clinical protocol, technical procedures for the development and implementation of a multicentre prospective cohort study to investigate preterm birth and other maternal and perinatal complications; a nested case-control from a multicentre international cohort to identify clinical and metabolomics predictors for sPTB; two articles addressing incidence, clinical and epidemiological risk factors and maternal and perinatal outcomes associated with sPTB in a Brazilian multicentre cohort of low-risk nulliparous pregnant women. **Results:** According to the EMIP secondary analyses, the greater the rate of weight gain, the higher the predicted probability for all preterm birth subtypes regardless the initial BMI, except in normal BMI or overweight women and sPTB. Three clusters of women with preterm birth were identified; cluster one of women without any pre-defined conditions, cluster two with mixed conditions and cluster three with women who had preeclampsia, eclampsia, HELLP syndrome and/or fetal growth restriction. Maternal and perinatal outcomes did not differ between clusters. Metabolomics is an achievable and promising technique to investigate the pathophysiology and to identify biomarkers of complex disease such as sPTB. Metabolomics using gas chromatography-mass spectrometry identified more than 140 metabolites in serum samples of nulliparous pregnant women and three of them were significantly associated with sPTB in samples from Cork, Ireland. A predictive model associating metabolites and clinical

markers showed an area under ROC curve of 0.73 for sPTB. The incidence of sPTB was 6.7% in the Brazilian multicentre cohort study of nulliparous women and use of alcohol and cervical length were the factors significantly associated with its occurrence. Perinatal adverse outcomes were much more frequent in preterm birth cases, especially pi-PTB, than term birth cases. **Conclusion**: Gestational weight gain is a modifiable factor associated with the predicted probability for preterm birth. A substantial number of women does not present conditions potentially associated with preterm birth. Omics science studies might be a reasonable approach to investigate the aetiology and predictive markers for maternal and perinatal complications. Metabolomic studies addressing the prediction for sPTB, preeclampsia, gestational diabetes mellitus and fetal growth restriction show promising findings, although they still require repeated validations and reproducibility. The development of a multicenter study to investigate sPTB requires qualified human resources, adequate infrastructure, institutional commitment and the involvement of funding and research agencies. The predictive model for sPTB in nulliparous women showed a good performance, although further validation is required. The cervical length remains a remarkable clinical marker to be better explored in our population, as related preventative interventions to reduce the increased perinatal adverse outcomes associated with sPTB. Evidence-based resolution of pregnancy is essential in pi-PTB cases.

Key words: premature obstetric labor, premature rupture fetal membranes, risk factors, mass screening, prenatal care, perinatology, metabolomics.

SÍMBOLOS, SIGLAS E ABREVIATURAS

® – Marca Registrada

°C – Grau Celsius

µL – *microlitre*

2-D – *Two dimensional*

3-D – *Three dimensional*

AA – *Aminoacids*

ACS – *Antenatal corticosteroids*

ADA – *American Diabetes Association*

ADAM12 – *Desintegrin and metalloprotease 12*

AF – Amniotic fluid

AGA – *Adequate for gestational age*

ALPS – *Antenatal Late Preterm Steroids*

AMDIS – *Automated Mass Spectral Deconvolution and Identification System*

ANC – *Antenatal care*

APO – *Adverse perinatal outcome*

AUC – *Area under the curve*

BM – *Bowel movement*

BMGF – *Bill and Melinda Gates Foundation*

BMI – *Body mass index*

BNSRPH – *Brazilian Network for Studies on Reproductive and Perinatal Health*

CAGE – *Cap analysis of gene expression*

CAS – *Chemical Abstracts Service*

CDC – *Center for Diseases Control*

CE – *capillary electrophoresis*

CEP – Comitê de Ética em Pesquisa

CI – *Confidence interval*

CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico

CNS – Conselho Nacional de Saúde

CONEP – Comitê Nacional de Ética em Pesquisa

CPAP – *Continuous positive airway pressure*

CRF – *Case-report form*

C-section – *Caesarean section*

CV – *cervicovaginal*

DNA – *Deoxyribonucleic acid*

EMIP – Estudo multicêntrico de investigação em prematuridade no Brasil

FAME – *Fatty acid methyl esterification*

FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo

FASD – *Fetal alcohol spectrum disorder*

FDA – *United States Food and Drug Admnistration*

FDR – *False Discovery rate*

fFN – *Fetal fibronectin*

FGR – *Fetal growth restriction*

FPM – *Fasting plasma glucose*

g – grams/gramas

GA – *Gestational age*

GC – *gas chromatography*

GC-MS – *Gas chromatography-mass spectrometry*

GDM – Gestational diabetes mellitus

GROW – *Gestation Related Optimal Weight*

HDI – *Human development index*

HELLP – *Haemolysis, elevated liver enzymes levels, and low platelet levels*

HIP – *Hyperglycaemia in pregnancy*

HIPAA – *Health Insurance and Accountability Act*

H-NMR – *proton nuclear magnetic resonance spectroscopy*

HPa – *Hectopascal*

HSROC – *Hierarchical summary receiver operator characteristic curve*

IAI – Intra-amniotic inflammation/infection

IATA – *International Air Transport Association*

IBM – *International Business Machines Corporation*

IGFPB-1 – *Insulin-like growth factor-binding protein 1*

IL-6 – *Interleukin-6*

IMC – Índice de massa corpórea

IMPROvED – *Improved Pregnancy Outcomes by Early Detection*

IOM – *Institute of Medicine*

IRB – *Institutional Review Board*

IU – *International unit*

Kg/m^2 – *Kilogram per square metre*

lb – *libra/pound*

LBW – *Low birth weight*

LC-MS – *Liquid chromatography-mass spectrometry*

LGA – *Large for gestational age*

LMP – *Last menstrual period*

LMW – *Low molecular weight*

LNBio – *National Laboratory of Biosciences*

m/z – *mass to charge ratio*

MCF – *Methyl chloformate*

mg – milligrams

min – *minutes*

mL — *millilitre*

mm — millimetres

MPSS — *Massively parallel signature sequencing*

mRNA — *Messenger ribonucleic acid*

MS — *mass spectrometry*

ng/mL — nanogram per millilitre

NICU — *Neonatal intensive care unit*

NIST — *National Institute of Standards and Technology*

NMR — *Nuclear magnetic resonance*

OR — *Odds ratio*

oz — *ounce*

PAMG-1 — *Placental alpha microglobulin-1*

PAPP-A — *pregnancy-associated plasma protein*

PE — *Preeclampsia*

PECEP — *Pesario cervical para evitar prematuridad*

phIGFBP-1 — *Insulin-like growth factor binding protein*

PI — *Principal Investigator*

pi-PTB — *Provider-initiated preterm birth*

PlGF — *placental growth factor*

PLS-DA — *Sparse partial least squares discriminant analysis*

PPE — Parto prematuro espontâneo

pPROM — *Preterm premature of membranes*

P-PROM — *Preterm premature of membranes*

PPSUS — Programa de pesquisa para o SUS

Preterm SAMBA — *Preterm Screening and Metabolomics in Brazil and Auckland*

PRISMA — *Preferred Reporting Items for Systematics Reviews and Meta-Analyses*

PROM-PTB — *Premature of membranes preterm birth*

PSU — *Primary sampling unit*

PTB — *Preterm birth*

PTL — Preterm labor

QC — *Quality Control*

QQQ-MS — *Triple quad mass spectrometry*

QUADAS-2 — Quality Assessment of Diagnostic Accuracy Studies

RNA — *Ribonucleic acid*

ROC — *Receiver operating characteristic*

rpm — *revolutions per minute*

RR — *Relative risk*

RWG — *Rate of weight gain*

SAGE — *Serial analysis of gene expression*

SAS — *Statistical Analysis System*

SCOPE — *Screening of Pregnancy Endpoints*
SGA — *Small for gestational age*
SINASC — Sistema de informações sobre nascidos vivos
SOFA — *Sequential Organ Failure Assessment*
SOP — *Standard operating procedure*
SPREC — *Standard Preanalytical Coding*
SPSS — *Statistical Package for the Social Sciences*
sPTB — *Spontaneous preterm birth*
STROBE — *Strengthening the Reporting of Observational studies in Epidemiology*
SUS — Sistema Único de Saúde
Unicamp — Universidade Estadual de Campinas
UPLC-MS — *Ultra-performance liquid chromatography tandem mass spectrometry*
US — *Ultrasound*
US$ — *United State Dolars*
USA — *United States of America*
USD — *United States Dolars*
v/v — *volume per volume*
WG — *Weight gain*
WGR — *Weight gain rate*
WHO — *World Health Organization*
wk — *week*
y — *years*
YLL — *Years of life lost*

SUMÁRIO

1. INTRODUÇÃO

A introdução dessa tese foi transformada em uma revisão narrativa introdutória sobre o tema e foi submetida a publicação na revista *The Scientific World Journal,* e cujo texto aparece a seguir.

26/11/2018 Gmail - 1701398: Acknowledging Receipt

Gmail **Renato Souza <doutorrenatosouza@gmail.com>**

1701398: Acknowledging Receipt

The Scientific World Journal <eslam.omran@hindawi.com> 25 November 2018 at 23:00
To: cecatti@unicamp.br
Cc: eslam.omran@hindawi.com, renatotsouzasp@gmail.com

Dear Dr. Cecatti,

The Review Article titled "A comprehensive integrative review of factors associated with preterm birth, related perinatal outcomes, prevention and its prediction including metabolomic markers," by Renato T Souza and Jose Guilherme Cecatti has been received and assigned the number 1701398.

All authors will receive a copy of all the correspondences regarding this manuscript.

Thank you for submitting your work to The Scientific World Journal.

Best regards,

--
****
Eslam Omran
Editorial Office
Hindawi
http://www.hindawi.com
****

REVIEW

A comprehensive integrative review of factors associated with preterm birth, related perinatal outcomes, prevention and its prediction including metabolomic markers

Renato T Souza [1]

Jose Guilherme Cecatti [1]

1. Department of Obstetrics and Gynecology, University of Campinas, School of Medicine, Brazil.

Correspondence:

José G Cecatti

Department of Obstetrics and Gynecology

University of Campinas

Brazil

E-mail: cecatti@unicamp.br

Abstract

Preterm birth is a major maternal complication implicating in a great burden for perinatal, neonatal, infant and adult health. Lack of knowledge regarding its aetiology and development is known, resulting in poor screening, prediction and preventive methods. This current integrative review discuss the current knowledge regarding some risk factors for preterm birth, the differences between screening and predicting methods, the limitations of some current preventive interventions, the importance of applying standardized concepts for exposures and outcomes and why it is important to develop more accurate and reproducible methods for predicting preterm birth. In addition, it introduces the concept of metabolomics technology and how it has become a promising approach for identifying biomarkers for spontaneous preterm birth.

1. Definition and impact of prematurity

> *"[…] I went into premature labor at 24 weeks 6 days. I had been really uncomfortable the night before and thought I needed to go to the bathroom. Well the next day it was still bad so I called the doctor on call. She told me I probably just needed to have a BM but if it would make me feel better, I could come in. My husband and I went in just to get checked out. They never actually checked me until I started bleeding (the doctor was in a c-section when I got to the hospital). They gave me the steroid shots and magnesium to stop the contractions. It didn't work and I was 10cm just a couple hours later. She was still breach so they did an emergency c-section. Our beautiful daughter, Charlie, was born on April 14, 2018 weighing 1lb 9.4oz and 12.21" long. She was intubated due to her lungs being underdeveloped, but the team said everything else looked good. She was doing great, but on her 4th day here she suddenly took a turn for the worse. Basically, her heart gave out and they were not able to resuscitate her. We had her for 4 days and then the Lord called her home. She is my first biological child and now I fear to try again. After seeing a specialist, we were told I have an incompetent cervix and that is why I went into labor and displayed so quickly. I also have a bicornuate uterus. The doctor said if we decide to try again, I would have a cerclage and the shots. She gave us a 50-70% chance of making it to 36 weeks. They would do a cesarean at 36 weeks due to the way they cut me inside during the emergency. […]"* [1]

The birth of a preterm baby may have diverse negative consequences for the baby, (his or her) neonatal life, childhood or adult life, family, healthcare system/service, and the entire society. The experience of a mother describing how she accompanied her daughter progress with complications and death due to prematurity, led us to address topics related to preterm birth. The focus of such approach will be factors associated with preterm birth, perinatal outcomes and ways to predict this outcome. This is supported by the hypothesis that it is possible to better understand and predict the preterm birth process, creating opportunities for increased effectiveness in preventing the condition.

It took several decades to consolidate the definition of preterm birth. At the beginning of the 20th century, newborn infants weighing less than 2.500g at birth were considered preterm, based primarily on neonatal behavior and progression to neonatal mortality, intracranial hemorrhage and other morbid conditions [2]. In 1950, a group of experts of the World Health Organization (WHO) published a technical report defining preterm newborns as those weighing less than 2.500g at birth or those born at less than 37 weeks of gestation [3]. In this document, the WHO had already established two

priorities for the promotion of research and specific programs aimed at minimizing the consequences of preterm birth: prevention and preterm infant care.

Preterm birth may be classified according to clinical presentation or motivator: spontaneous, when due to spontaneous preterm labor (contractions, cervical effacement and dilatation) or preterm rupture of membranes; and therapeutic, when theoretically there is a maternal and/or fetal condition that poses risk to the mother or fetus, generating sufficient motivation for resolution at a preterm gestational age [4]. Furthermore, iatrogenic preterm birth is defined as birth due to therapeutic intervention without the existence of sufficient risks to justify any intervention, i.e., due to convenience, maternal desire or simply without scientific evidence for premature resolution [5]. For each of the three subtypes of preterm births (spontaneous preterm labor, preterm premature rupture of membranes - P-PROM - or therapeutic) there are different risk factors and associated maternal and perinatal outcomes [6–8].

Therefore, at least the distinction between spontaneous and therapeutic preterm birth is highly recommended when studying the determinants and consequences of preterm birth. The recognition that not every preterm birth occurs because of the same determinants was an early step in studying causes and developing preventive strategies. Preterm birth is also categorized according to gestational age at birth, and is divided into: late preterm (between 34 weeks + 0 days and 36 weeks + 6 days), moderately preterm (32+0 weeks – 33+6 weeks), very preterm (28+0 weeks – 31+6 weeks) and extremely preterm (<28weeks) [5,9].

Pregnancy of a singleton or multiple fetuses (twins) confers great differences not only in terms of the incidence of preterm birth, but also concerning its associated factors and maternal and perinatal outcomes [10]. A study evaluating offical data of the Information System of Live Births (SINASC) from 2011 to 2014 in Brazil, shows that about 53% of twin pregnancies progress to preterm deliveries [11]. Furthermore, there is an increase in complications, such as maternal near-miss events, maternal mortality, perinatal mortality, preeclampsia, postpartum hemorrhage [10–12]. The increased incidence of complications due to multiple pregnancies associated with a higher rate of twin pregnancies in the last decades denote the importance of this type of pregnancy in preterm birth and maternal and perinatal health [10,13]. Twin pregnancy is not the focus

of this review, since an adequately designed and appropriate approach would be required for this type of pregnancy to evaluate its associated factors, preventive and predictive methods for preterm birth and respective perinatal outcomes [10].

A study by the World Health Organization (WHO) estimated that around 15 million preterm births occur annually worldwide [4,14], representing a rate of 10.3% of all deliveries. International data from 1990 to 2010 in 65 countries of Europe, Australasia, and the Americas showed that the absolute number of preterm births and preterm infant rates increased during this period [4]. Countries from North Africa, Sub-Saharan Africa and Asia represent little more than 70% of deliveries and 80% of preterm births across the world. Furthermore, only five countries -India, China, Nigeria, Bangladesh, and Indonesia – account for almost half of preterm births worldwide [14]. Around 17% of preterm births occur in the Americas (North America, Latin America and Caribbean), Europe and Oceania. However, these regions have the highest proportion of extreme preterm births [14]. Preterm birth represents a huge public health issue in all contexts and countries, either in low-income or high-income countries [15,16].

Complications due to preterm births account for more than one-third of neonatal deaths worldwide, representing over 1 million newborn infants who died in the first month of life in 2010. The impact of complications due to preterm birth still has repercussions for childhood health indicators. It is the second cause of death until age 5 years globally, and the first cause of death in mid-income and high-income countries [4].

Since the 1950s many advances have been made in the number of options and level of scientific evidence-based preventive measures for neonatal complications due to preterm birth. Examples include measures of tertiary prevention such as the use of tocolytics and corticotherapy for the prevention of hyaline membrane, intraventricular hemorrhage and necrotizing enterocolitis; magnesium sulfate for the prevention of cerebral palsy in cases of imminent preterm delivery; and antibiotic therapy for the prevention of neonatal sepsis and for prolonging the latent phase in cases of P-PROM [17–21]. Although these measures have a short- and long-term impact on perinatal morbidity and mortality, they are usually only adopted when preterm birth has already begun and its occurrence is imminent. Earlier identification of these cases while still in the

asymptomatic phase, could theoretically increase the window of opportunity for preventive interventions and bring better perinatal outcomes [22,23].

There were also advances in the identification and institution of early therapies for neonatal complications such as neonatal sepsis, hypothermia, visual, cerebral (intra and periventricular hemorrhage), auditory and/or neuropsychomotor impairment, providing the newborn infant with the possibility of earlier neonatal follow-up and better long-term results [24,25]. The advent of CPAP, for example, which is the English abbreviation for continuous positive airway pressure, mechanical ventilation, use of exogenous surfactant in the 70s and refinement of oxygen saturation targets in neonatal oxygen therapy in the last decade, resulted in significant improvement in neonatal survival, especially for extremely preterm infants [24]. When estimating the potential “years of life lost” due to premature death - *YLL*, in Brazil, neonatal complications arising from preterm birth fell to second place in 1990, to sixth in 2005, then dropped to tenth place in 2015, reducing approximately 50 and 40% of years lost in the respective periods [26]. Advances in tertiary and quaternary prevention, which correspond to a decrease in complications or adverse events after the emergence of disease or its sequelae, do not seem to be equally accompanied by primary or secondary intervention. Difficulty lies, in part, in the lack of knowledge of the pathophysiology of preterm birth and its risk factors, limiting the development of preventive measures and effective prediction models.

2. Risk factors for preterm birth and prediction

Risk factor is a term used to designate conditions, characteristics, habits or markers that, when present, increase the probability or likelihood of a determined injury. Risk, therefore, is related to the appearance of a condition [27,28]. Fixed risk factors are gender, ethnicity or age. Modifiable risk factors are weight, body mass index (BMI), smoking, alcoholism or use of a condom, for example [27]. They may have different strengths of association with the risk for a determined condition, depending on the combination of other factors, time of exposure or even the population studied [28,29].

An example of a combination of factors is BMI and gestational weight gain. The Institute of Medicine (IOM) categorized BMI into low weight (BMI <18.5 kg/m^2), normal (BMI 18.5-24.9 kg/m^2), overweight (BMI 25.00-29.9 kg/m^2) and obesity (BMI ≥30.0 kg/m^2) [30]. A study evaluating data from a prospective cohort with more than 45 thousand North-American pregnant women showed that BMI and gestational weight gain seem to have different impacts on risk for different subtypes of preterm births, depending on the category of initial BMI and respective weight gain [31]. Nevertheless, in this study gestational weight gain was calculated by subtracting the initial weight from the last weight before childbirth. This method does not consider that women with preterm delivery had less weeks of gestation to gain weight, mainly in the third trimester, period of highest rate of weight gain according to the IOM [30]. This results in a biased comparison of weight gain, for example, between a woman delivering at 28 weeks and another woman delivering at 41 weeks. In this case, the use of weight gain rate per week, would be highly recommended. A systematic review evaluating 39 studies including data of almost one million and 800 thousand women highlights the lack of homogeneity in categorizing initial BMI and defining outcome according to subtypes of prematurity and estimate of gestational age [32]. Studies on circumventing these limitations are still scarce, although necessary to better understand the role of BMI and gestational weight gain in the risk for different subtypes of prematurity.

Didactically, risk factors for preterm delivery may be classified as clinical, semiological, microbiological, ultrasonographic and biochemical [33]. Enviromental, social and genetical factos are also included [30]. According to some systematic reviews, the main clinical risk factors for preterm birth, i.e., those that have a higher independent association with preterm delivery, are history of previous preterm delivery, smoking and multiple pregnancy [4,8,34]. A history of previous preterm birth is the most important risk factor for preterm birth. A previous preterm delivery increases 3-fold to 4-fold the risk of having a new preterm delivery [8,35–37]. Availability of information is relatively accessible, since it is collected from basic obstetric clinical history, preferentially detailing how and at which gestational age the preterm birth occurred [35–37]. The earlier the preterm delivery, the higher the risk for a new case of preterm delivery; the number of recurrences was also associated with a 5-fold to 6-fold increase in the chance of having a

new preterm delivery [37]. However, a limitation of this risk marker is that it cannot be applied to nulliparous women.

Smoking is a modifiable risk factor. It is associated with an incidence of preterm birth that is 3-fold to 4-fold higher in smokers than in non-smokers [34,38]. Risk seems to be dose-dependent, i.e., the higher the number of cigarettes, the higher the risk. In addition, it is also associated with passive smokers, i.e., women living in areas of exposure to cigarette smoke [39,40].

It is important to emphasize that a condition that is associated with an outcome may not always be considered a risk factor for the condition. Exposure prior to the appearance of disease, its removal or reduction is a characteristic associated with a lower incidence of disease. Dose-dependence and measure of exposure need to be considered in risk relation. These characteristics are preponderant in the application of risk factors as predictors. Flecther and cols [28] described (p.54):

> *"[...] cumulative doses of exposure to the sun constitute a risk factor for non-melanoma skin cancer, while episodes of severe solar burns are the best predictors for melanoma. The choice of the appropriate measure of exposure to a risk factor is based on all that is known about the clinical and biological effects from exposure, on pathophysiology of the disease and any epidemiologival studies."*

The great challenge lies in the limited knowledge of the patophysiology and etiology of preterm birth. There are some propositions concerning the mechanisms involved in preterm birth. A hypothesis by Behrman et al [41], highlighted the role of uterine distension, decidual hemorrhage or thrombosis, inflammatory or infectious processes, activation of the hypothalamus-pituitary-adrenal axis and stress, which alone or in conjunction, may lead to pro-inflammatory activation of the decidua and membranes. Prostaglandins and metalloproteinases, in turn, along with other inflammatory agents, may promote cervical remodeling and/or uterine contractions leading ultimately to preterm labor and/or preterm premature rupture of membranes [41]. In contrast, Menon et al [42] categorized risk factors as static and dynamic, also proposing a complex and not fully clear interaction between diverse inflammatory, immunological, environmental, epigenetic mechanisms, among others, that culminate in senescence and "weakening" of amniotic membranes, decidual and myometrial

activation, cervical effacement and, finally, preterm birth. Multiple markers involved in these mechanisms are studied as potential predictors of preterm birth.

Systematic reviews have identified diverse studies evaluating these different biological and biophysical markers, highlighting fetal fibronectin l (fFN) and insulin-like growth factor binding protein (phIGFBP-1), binding proteins present between the chorion of the amniotic membrane and the maternal decidua, and cervical length measurement in the second trimester of pregnancy by transvaginal ultrasound. Systematic reviews have concluded that those markers are not sufficiently accurate to be useful in clinical prediction of preterm birth, especially in asymptomatic women [33,43–45].

A Dutch prospective cohort study, including nearly 12 thousand women, assessed the performance of cervical length measurement in the prediction of preterm birth [46]. Measurement of the cervix was performed between 16 and 22 weeks of gestation. It was shown to be poor and did not vary significantly between nulliparous and multiparous women and among women considered to be at low or high risk. The area under the ROC curve ranged from 0.56 to 0.61 for the multiparous group and low-risk nulliparous group, respectively, i.e., the method fails to identify around 40 to 50% of women who will have preterm delivery.

Fetal fibronectin in vaginal secretion does not show much superior results in asymptomatic women. A cohort study from the United Kingdom analyzed the performance of fFN collected from cervicovaginal secretion as a predictor of spontaneous preterm delivery at less than 34 weeks of gestation [47]. Almost 1,500 women were included and vaginal secretion was collected from 22 to 28 weeks. The study showed that levels above 50 ng/ml have a sensitivity of 46.5% and a specificity of 88.7%. The higher the cut-off point for fFN in vaginal secretion, the higher the negative predictive value (NPV) and specificity of fFN. When the cut-off point was 500 ng/ml, specificity and NPV were higher than 90%. However, its clinical application is still limited, since it is expected that a large part of the population does not have such high levels of fFN during this phase of gestation and the test has a very low sensitivity with this cut-off point, i.e., many women with preterm delivery do not achieve such high fFN levels in vaginal secretion during this period.

Other propositions have attempted to address the association between multiple factors involved in the development of preterm birth. A group of experts proposed a classification of women at risk for preterm delivery, according to phenotypes [5,48]. Empirically, those authors defined that the development of preterm birth is not exclusive to a single group of women who necessarily have similar characteristics and risk factors. On the contrary, probably different groups of women have conditions in common that are associated with preterm birth and their different subtypes. Conditions that potentially define phenotypes of preterm birth were divided into maternal, fetal and placental conditions. Conditions are not based on risk factors, but depend on conditions present in the index pregnancy that determine the occurrence of preterm birth. The application of this new classification could help understand the associations between determinants of preterm birth, measure the benefits of preventive measures and identify conditions that achieve the highest impact from these measures, and ultimately, understand the subgroups of women that are at higher risk for different subtypes of preterm birth.

The authors applied this concept through a secondary analysis of an international multicenter cohort study named INTERGROWTH 21st [49]. Slightly more than 50 thousand women had estimates of gestational age calculated by obstetric ultrasound and 5,828 women had preterm deliveries (10.5%). A cluster analysis of preterm births was conducted, grouped or not, according to one or more of 6 maternal conditions, 7 fetal conditions and 3 placental conditions. Finally, twelve clusters were identified, drawing attention to cluster 1 in which 1.747 women (30%) had none of the 16 predefined conditions. Over 80% of women from this cluster had preterm births either by preterm labor or P-PROM. On the other hand, the majority of women were divided into 11 clusters, charaterized by major conditions such as preeclampsia/eclampsia, chorioamnionitis, twin pregnancies or bleeding at the beginning of pregnancy, etc., showing that it is possible to identify determining factors in subgroups of women with preterm birth, helping to understand the etiology and identify women at higher risk. Nevertheless, this concept still requires reproducibility. Validation of cluster determination, along with their predefining conditions in other populations, is necessary.

Thus, we are faced with the need to better explore risk models for preterm birth, identify risk factors and their associations, helping to determine etiological theories and develop predictive models that are efficient at predicting spontaneous preterm birth.

3. Prevention of preterm birth

According to Geoffrey Rose [29], there are two prevention strategies: one based on individual preventive measures through the identification of individuals at higher risk of developing the condition; and the other based on measures of the general population, irrespective of the existence of risk factors. Available access to prenatal care, qualified childbirth and postpartum care, incentive programs for healthy lifestyle habits and protection of a woman's right to health care are important strategies that may have an impact on maternal and perinatal health indicators, including preterm birth [50]. A good example of exposure that has preventive measures based on both strategies is smoking. Around 50% of American pregnant women stop smoking in the first trimester of pregnancy [51]. Individual policies such as counseling, stimulation of pharmacologic replacement of nicotine, psychological support and even financial incentives have an impact on the prevention of adverse perinatal outcomes. Population policies such as dissociating the image of the cigarette as a healthy and socially desirable habit through campaigns in the media, increase in taxes for the tobacco industry and laws that restrict areas where smoking is allowed also demonstrated a beneficial effect [51]. A systematic review including clinical trials testing different strategies for cessation of smoking showed that interventions reduced preterm births by approximately 15% [51]. Although continuous effort and specific public policies are necessary, this is a good example of how identifying the risk associated with prevention strategies may result in more cost-effective and better maternal and perinatal outcomes [51–53].

The identification of factors associated with a higher risk of developing spontaneous preterm birth may be useful both for understanding its pathophysiology and identifying women at higher risk who might benefit from prevention strategies. In the latter case, it may also possible to distinguish between screening for risk and prediction of preterm birth. Although both methods use risk factors as a basis for their models or algorithms, the method employed and its practical application may be quite distinct.

For example, women with transvaginal ultrasound assessment of cervical length between 20 and 25mm, measured in the second trimester by standardized technique [54] had an incidence of preterm birth ranging from 22% to 32% [55]. This incidence may reach 56% in cervical length lower than 5mm [55]. The increased incidence in women with a cervix lower than 25mm, in comparison to the general population, confers a 4-fold to 5-fold higher risk for preterm birth. Observational studies in different populations confirm this inverse association between uterine cervix measurement in the second trimester and prevalence of spontaneous preterm birth [46,55,56]. Therefore, a value lower than 25 mm was considered a “short” uterine cervix and a value higher than 25mm was considered a “normal” uterine cervix [46,55]. Based on uterine cervix measurement to stratify women at higher risk, several clinical trials have tested preventive interventions for spontaneous preterm birth and its associated to adverse perinatal events, initially comparing natural micronized progesterone (vaginal tablet) or hydroxyprogesterone caproate (intramuscular injection) with placebo. Systematic reviews with meta-analysis showed that the use of vaginal progesterone seems to be beneficial for the reduction in preterm birth at less than 37, 34 and 28 weeks and neonatal morbid conditions [57,58]. However, differences in reduction rates of different morbid conditions or even preterm birth may be attributed to different selection criteria for women included in clinical trials.

The OPPTIMUM study, for example, a British multicenter study including 65 centers in the United Kingdom and one in Sweden, published in 2016 (after the systematic review), aimed to evaluate not only the benefit of progesterone in reducing prematurity and neonatal morbidity, but also its long-term effect on the child [59]. The study selected women with singleton pregnancies at high risk for preterm birth based on: history of previous preterm birth, gestational loss in the second trimester or preterm premature rupture of membranes or cervical procedure and positive vaginal fetal fibronectin. A year after the beginning of the clinical trial, researchers decided to include women at “mid-high” risk, defined by these women as having negative fetal fibronectin, but with a history of spontaneous preterm birth at less than 34 weeks or uterine cervix measuring less than 25mm in the second trimester. This double-blind controlled study randomized more than 600 women in each group (vaginal progesterone 200mg *vs* placebo) and demonstrated that progesterone was not beneficial for reducing preterm birth or the majority of

perinatal morbid conditions such as pulmonary bronchodysplasia, neonatal infection, necrotizing enterocolitis or neurological development and neurocognitive score at 2 years of age. However, it showed a reduction in neonatal death (non-adjusted Odds ratio of 0.17 [0.06 – 0.49], p-value of 0.0009) and for cerebral alterations on ultrasound (non-adjusted Odds ratio of 0.50 [0.31 – 0.84], p-value of 0.008). The authors of this study concluded that subgroups of women who might benefit from progesterone, are not easily identified by current screening strategies. This should encourage studies on new prevention strategies and also those aimed at identifying women that may be potentially eligible for receiving this treatment.

Another technique studied for decades is cerclage which is primarily based on suture of the uterine cervix or isthmus-cervical region to prevent early effacement/dilatation of the cervix. Shirodkar technique [60], described in 1953, and a technique by McDonald [61] in 1957 are the basis for all the subsequently described variations. This technique was initially proposed for cases with a history of cervical insufficiency, a known cause of late abortion and extreme prematurity. A systematic review of Cochrane systematic review with 15 clinical trials showed advantages in prolonging pregnancy, decreasing neonatal morbidity and prematurity rate when indicated in women with a history of cervical insufficiency [62]. The advent of cervical measurement in the second trimester, associated with a history of preterm birth, seems to have improved the identification of women benefiting from cerclage to prevent preterm births, particularly in cases in which there is still no history of recurrent pregnancy loss [63]. This shows that the search for an association of risk factors in the prevention of preterm birth may still be very useful, even in situations where a good solution was apparently found, as for cervical incompetence and cerclage.

It is also worth mentioning that another intervention was studied for preterm birth prevention in high-risk women. A pessary, a device made of firm silicone in the shape of a convex ring is inserted into the posterior vaginal fornix, fastened to the cervix. The theoretical mechanism for the prevention of preterm birth is based on: 1) a change in the axis of forces resulting from the uterine body and isthmus that act on the cervix, and 2) a potential closure of the cervix with consequent strengthening of the cervical canal and immunologic barrier of the cervix, preserving the amniotic membranes from contact with

the vaginal environment [64]. Although the subject has been studied since the middle of the twentieth century, the identification of women who actually benefit from this intervention remains a challenge. The PECEP (*Pesario Cervical para Evitar Prematuridad)* study published in 2012 was the first randomized study using the pessary (*versus* expectant management) to prevent preterm birth. Selecting pregnant women at high risk based on cervical length measurement in the second trimester, with slightly more than 190 women per group, the study showed that the incidence of preterm births below 34 weeks decreased by 80%. Subsequent studies demonstrated conflicting results and did not confirm such a reduction in the incidence of preterm births observed by Goya *et al* [64]. However, the selection of eligible women and the association with other interventions, such as progesterone, is heterogeneous among studies [65–68].

Despite the advances/benefits resulting from a combination of screening for risk and interventions, e.g. progesterone, pessary and cerclage in women selected on the basis of risk factors, there still seems to exist limitations and heterogeneity in screening. Better results from the use of these measures may be potentially hindered. Which women might actually benefit from the use of progesterone during prenatal care? Or, which women might not benefit from any preventive intervention? Furthermore, there is no consensus over which level of risk estimate a woman would be really considered at high risk. Improved identification of women at high (or low) risk for preterm birth with the development of prediction models that have good discriminatory performance may be quite relevant to advance the investigation of the benefits of using (or not) progesterone, pessary or any other form of preterm birth prevention.

4. Risk assessment and prediction of preterm birth

The description of these prevention studies along with their interpretations are important to highlight the fundamental role of adequate screening of women who may benefit from prevention strategies. Distinctions must be made regarding the risk assessment model and a predictor model for an outcome. This distinction may actually help understand the clinical application of a screening strategy for women at high risk for preterm delivery.

As an example of marker of risk, the cervix is known to be independently associated with a higher risk for preterm birth [55,56]. Although this may be useful for implementation of differentiated care, suggesting screening and interventions for the subgroup of women with a short cervix, this practice is fragile in terms of the population and has a low impact on prevention [69]. The reason for this is because despite a higher risk for preterm birth, a woman with a short cervix has the highest odds of having a term birth. Furthermore, the shortening process of the cervix may not occur early in the recommended screening phase (second trimester, between 18 and 24 weeks). In summary, the cervix is a marker of low sensitivity (a considerable proportion of women with a short cervix are likely to deliver at term). At the same time, the marker has a low rate in general population, since a cervix of 25mm corresponds to a 5^{th} and 3^{rd} percentile in the population curve of cervical measurement [55]. A cohort with almost 3 thousand pregnant women evaluating the performance of 28 markers in the second trimester of pregnancy shows that a short cervix has a sensitivity of 36.8% for preterm birth at less than 35 weeks. This means that almost two-thirds of women with preterm birth below this gestational age would not be screened by this criterion, resulting in elevated false-negative rates of the method. Therefore, despite the positive association with preterm birth, a short cervix seems to be an inappropriate marker to compose predictive models, resulting in low efficacy when employed in clinical practice [46,55,56]. Even serial measurements of the cervix, based on the theory that shortening of the cervix over the weeks could be a better predictor of preterm birth, showed a worse predicitive performance than a single measurement [70].

A prospective observational study included more than 9 thousand nulliparous pregnant women from 8 North-American centers evaluating the performance of fetal fibronectin and transvaginal measurement of the uterine cervix in predicting spontaneous preterm birth [71]. The area under the ROC curve was 0.59 for fetal fibronectin equal to or higher than 50 ng/dL and 0.67 for a cervix lower than 25mm. The model containing both variables had an area under the ROC curve of 0.67. The authors concluded that the performance was poor and of low clinical utility.

In summary, systematic reviews have concluded that there are no markers in the literature that can be applied in clinical practice to predict spontaneous preterm birth with

a good performance [72] and that enable new preventive approaches and studies in this area.

5. Metabolomics and Preterm Birth

The term "omics sciences" is applied to the field of knowledge that focuses on genomic studies, gene identification, DNA sequence polymorphisms, genes and the genoma; transcriptomic, focused on the study of gene expression - RNAs; proteomic, when proteins/enzymes are identified and quantified; or metabolomics, when metabolites, a product of this chain interaction [73–76]. The application of each technique to investigate markers or pathophysiology of diseases, primarily those involving complex mechanisms that are still not fully elucidated, is basically dependent on the objectives and resources available. Actually, an integrated application of the various methods may be the option [75]. The main advantage of metabolomics is that is seems to be closer to disease phenotype, presenting the result of the final pathway of interactions between genes, RNAm and proteins. According to Dettmer, Aronov & Hammock [77]:

> "*Genomi tells what can happen, transcriptomi what appears to be happening, proteomi what makes it happen, and metabolomi what has happened and what is happening*"

Metabolomics is the science that studies metabolites, small molecules present in different chains of the metabolism of an organism [78]. These small molecules may be substrates, products and cofactors of intracellular and extracellular chemical reactions such as aminoacids, biliary acids, carbohydrates, lipids, vitamins and others [79]. The group of metabolites in a certain sample or organism are termed metabolome. Diverse techniques are applied to identify and quantify metabolites such as mass spectrometry coupled to liquid or gas chromatography or magnetic resonance imaging. Furthermore, diverse configurations or variants may be used to obtain a better performance, depending on the metabolite of interest, its polarity, mass spectrum to be studied or other physical chemical characteristics of the metabolites and samples to be analyzed. Technological advances in instruments for data acquisition and bioinformatics have provided sufficient aid, so that metabolomics is able to identify and analyze hundreds or even millions of metabolites in a certain biological sample. Studies on diverse applications in biological

samples demonstrate a high sensitivity in the detection and measurement of metabolites [78].

By identifying and quantifying metabolites, this technique is capable of showing the fingerprint of metabolic interactions of the organism in a certain sample at a certain time. Metabolomics is a technique known as hypothesis-free, i.e., that does not require an initial hypothesis. Instead of testing a certain hypothesis, the technique may generate novel hypotheses through its results when elucidating markers and biological pathways involved in the process of disease development, which may not have been clarified [75,77,78]. It may be a relevant complementary tool for the construction of knowledge in diseases where pathophysiology has yet to be fully elucidated and possibly involves multiple complex genetic and environmental interactions, such as preterm delivery, preeclampsia and fetal growth restriction [78,79].

Metabolomics has been applied in biological samples for the investigation of processes ranging from embryogenesis to the emergence of complex diseases such as cancer, Parkinson's disease, diabetes and depression [80]. In the area of maternal and perinatal health care, it has been mainly applied to identify biomarkers, which are clinically useful for the performance of diagnostic or prognostic prediction [75,79,81].

For example, serum samples collected at 15 weeks of gestation from a group of approximately 39 nulliparous pregnant women with a history of preeclampsia were analyzed and compared to 40 pregnant women without complications. An untargeted technique, which is not aimed at identifying a class or specific type of metabolite, was applied in a nested case-control study performed from a cohort study and 45 metabolites had significantly different levels in both groups in the identification phase, and were characterized as potential biomarkers. For validation, 14 metabolites were selected to compose the final model, resulting in an area under the ROC curve of 0.92 and an odds ratio of 23 (95%CI; 7-73) [82]. Another study using samples of a similar number of women who progressed to preeclampsia and samples collected a short time earlier (between 11 and 14 weeks) showed more modest results, albeit still promising. The model containing 4 metabolites has a detection rate of only 50%, assuming a false-positive rate of 10%, with an area under the ROC curve of 0.81 for cases of preeclampsia [83]. Few studies on the identification of biomarkers to compose prediction models for preterm birth have been

published until now and some narrative reviews of the subject have described a great heterogeneity in the methodology employed [75,79,81]. To date, there are no systematic reviews that analyze the performance of metabolomics in predicting spontaneous preterm delivery.

Diverse reflections on the most effective method for investigating preterm birth using metabolomic markers should be made. First, there is the type of sample used (urine, blood, amniotic fluid, hair, vaginal secretion). Then, there is the time for sample collection (during clinical presentation of preterm birth or in the early phase of pregnancy when there is no symptom). Furthermore, metabolomics demands a high methodological rigor in the collection and storage of biosamples, since this is a highly sensitive method for identifying small low-weight molecules; various types of "noise," or interference in data acquisition, may hinder the identification of these molecules. Heterogeneity in sample collection and storage may be the cause. In addition, a well-delineated study design, with well-defined outcomes, following clear classifications, associated with sequential validations of findings are crucial for reliability and reproducibility of this technique. Finally, still in the phase of data analysis, caution to some important considerations is emphasized. For example, usually hundreds or even thousands of metabolites are analyzed at the same time in a sample. Since the number of variables (metabolites) is much higher than the number of samples (individuals), analysis is very susceptible to significantly false results. To correct this effect, Bonferroni correction may be used, attenuating the significance of p-value according to the number of variables (metabolites) analyzed. The FDR (*False Discovery Rate*) concept may also be applied to control the number of false-positive conclusions, and only the "most promising" variables receive significance [84]. This technique was proposed by Benjamini & Hochberg [84] in the 1990s and is based on the proportion between the true null hypothesis (H0) and the rejected null hypothesis, decreasing the possibility of markers considered to be statistically discriminatory. Actually, these markers are not discriminatory. These are only two examples of methodological care required in the phase of data analysis.

Many questions need to be answered concerning the mechanisms involved in the development of preterm birth: Why do some women have early cervical remodeling (with evident short cervix at transvaginal ultrasound in the second trimester) and others do not?

Which and how are the interactions between different risk factors including infection, vaginal bleeding and body mass index and how can they determine preterm birth? In theory, metabolomics depicts the final pathway resulting from these interactions and seems to be a useful approach not only to predict spontaneous preterm birth, but also to elucidate the diverse mechanisms involved.

6. Conclusion

To adequately address the investigation of preterm birth, its associated factors and perinatal outcomes, a robust methodological approach is required, using judicious and standardized definitions of exposures and outcomes. Based on this premise, a multifaceted comprehensive approach, albeit integrated, was proposed for data exploration on factors associated with preterm birth, its prediction and perinatal outcomes, capable of generating new knowledge of the theme.

It is expected that the results of this approach may contribute to the prediction of the most effective performance and better understanding of the factors associated with spontaneous preterm birth and consequent adverse perinatal results, collaborating with the development and application of public policies to prevent preterm birth and its perinatal consequences.

References

1. March of Dimes Forum: Lost my daughter and afraid to try again [Internet]. 2018 [Accessed 2018 Nov 20]. Available from: http://share.marchofdimes.org/forum/loss-and-grief/f/loss-and-grief/11412/lost-my-daughter-and-afraid-to-try-again#.W_CbOehKhPY

2. Hess J. A city-wide plan for the reduction of deaths associated with and due to prematurity. Proceedings of the fifth Annual Meeting of the American Academy of Pediatrics. J Pediatr. 1935;6:104–21.

3. EXPERT group on prematurity; final report, Geneva, 17-21 April 1950. World Health Organ Tech Rep Ser. 1950 Oct;27:1–11.

4. Blencowe H, Cousens S, Chou D, Oestergaard M, Say L, Moller A-B, et al. Born Too Soon: The global epidemiology of 15 million preterm births. Reprod Health. 2013 Nov 15;10(Suppl 1):S2.

5. Goldenberg RL, Gravett MG, Iams J, Papageorghiou AT, Waller SA, Kramer M, et al. The preterm birth syndrome: issues to consider in creating a classification system. Am J Obstet Gynecol. 2012 Mar;206(2):113–8.

6. Morisaki N, Togoobaatar G, Vogel J, Souza J, Rowland Hogue C, Jayaratne K, et al. Risk factors for spontaneous and provider-initiated preterm delivery in high and low Human Development Index countries: a secondary analysis of the World Health Organization Multicountry Survey on Maternal and Newborn Health. BJOG. 2014 Mar;121 Suppl:101–9.

7. Souza RT, Cecatti JG, Passini R, Tedesco RP, Lajos GJ, Nomura ML, et al. The Burden of Provider-Initiated Preterm Birth and Associated Factors: Evidence from the Brazilian Multicenter Study on Preterm Birth (EMIP). PLoS One. 2016 Jan 2;11(2):e0148244.

8. Passini Jr. R, Cecatti JG, Lajos GJ, Tedesco RP, Nomura ML, Dias TZ, et al. Brazilian multicentre study on preterm birth (EMIP): prevalence and factors associated with spontaneous preterm birth. PLoS One. 2014/10/10. 2014;9(10):e109069.

9. Kramer MS, Papageorghiou A, Culhane J, Bhutta Z, Goldenberg RL, Gravett M, et al. Challenges in defining and classifying the preterm birth syndrome. Am J Obstet Gynecol. 2012 Feb;206(2):108–12.

10. Fuchs F, Senat M-V. Multiple gestations and preterm birth. Semin Fetal Neonatal Med. 2016 Apr 1;21(2):113–20.

11. Santana D, Souza R, Surita F, Argenton J, Silva C, Cecatti J. Twin Pregnancy in Brazil: A Profile Analysis Exploring Population Information from the National Birth E-Registry on Live Births. Biomed Res Int. 2018;Ahead of p.

12. Santana DS, Silveira C, Costa ML, Souza RT, Surita FG, Souza JP, et al. Perinatal outcomes in twin pregnancies complicated by maternal morbidity: evidence from the WHO Multicountry Survey on Maternal and Newborn Health. BMC Pregnancy Childbirth. 2018 Nov 20;18(1):449.

13. Vogel JP, Torloni MR, Seuc A, Betrán AP, Widmer M, Souza JP, et al. Maternal and perinatal outcomes of twin pregnancy in 23 low- and middle-income countries. Bhutta ZA, editor. PLoS One. 2013 Aug 1;8(8):e70549.

14. Chawanpaiboon S, Vogel JP, Moller A-B, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. Lancet Glob Heal. 2018 Oct;

15. Harrison MS, Goldenberg RL. Global burden of prematurity. Semin Fetal Neonatal Med. 2016 Apr 1;21(2):74–9.

16. Frey HA, Klebanoff MA. The epidemiology, etiology, and costs of preterm birth. Semin Fetal Neonatal Med. 2016 Apr 1;21(2):68–73.

17. Smith V, Devane D, Begley CM, Clarke M, Higgins S. A systematic review and quality assessment of systematic reviews of randomised trials of interventions for preventing and treating preterm birth. Eur J Obstet Gynecol Reprod Biol. 2009 Jan;142(1):3–11.

18. Roberts D, Brown J, Medley N, Dalziel SR. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. Cochrane Database Syst Rev. 2017 Mar 21;3:CD004454.

19. Mackeen AD, Seibel-Seamon J, Muhammad J, Baxter JK, Berghella V. Tocolytics for preterm premature rupture of membranes. Cochrane Database Syst Rev. 2014 Feb 27;(2):CD007062.

20. Kenyon S, Boulvain M, Neilson JP. Antibiotics for preterm rupture of membranes.

Cochrane Database Syst Rev. 2013 Dec 2;(12):CD001058.

21. Shepherd E, Salam RA, Middleton P, Makrides M, McIntyre S, Badawi N, et al. Antenatal and intrapartum interventions for preventing cerebral palsy: an overview of Cochrane systematic reviews. Cochrane Database Syst Rev. 2017 Aug 8;8:CD012077.

22. Lawn JE, Kinney M V, Belizan JM, Mason E, McDougall L, Larson J, et al. Born Too Soon: Accelerating actions for prevention and care of 15 million newborns born too soon. Reprod Health. 2013 Nov 15;10(Suppl 1):S6.

23. Georgiou HM, Di Quinzio MKW, Permezel M, Brennecke SP. Predicting Preterm Labour: Current Status and Future Prospects. Dis Markers. 2015;2015.

24. Glass HC, Costarino AT, Stayer SA, Brett CM, Cladis F, Davis PJ. Outcomes for extremely premature infants. Anesth Analg. 2015 Jun;120(6):1337–51.

25. Locatelli A, Consonni S, Ghidini A. Preterm Labor: Approach to Decreasing Complications of Prematurity. Obstet Gynecol Clin North Am. 2015;42(2):255–74.

26. França EB, Passos VM de A, Malta DC, Duncan BB, Ribeiro ALP, Guimarães MDC, et al. Cause-specific mortality for 249 causes in Brazil and states during 1990–2015: a systematic analysis for the global burden of disease study 2015. Popul Health Metr. 2017 Dec 22;15(1):39.

27. Offord DR, Kraemer HC. Risk factors and prevention. Evid Based Ment Health. 2000 Aug 1;3(3):70–1.

28. Fletcher R, Fletcher S, Fletcher G. Risco: Princípios Básicos. In: Epidemiologia Clínica: Elementos Essenciais. 5th ed. Porto Alegre: Artmed; 2014. p. 280.

29. Rose G. Sick individuals and sick populations. Int J Epidemiol. 2001 Jun 1;30(3):427–32.

30. The National Academies Press. Institute of Medicine (IOM), National Research Council (NRC). Weight Gain During Pregnancy: Reexamining the Guidelines. Washington DC; 2009.

31. Masho SW, Bishop DL, Munn M, Ogden C, Carroll M, Curtin L, et al. Pre-pregnancy BMI and weight gain: where is the tipping point for preterm birth? BMC Pregnancy Childbirth. 2013 Dec 24;13(1):120.

32. Torloni MR, Betrán AP, Daher S, Widmer M, Dolan SM, Menon R, et al. Maternal BMI and preterm birth: a systematic review of the literature with meta-analysis. J Matern Fetal Neonatal Med. 2009 Nov;22(11):957–70.

33. Honest H, Hyde CJ, Khan KS. Prediction of spontaneous preterm birth: no good test for predicting a spontaneous preterm birth. Curr Opin Obstet Gynecol. 2012 Dec;24(6):422–33.

34. Koullali B, Oudijk MA, Nijman TAJ, Mol BWJ, Pajkrt E. Risk assessment and management to prevent preterm birth. Semin Fetal Neonatal Med. 2016 Feb 18;21(2):80–8.

35. Phillips C, Velji Z, Hanly C, Metcalfe A. Risk of recurrent spontaneous preterm birth: a systematic review and meta-analysis. BMJ Open. 2017 Jun 5;7(6):e015402.

36. Baer RJ, Yang J, Berghella V, Chambers CD, Coker TR, Kuppermann M, et al. Risk of preterm birth by maternal age at first and second pregnancy and race/ethnicity. J Perinat Med. 2018 Jul 26;46(5):539–46.

37. McManemy J, Cooke E, Amon E, Leet T. Recurrence risk for preterm delivery. Am J Obstet Gynecol. 2007 Jun;196(6):576.e1-576.e7.

38. Shah NR, Bracken MB. A systematic review and meta-analysis of prospective studies on the association between maternal cigarette smoking and preterm delivery. Am J Obstet Gynecol. 2000 Feb;182(2):465–72.

39. Florek E, Piekoszewski W, Hubert A, Kornacka MK. Influence of active and passive smoking during pregnancy on newborn health. In: Handbook of Smoking and Health. Laboratory of Environmental Research, Department of Toxicology, University of Medical Sciences, Poznan, Poland: Nova Science Publishers, Inc.; 2011. p. 264–307.

40. Huang S-H, Weng K-P, Huang S-M, Liou H-H, Wang C-C, Ou S-F, et al. The effects of maternal smoking exposure during pregnancy on postnatal outcomes: A cross sectional study. J Chinese Med Assoc. 2017;80(12):796–802.

41. Behrman R, Butler A, editors. Institute of Medicine (US) Committee on Understanding Premature Birth and Assuring Healthy Outcomes. In: Preterm Birth: Causes, Consequences, and Prevention. Washington (DC): National Academies Press (US); 2007.

42. Menon R. Oxidative stress damage as a detrimental factor in preterm birth pathology. Front Immunol. 2014;5:567.

43. Conde-Agudelo A, Romero R. Cervical phosphorylated insulin-like growth factor binding protein-1 test for the prediction of preterm birth: a systematic review and metaanalysis. Am J Obstet Gynecol. 2016 Jan;214(1):57–73.

44. Hee L. Likelihood ratios for the prediction of preterm delivery with biomarkers. Acta Obstet Gynecol Scand. 2011 Nov;90(11):1189–99.

45. Goldenberg RL, Iams JD, Mercer BM, Meis PJ, Moawad A, Das A, et al. The Preterm Prediction Study: toward a multiple-marker test for spontaneous preterm birth. Am J Obstet Gynecol. 2001 Sep;185(3):643–51.

46. van der Ven J, van Os MA, Kazemier BM, Kleinrouweler E, Verhoeven CJ, de Miranda E, et al. The capacity of mid-pregnancy cervical length to predict preterm birth in low-risk women: a national cohort study. Acta Obstet Gynecol Scand. 2015 Nov;94(11):1223–34.

47. Abbott DS, Hezelgrave NL, Seed PT, Norman JE, David AL, Bennett PR, et al. Quantitative fetal fibronectin to predict preterm birth in asymptomatic women at high risk. Obstet Gynecol. 2015 May;125(5):1168–76.

48. Villar J, Papageorghiou AT, Knight HE, Gravett MG, Iams J, Waller SA, et al. The preterm birth syndrome: a prototype phenotypic classification. Am J Obstet Gynecol. 2012 Feb;206(2):119–23.

49. Barros FC, Papageorghiou AT, Victora CG, Noble JA, Pang R, Iams J, et al. The Distribution of Clinical Phenotypes of Preterm Birth Syndrome: Implications for Prevention. JAMA Pediatr. 2015 Jan 5;169(3):220–9.

50. Requejo J, Merialdi M, Althabe F, Keller M, Katz J, Menon R. Born Too Soon: Care during pregnancy and childbirth to reduce preterm deliveries and improve health outcomes of the preterm baby. Reprod Health. 2013 Jan;10 Suppl 1(Suppl 1):S4.

51. Lumley J, Chamberlain C, Dowswell T, Oliver S, Oakley L, Watson L. Interventions for promoting smoking cessation during pregnancy. In: Chamberlain C, editor. Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd; 2009. p.

CD001055.

52. Scherman A, Tolosa JE, McEvoy C. Smoking cessation in pregnancy: a continuing challenge in the United States. Ther Adv Drug Saf. 2018 Aug 28;9(8):457–74.

53. Honest H, Forbes C, Durée K, Norman G, Duffy S, Tsourapas A, et al. Screening to prevent spontaneous preterm birth: systematic reviews of accuracy and effectiveness literature with economic modelling. Health Technol Assess (Rockv). 2009 Sep;13(43):1–627.

54. To MS, Skentou C, Chan C, Zagaliki A, Nicolaides KH. Cervical assessment at the routine 23-week scan: standardizing techniques. Ultrasound Obstet Gynecol. 2001 Mar;17(3):217–9.

55. Iams JD, Goldenberg RL, Meis PJ, Mercer BM, Moawad A, Das A, et al. The length of the cervix and the risk of spontaneous premature delivery. National Institute of Child Health and Human Development Maternal Fetal Medicine Unit Network. N Engl J Med. 1996 Feb 29;334(9):567–72.

56. Berghella V, Roman A, Daskalakis C, Ness A, Baxter JK. Gestational age at cervical length measurement and incidence of preterm birth. Obstet Gynecol. 2007 Aug;110(2 Pt 1):311–7.

57. Romero R, Conde-Agudelo A, Da Fonseca E, O'Brien JM, Cetingoz E, Creasy GW, et al. Vaginal progesterone for preventing preterm birth and adverse perinatal outcomes in singleton gestations with a short cervix: a meta-analysis of individual patient data. Am J Obstet Gynecol. 2018 Feb;218(2):161–80.

58. Dodd JM, Jones L, Flenady V, Cincotta R, Crowther CA. Prenatal administration of progesterone for preventing preterm birth in women considered to be at risk of preterm birth. Cochrane Database Syst Rev. 2013 Jul 31;(7):CD004947.

59. Norman JE, Marlow N, Messow C-M, Shennan A, Bennett PR, Thornton S, et al. Vaginal progesterone prophylaxis for preterm birth (the OPPTIMUM study): a multicentre, randomised, double-blind trial. Lancet. 2016 May 21;387(10033):2106–16.

60. Shirodkar VN. Some observations on non-malignant conditions of the cervix. J Obstet Gynaecol India. 1953;3:287–9.

61. McDonald I. Suture of the cervix for inevitable miscarriage. J Obstet Gynaecol Br Emp. 1957 Jun;64(3):346–50.

62. Eleje GU, Ikechebelu JI, Eke AC, Okam PC, Ezebialu IU, Ilika CP. Cervical cerclage in combination with other treatments for preventing preterm birth in singleton pregnancies. Cochrane Database Syst Rev. 2017;2017(11).

63. American College of Obstetricians and Gynecologists. ACOG Practice Bulletin No.142: Cerclage for the management of cervical insufficiency. Obstet Gynecol. 2014 Feb;123(2, PART 1):372–9.

64. Goya M, Pratcorona L, Merced C, Rodó C, Valle L, Romero A, et al. Cervical pessary in pregnant women with a short cervix (PECEP): an open-label randomised controlled trial. Lancet. 2012 May 12;379(9828):1800–6.

65. Cruz-Melguizo S, San-Frutos L, Martínez-Payo C, Ruiz-Antorán B, Adiego-Burgos B, Campillos-Maza JM, et al. Cervical Pessary Compared With Vaginal Progesterone for Preventing Early Preterm Birth: A Randomized Controlled Trial. Obstet Gynecol. 2018 Oct;132(4):907–15.

66. Saccone G, Maruotti GM, Giudicepietro A, Martinelli P, Italian Preterm Birth Prevention (IPP) Working Group. Effect of Cervical Pessary on Spontaneous Preterm Birth in Women With Singleton Pregnancies and Short Cervical Length: A Randomized Clinical Trial. JAMA. 2017 Dec 19;318(23):2317–24.

67. Nicolaides KH, Syngelaki A, Poon LC, Picciarelli G, Tul N, Zamprakou A, et al. A Randomized Trial of a Cervical Pessary to Prevent Preterm Singleton Birth. N Engl J Med. 2016 Mar 17;374(11):1044–52.

68. Dugoff L, Berghella V, Sehdev H, Mackeen AD, Goetzl L, Ludmir J. Prevention of preterm birth with pessary in singletons (PoPPS): randomized controlled trial. Ultrasound Obstet Gynecol. 2018 May;51(5):573–9.

69. Kyvernitakis I, Maul H, Bahlmann F. Controversies about the Secondary Prevention of Spontaneous Preterm Birth. Geburtshilfe Frauenheilkd. 2018 Jun;78(6):585–95.

70. Conde-Agudelo A, Romero R. Predictive Accuracy of Changes in Transvaginal Sonographic Cervical Length Over Time for Preterm Birth: A Systematic Review and Meta-Analysis. Am J Obstet Gynecol. 2015 Jun 9;

71. Esplin MS, Elovitz MA, Iams JD, Parker CB, Wapner RJ, Grobman WA, et al. Predictive Accuracy of Serial Transvaginal Cervical Lengths and Quantitative Vaginal Fetal Fibronectin Levels for Spontaneous Preterm Birth Among Nulliparous Women. JAMA. 2017 Mar 14;317(10):1047.

72. Conde-Agudelo A, Papageorghiou A, Kennedy S, Villar J. Novel biomarkers for the prediction of the spontaneous preterm birth phenotype: a systematic review and meta-analysis. BJOG An Int J Obstet Gynaecol. 2011 Aug;118(9):1042–54.

73. Li S, Dunlop AL, Jones DP, Corwin EJ. High-Resolution Metabolomics: Review of the Field and Implications for Nursing Science and the Study of Preterm irth. Biol Res Nurs. 2016 Jan;18(1):12–22.

74. Dettmer K, Hammock BD. Metabolomics--a new exciting field within the omics sciences. Environ Health Perspect. 2004 May;112(7):A396-7.

75. Romero R, Espinoza J, Gotsch F, Kusanovic J, Friel L, Erez O, et al. The use of high-dimensional biology (genomics, transcriptomics, proteomics, and metabolomics) to understand the preterm parturition syndrome. BJOG An Int J Obstet Gynaecol. 2006 Dec 19;113:118–35.

76. Patti GJ, Yanes O, Siuzdak G. Innovation: Metabolomics: the apogee of the omics trilogy. Nat Rev Mol Cell Biol. 2012 Apr;13(4):263–9.

77. Dettmer K, Aronov PA, Hammock BD. Mass spectrometry-based metabolomics. Mass Spectrom Rev. 2007 Jan;26(1):51–78.

78. Kell DB. Metabolomics and systems biology: making sense of the soup. Curr Opin Microbiol. 2004 Jun;7(3):296–307.

79. Horgan RP, Clancy OH, Myers JE, Baker PN. An overview of proteomic and metabolomic technologies and their application to pregnancy research. BJOG. 2009 Jan;116(2):173–81.

80. Putri SP, Nakayama Y, Matsuda F, Uchikata T, Kobayashi S, Matsubara A, et al. Current metabolomics: practical applications. J Biosci Bioeng. 2013 Jun;115(6):579–89.

81. Dessì A, Marincola FC, Fanos V. Metabolomics and the great obstetrical syndromes - GDM, PET, and IUGR. Best Pract Res Clin Obstet Gynaecol. 2014 Aug 21;29(2):156–64.

82. Kenny LC, Black MA, Poston L, Taylor R, Myers JE, Baker PN, et al. Early pregnancy prediction of preeclampsia in nulliparous women, combining clinical risk and biomarkers: the Screening for Pregnancy Endpoints (SCOPE) international cohort study. Hypertension. 2014 Sep;64(3):644–52.

83. Odibo AO, Goetzinger KR, Odibo L, Cahill AG, Macones GA, Nelson DM, et al. First-trimester prediction of preeclampsia using metabolomic biomarkers: a discovery phase study. Prenat Diagn. 2011 Oct;31(10):990–4.

84. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J R Stat Soc. 1995;57(1):289–300.

2. OBJETIVOS

2.1. Objetivo Geral

Desenvolver uma abrangente abordagem aos fatores clínicos e epidemiológicos associados ao parto prematuro, seus preditores metabolômicos e respectivos desfechos perinatais.

2.2. Objetivos Específicos

2.2.1. Avaliar a associação do índice de massa corpórea e ganho de peso gestacional com os diferentes subtipos de parto prematuro e com desfechos perinatais.

2.2.2. Identificar fenótipos maternos relacionados ao parto prematuro e suas respectivas condições associadas, características maternas e desfechos maternos e perinatais.

2.2.3. Avaliar a aplicação da ciência ômica nos estudos em saúde materna e perinatal, com enfoque na metabolômica e na predição das principais complicações na gestação.

2.2.4. Desenvolver um método padronizado para revisar sistematicamente os estudos em predição de prematuridade espontânea através de marcadores metabolômicos.

2.2.5. Desenvolver o método e procedimentos utilizados em um estudo multicêntrico para investigar a predição de parto prematuro e outras complicações maternas e perinatais.

2.2.6. Implementar e desenvolver um estudo para predição de parto prematuro e outras complicações maternas e perinatais, fornecendo subsídios metodológicos e descrevendo detalhes sobre os aspectos práticos dessa implementação e estratégias para a solução de dificuldades encontradas.

2.2.7. Identificar um conjunto de marcadores metabolômicos, clínicos e/ou sociodemográficos preditivos de parto prematuro.

2.2.8. Avaliar a incidência e potenciais fatores de risco associados à ocorrência de parto prematuro espontâneo.

2.2.9. Avaliar a ocorrência de desfechos perinatais adversos associados à prematuridade.

3. MÉTODO

Para abordar mais alargada e profundamente os fatores associados ao parto prematuro e seus possíveis preditores, principalmente os potenciais e inovadores marcadores metabolômicos, foram utilizados e desenvolvidos mais de um projeto de pesquisa, gerando maior complexidade na elaboração de uma linha única de descrição de método. Fundamentalmente, houve no decorrer dessa tese o desenvolvimento completo de um modelo metodológico para identificação e validação de marcadores clínicos e metabolômicos, incluindo desde a concepção do desenho de um estudo em duas fases (fase de identificação e de validação de marcadores) até a implementação de um grande estudo de coorte multicêntrico no Brasil para estudar complicações maternas e perinatais. Outras análises e artigos, embora secundários, foram elaborados ou estão planejados para complementar a abordagem analítica sobre os fatores associados à prematuridade, assim como para registrar e tornar público o método e *expertise* desenvolvidos no decorrer desse caminho. Para a revisão sistemática, um artigo próprio para seu método é apresentando também como produto da tese, descrevendo pormenorizadamente os procedimentos empregados. O protocolo de revisão sistemática foi registrado na plataforma PROSPERO (PROSPERO 2018 CRD42018100172) (1).

Com isso, diferentes métodos e projetos de pesquisa foram utilizados para compor essa tese, que inclui artigos de descrição de protocolo, revisão narrativa, revisão sistemática, de análise secundária e de análise primária com dados originais. Para as análises primárias e secundárias com dados originais, foram utilizados dados dos seguintes estudos observacionais: do Estudo Multicêntrico de Investigação em Prematuridade no Brasil – EMIP, do *Preterm Screening and Metabolomics in Brazil and Auckland* – Preterm SAMBA e do *Screening of Pregnancy Endpoints* – SCOPE. Apesar do método de cada análise ser detalhadamente descrito nos respectivos artigos, serão descritos nessa sessão alguns aspectos relevantes dos métodos adotados de acordo com a fonte dos dados (Estudos EMIP, Preterm SAMBA ou SCOPE) e com o tipo de artigo (revisão sistemática, narrativa ou de protocolo). Os estudos EMIP e Preterm SAMBA foram desenvolvidos dentro da Rede Brasileira de Estudos em Saúde Reprodutiva e Perinatal, criada e coordenada por investigadores do Departamento de

Tocoginecologia da Faculdade de Ciências Médicas da Unicamp. Essa Rede foi criada em meados de 2008 com o objetivo de fomentar e organizar profissionalmente os estudos em saúde materna e perinatal nos serviços acadêmicos universitários do Brasil (2). Já tendo feito pouco mais de 10 anos desde sua criação, a Rede organizou e implementou vários estudos multicêntricos, observacionais e ensaios clínicos, em tópicos relevantes da área. Além disso, tem proporcionado a capacitação de muitos docentes e jovens pesquisadores em diversas regiões do país, contribuindo ainda mais com o avanço científico e tecnológico em nossa área e para a implementação de novos estudos multicêntricos contando a participação de pessoal qualificado (2). O financiamento de estudos da Rede soma atualmente pouco mais de 4 milhões de reais que ajudaram não só no desenvolvimento dos projetos de pesquisa, mas também a estruturar os centros participantes e a aumentar a interação com outros parceiros internacionais, que tem sido fundamental para a manutenção de suas atividades e de novas colaborações em estudos internacionais. Os estudos em que essa tese se baseia são todos provenientes diretamente das atividades da Rede e são, sem dúvida, um trabalho conjunto de equipe(s).

Estudo Multicêntrico de Investigação em Prematuridade no Brasil - EMIP

O estudo EMIP teve o objetivo de avaliar de forma abrangente a prevalência de partos prematuros e seus fatores associados no Brasil. Foi um estudo multicêntrico de corte transversal com componente caso-controle aninhado que foi desenvolvido em 20 maternidades do Sudeste, Sul e Nordeste do país, consideradas “de referência” em suas sub-regiões. O método do estudo já foi publicado anteriormente (3,4). Resumidamente, o EMIP explorou a ocorrência de prematuridade de acordo com os seus três subtipos: espontânea, (devida ao trabalho de parto espontâneo), ruptura prematura pré-termo de membranas e terapêutica (quando houve uma condição materna ou fetal que na avaliação do prestador de cuidado à saúde, colocava em risco a continuidade da gestação e que, por isso, motivou a resolução da gestação em idade gestacional pré-termo). Parto prematuro foi definido como aquele que ocorreu antes de 37 semanas de gestação, estimada ou pela data da última menstruação, pelo ultrassom, por ambos ou, enfim, pelo método de escore de New Ballard (5).

A equipe de assistentes de pesquisa realizou uma vigilância de todos os partos prematuros ocorridos de abril de 2011 a julho 2012, incluindo mulheres com gestações de feto único e gestações múltiplas. Mulheres que tiveram parto a termo logo após os casos de parto prematuro foram convidadas a participar para compor o grupo controle, até que o tamanho amostral para o grupo controle fosse atingido. O cálculo amostral resultou na necessidade de haver ao menos 1.054 sujeitos em cada um dos quatro subgrupos (3 de casos e 1 de controle), ao considerar uma prevalência de prematuridade de 6,5% em 2006. Os dados foram obtidos através de entrevista com a mulher ainda na internação pós-parto e de revisão de dados do prontuário dela e do recém-nascido e do cartão de pré-natal. Os dados do recém-nascido foram registrados até 56 dias após o parto ou até a alta, o que ocorresse primeiro. Foi utilizada uma ficha de coleta de dados contendo mais de 300 variáveis abrangendo dados clínicos, sociodemográficos, sobre as características de assistência ao pré-natal e ao parto prestadas, sobre a ocorrência de complicações ou morbidade durante a gestação ou puerpério e de desfechos maternos e perinatais (Anexo 1). Essas informações, então, foram repassadas a um sistema *online* OpenClínica® de banco de dados.

Diversos procedimentos como checagem manual dos dados, visitas de monitoramento in loco e análise de consistência interna dos dados (conferência das frequências e do cruzamento de variáveis, etc.) foram realizados para assegurar confiabilidade aos dados. O acesso ao banco de dados era restrito e hierárquico, conforme o papel desenvolvido pelos diferentes pesquisadores. Todas participantes assinaram termo de consentimento livre e esclarecido e tiveram participação voluntária. O estudo teve aprovação ética dos respectivos Comitês de Ética em Pesquisa (CEP) locais e foi referendado pelo Comitê Nacional de Ética em Pesquisa – CONEP (Anexo 2), seguindo os preceitos éticos das normas regulamentadoras de pesquisas envolvendo seres humanos previstos da Resolução 196/96 do Conselho Nacional de Saúde e na Declaração de Helsinki. A identidade das participantes foi e é mantida confidencial. Esse projeto foi contemplado com auxílio à pesquisa no contexto do programa PPSUS financiado conjuntamente pelo CNPq e Fapesp.

***Preterm Screening and Metabolomics in Brazil and Auckland* – Preterm SAMBA**

O projeto de pesquisa Preterm SAMBA é uma iniciativa com colaboração internacional entre pesquisadores do Departamento de Tocoginecologia da FCM/Unicamp e pesquisadores da Universidade de Auckland, Auckland, Nova Zelândia, e University College Cork, em Cork, Irlanda. O estudo tem como objetivo principal desenvolver um teste preditivo para parto prematuro espontâneo. Para isso, o projeto contou com dois desenhos de estudo: um componente caso-controle desenhado para desenvolver um modelo preditor de parto prematuro espontâneo e um componente de coorte prospectivo para validação do modelo preditor. A fase de desenvolvimento foi realizada utilizando-se dos dados e amostras do Estudo SCOPE, enquanto para a fase de validação, foi planejado, implementado e desenvolvido um estudo de coorte com gestantes nulíparas de baixo risco de 5 maternidades brasileiras. Os detalhes metodológicos, técnicos, operacionais e sobre o desenvolvimento desse estudo de coorte são apresentados em dois artigos como produtos dessa tese. Em suma, gestantes elegíveis foram incluídas antes de 21 semanas de gestação e realizaram até três visitas do estudo: obrigatória entre 19 e 21 semanas e opcional entre 27 e 29 semanas e entre 37 e 39 semanas. Dados clínicos, sociodemográficos, epidemiológicos, antropométricos, nutricionais, sobre hábitos de vida e amostras biológicas (sangue e cabelo) foram coletados na primeira visita. Na segunda e terceira visitas, apenas dados clínicos e antropométricos. Após o parto, desfechos primários (ocorrência de parto prematuro), e secundários (pré-eclâmpsia, restrição do crescimento fetal e diabetes mellitus gestacional) foram sistematicamente coletados.

Para identificar os fatores de risco clínicos para a ocorrência de parto prematuro espontâneo e avaliar a ocorrência de desfechos perinatais adversos associado à prematuridade, utilizamos os dados desta coorte brasileira do componente de validação do estudo Preterm SAMBA. O estudo foi aprovado no CEP do centro coordenador (Anexo 3) e em todos os centros participantes.

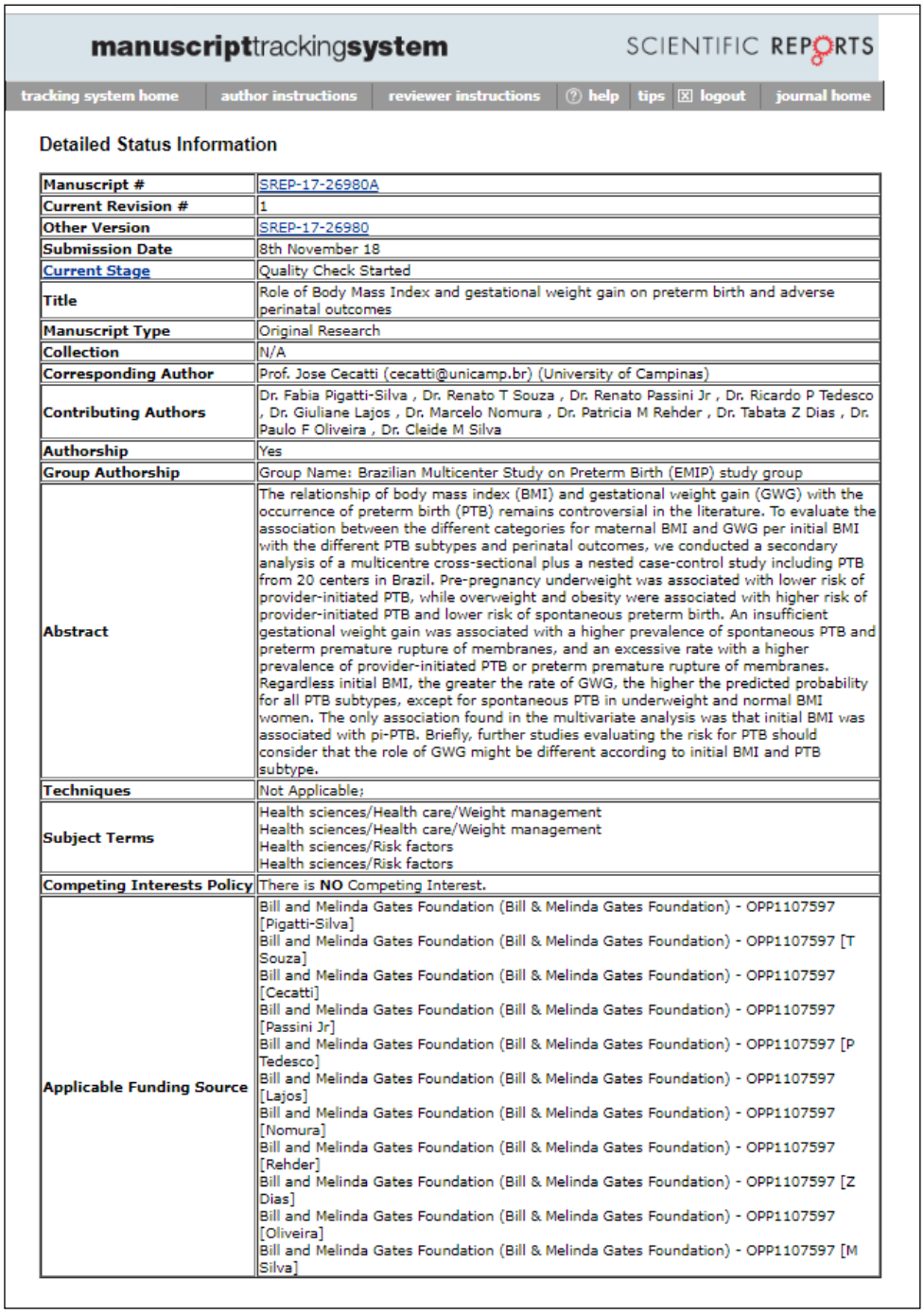
***Screening of Pregnancy Endpoints* - SCOPE**

O primeiro componente do projeto, desenvolvimento do modelo preditor, ou de descobrimento, consiste em um estudo caso-controle com mulheres que

participaram do estudo SCOPE (6,7), um estudo de coorte prospectivo multicêntrico que recrutou 5.690 mulheres nulíparas de baixo risco entre novembro de 2004 e agosto de 2008 de diferentes regiões - Nova Zelândia, Austrália, Irlanda e Reino Unido, criando um banco de dados e de material biológico dessas mulheres e recém-nascidos (sangue, cabelo, urina, sangue de cordão umbilical, etc.). Onze instituições financiaram essa coorte internacional que teve aprovação ética em todos os respectivos centros participantes (6). Para essa análise, estabelecemos um estudo caso-controle composto por mulheres de Cork, Irlanda, e Auckland, Nova Zelândia, que tiveram partos prematuros espontâneos antes de 37 semanas. Amostras de soro colhidas às 15 e 20 semanas de gestação e armazenadas em freezer a -80°C foram utilizadas para análise em espectrometria de massa acoplada a cromatografia gasosa.

4. RESULTADOS

4.1. Artigo *Role of Body Mass Index and gestational weight gain on preterm birth and adverse perinatal outcomes*



manuscripttrackingsystem SCIENTIFIC REPORTS

tracking system home | author instructions | reviewer instructions | help | tips | logout | journal home

Detailed Status Information

Manuscript #	SREP-17-26980A
Current Revision #	1
Other Version	SREP-17-26980
Submission Date	8th November 18
Current Stage	Quality Check Started
Title	Role of Body Mass Index and gestational weight gain on preterm birth and adverse perinatal outcomes
Manuscript Type	Original Research
Collection	N/A
Corresponding Author	Prof. Jose Cecatti (cecatti@unicamp.br) (University of Campinas)
Contributing Authors	Dr. Fabia Pigatti-Silva , Dr. Renato T Souza , Dr. Renato Passini Jr , Dr. Ricardo P Tedesco , Dr. Giuliane Lajos , Dr. Marcelo Nomura , Dr. Patricia M Rehder , Dr. Tabata Z Dias , Dr. Paulo F Oliveira , Dr. Cleide M Silva
Authorship	Yes
Group Authorship	Group Name: Brazilian Multicenter Study on Preterm Birth (EMIP) study group
Abstract	The relationship of body mass index (BMI) and gestational weight gain (GWG) with the occurrence of preterm birth (PTB) remains controversial in the literature. To evaluate the association between the different categories for maternal BMI and GWG per initial BMI with the different PTB subtypes and perinatal outcomes, we conducted a secondary analysis of a multicentre cross-sectional plus a nested case-control study including PTB from 20 centers in Brazil. Pre-pregnancy underweight was associated with lower risk of provider-initiated PTB, while overweight and obesity were associated with higher risk of provider-initiated PTB and lower risk of spontaneous preterm birth. An insufficient gestational weight gain was associated with a higher prevalence of spontaneous PTB and preterm premature rupture of membranes, and an excessive rate with a higher prevalence of provider-initiated PTB or preterm premature rupture of membranes. Regardless initial BMI, the greater the rate of GWG, the higher the predicted probability for all PTB subtypes, except for spontaneous PTB in underweight and normal BMI women. The only association found in the multivariate analysis was that initial BMI was associated with pi-PTB. Briefly, further studies evaluating the risk for PTB should consider that the role of GWG might be different according to initial BMI and PTB subtype.
Techniques	Not Applicable;
Subject Terms	Health sciences/Health care/Weight management Health sciences/Health care/Weight management Health sciences/Risk factors Health sciences/Risk factors
Competing Interests Policy	There is **NO** Competing Interest.
Applicable Funding Source	Bill and Melinda Gates Foundation (Bill & Melinda Gates Foundation) - OPP1107597 [Pigatti-Silva] Bill and Melinda Gates Foundation (Bill & Melinda Gates Foundation) - OPP1107597 [T Souza] Bill and Melinda Gates Foundation (Bill & Melinda Gates Foundation) - OPP1107597 [Cecatti] Bill and Melinda Gates Foundation (Bill & Melinda Gates Foundation) - OPP1107597 [Passini Jr] Bill and Melinda Gates Foundation (Bill & Melinda Gates Foundation) - OPP1107597 [P Tedesco] Bill and Melinda Gates Foundation (Bill & Melinda Gates Foundation) - OPP1107597 [Lajos] Bill and Melinda Gates Foundation (Bill & Melinda Gates Foundation) - OPP1107597 [Nomura] Bill and Melinda Gates Foundation (Bill & Melinda Gates Foundation) - OPP1107597 [Rehder] Bill and Melinda Gates Foundation (Bill & Melinda Gates Foundation) - OPP1107597 [Z Dias] Bill and Melinda Gates Foundation (Bill & Melinda Gates Foundation) - OPP1107597 [Oliveira] Bill and Melinda Gates Foundation (Bill & Melinda Gates Foundation) - OPP1107597 [M Silva]

Original Research

Role of Body Mass Index and gestational weight gain on preterm birth and adverse perinatal outcomes

Fabia Pigatti Silva[1,+], Renato T Souza[1,+], Jose G Cecatti[1,*], Renato Passini Jr[1], Ricardo P Tedesco[2], Giuliane J Lajos[1], Marcelo L Nomura[1], Patricia M Rehder[1], Tabata Z Dias[1], Paulo F Oliveira[3], Cleide M Silva[3], for the Brazilian Multicenter Study on Preterm Birth (EMIP) study group [#]

[1] Department of Obstetrics and Gynecology, School of Medical Sciences, University of Campinas (UNICAMP), Campinas, SP, Brazil

[2] Department of Obstetrics and Gynecology, Jundiaí Medical School, Jundiaí, SP, Brazil

[3] Unit of Statistics, School of Medical Sciences, University of Campinas (UNICAMP), Campinas, Brazil

+these authors contributed equally to this work

[*]Corresponding author: cecatti@unicamp.br

[#] Membership of the Brazilian Multicentre Study on Preterm Birth study group is provided in the Acknowledgments.

Abstract

The relationship of body mass index (BMI) and weight gain (WG) with the occurrence of preterm birth (PTB) remains controversial in the literature. To evaluate the association between the different categories for maternal BMI and WG per initial BMI with the different PTB subtypes and perinatal outcomes, we conducted a secondary analysis of a multicentre cross-sectional plus a nested case-control study including PTB from 20 centers in Brazil. Pre-pregnancy underweight was associated with lower risk of provider-initiated PTB, while overweight and obesity were associated with higher risk of provider-initiated PTB and lower risk of spontaneous preterm birth. An insufficient gestational weight gain was associated with a higher prevalence of spontaneous PTB and preterm premature rupture of membranes, and an excessive rate with a higher prevalence of provider-initiated PTB or preterm premature rupture of membranes. Regardless initial BMI, the greater the rate of WG, the higher the predicted probability for all PTB subtypes, except for spontaneous PTB in underweight and normal BMI women. Briefly, further studies evaluating the risk for PTB should consider that the role of WG might be different according to initial BMI and PTB subtype.

INTRODUCTION

Preterm birth (PTB) is an increasing health concern and a major cause of neonatal mortality and long-term morbidity worldwide[1]. To develop effective strategies to reduce preterm birth, it is important to assess the causes and risk factors involved in its etiology. Body mass index (BMI) in pre- and early pregnancy and weight gain during pregnancy were already associated with preterm birth, however the exact role they play in determining preterm birth is still to be determined[2].

Obesity and overweight are also recognized as a growing global health problem[3]. The prevalence of overweight among adult women globally increased from 29.8% in 1980 to 38% in 2013, especially in middle-income countries[4]. For mothers, major adverse health outcomes are related with obesity as gestational diabetes, pregnancy-induced hypertension, preeclampsia, postpartum hemorrhage, and caesarean delivery[5]. These complications of pregnancy are known to contribute to medically indicated PTB and are more common in overweight and obese women[2,6]. On the other hand, low early pregnancy BMI has been consistently reported as a risk factor for preterm birth, especially for spontaneous preterm birth (sPTB), in comparison with women of any other weight status[7–9].

Considerably fewer studies have evaluated the association between gestational weight gain and preterm birth; in many cases with contradictory results, as well as the majority of them has been conducted in high-income countries that have different contexts, such as racial, cultural, and socioeconomic factors, compared to low and middle-income countries. In addition, these studies have generally failed to distinguish between the different preterm births subtypes (sPTB, PROM-PTB and pi-PTB) and the rate of gestational weight gain, limiting their ability to delineate the dose-response relationship between gestational weight gain and preterm birth subtypes[10–12]. Therefore, it is still necessary to evaluate the association between the early pregnancy´ BMI and gestational weight gain with the occurrence of preterm birth and perinatal outcomes, considering the rate of gestational weight gain as a modifier factor for adverse maternal and perinatal outcomes in developing world[13].

A retrospective cohort study with almost 9 thousand women delivering singleton babies between 2006 and 2009 in Lima, Peru, showed an independent association between the rate of gestational weight gain and preterm birth (especially sPTB), which varies depending on the pre-pregnancy BMI. This association was protective in underweight women, however in overweight and normal BMI women both very low rates and very high

rates of gestational weight gain were associated with an increased preterm birth rate. These results are important for public health and highlight the need for further studies to expand our knowledge on the determinants of preterm birth[10].

This study is part of the Brazilian Multicenter Study on Preterm Birth (EMIP), one of the most comprehensive epidemiological study on preterm birth in Brazil, conducted in 20 referral obstetrical facilities in different geographical regions of the country. The purpose of this analysis is to evaluate the association between pre-pregnancy or early pregnancy BMI and the gestational weight gain with the risk of preterm births and their subtypes. Secondly, we aim to assess the impact of the gestational weight gain and early pregnancy BMI on the severity of adverse perinatal outcomes among preterm births.

RESULTS

From all the 33,740 births surveyed by EMIP study, 4,150 were preterm births while 1,146 were selected to build the control group of term births (Figure 1). After excluding outliers' data and considering all preterm and term births, 4,506 (85%) had information about early/pre-pregnancy BMI and 4,193 (79.2%) had complete information to calculate the gestational weight gain (Figure 1). Although the majority of women had normal pre-pregnancy BMI (56.1%), approximately 85% were considered as having inadequacy of gestational weight gain, insufficient or excessive (data not shown). Additionally, more than one third of women were overweight or obese at the beginning of pregnancy (35.4%).

Table 1 shows the risk estimates for preterm birth according to maternal early/pre-pregnancy BMI and adequacy of weight gain during pregnancy. Overweight and obesity were associated with higher risk for pi-PTB, however with lower risk for sPTB. Underweight was associated with a 40% lower risk for pi-PTB. An insufficient RWG during pregnancy, regardless the initial BMI, was associated with increased risk for sPTB (1.7 fold) and PROM-PTB (1.5 fold). Women with excessive RWG were more likely to have PROM-PTB (1.4 fold) and pi-PTB (2 fold).

Figures 2, 3, 4 and 5 show predicted probabilities for preterm birth subtypes for women respectively with underweight, normal BMI, overweight and obesity according to the RWG (per week). The greater the RWG of overweight and obese women, the higher the predicted probability for all subtypes of preterm birth. In women with underweight or normal BMI, the trend of increased probability according to higher RWG only remains for PROM-PTB and pi-PTB. The probability for spontaneous preterm birth in women with

underweight, however, remains bordering on stable regardless the RWG, while it decreases the greater the RWG in women with normal BMI.

Women with insufficient RWG had a proportionally higher prevalence of preterm below 28 weeks and between 28 and 33 weeks of gestation (Table 2). Newborns of women with insufficient RWG had higher proportion of NICU admission.

The multivariate analyses showed that fetal malformation, history of vaginal bleeding during pregnancy, maternal morbidity and multiple pregnancy were independently associated with any adverse perinatal outcome in women with preterm birth (Table 3). Adequacy of weight gain has not shown to be independently associated with APO, while initial BMI were poorly associated with pi-PTB.

DISCUSSION

EMIP study was a comprehensive survey on preterm birth in Brazil and, during the study progress, the prevalence of 6.5% was confirmed to be underestimated according to updated official data of the Brazilian Government (around 10% in 2011)[14]. Therefore, the surveillance of less than 37,000 deliveries was sufficient to achieve the number of participants in each group. The maternal characteristics of EMIP study has been already published elsewhere[15,16], but we highlight that more than one third of participant women were overweight or obese at the beginning of pregnancy. The high estimated rates of Brazilian women aged between 25-34 years that are overweight obese justify the concern with endemic obesity and overweight especially in middle and high-income countries. Traditionally, early BMI has been used as a proxy of nutritional status at the beginning of pregnancy[17], and has been largely studied as a risk factor for preterm birth [2,18,19]. Although conventional, early BMI is an unmodifiable marker for the index pregnancy. Therefore, the current analyses included the evaluation of the weight gain during pregnancy as it could possibly represent a more dynamic and modifiable nutritional status during pregnancy.

There are conflicting results in the literature regarding the risk of preterm birth and maternal early BMI[2,18,19]. In general, underweight are related with higher risk for sPTB and obesity with PROM and pi-PTB. However, many studies do not consider the preterm subtypes separately in the analyses, which is the main limitation for systematic reviews, as well as uniform categorization of BMI. According to a systematic review of maternal BMI and risk for PTB, although 39 studies published in 40 years on this topic, the lack of standardization of BMI limits the analyses and weakens the results and evidence[2]. The calculation of gestational weight gain is also another potential limitation on observational

studies, mainly in retrospective cohorts. Many studies calculate the total gestational weight gain, which complicates the comparison between a preterm and term delivery, not accounting for the expected lower weight gain in shorter gestational length. Two recent systematics reviews addressed the risks for adverse neonatal outcomes according to IOM gestational weight gain categories. Goldstein *et al*[20] and Kominiarek *et al*[21] showed that total weight gain below the recommendations were associated with higher risk for preterm birth OR 1.70 (CI 95% [1.32-2.20]) and OR 1.47 (CI 95% [1.31-1.64]), respectively.

Differently to what was previously described by IOM Guidelines[22], EMIP study results suggest that abnormal BMI and inadequacy of weight gain might have different effects on the risk for preterm birth subtypes. In our study, women with overweight and especially obesity were associated with higher risk for pi-PTB, however with lower risk for sPTB. Obesity and overweight are established risk factors for metabolic disorders such as gestational diabetes, hypertension, preeclampsia, polyhydramnios and others, which are associated with maternal complications and consequently with higher risk for pi-PTB[2,16,23]. Observational studies suggest that the myometrial function of obese women is affected due to an abnormal response of oxytocin, whose receptors are decreased in myometrial biopsies in obese women at term[24–27]. This alteration is associated with higher rates of postdates pregnancies and slower labour progression in obese women compared to non-obese. Additionally, there is a low-evidenced hypothesis regarding the effects of endogenous oxytocin in food intake and body weight. Higher levels of endogenous oxytocin and/or overexpression of oxytocin receptors might reduce food intake and increase energy expenditure, reducing body weight[28]. Considering that, these women would have higher risk for prematurity, but a protective mechanism for obesity.

On the other hand, a population-based cohort study of almost 1.6 million singleton deliveries in Sweden from 1992 through 2010 showed an increase risk for sPTB, especially extremely preterm, in overweight and obese pregnant women[29]. Obesity is characterized by inflammatory up-regulation, being associated with proinflammatories cytokines and adipokines and with alterations of hypothalamic-pituitary-adrenal axis, which is responsible for releasing corticotrophin-releasing hormone. In high values, it is known as a risk factor for premature rupture of membranes, preterm labour, eclampsia and pregnancy-induced hypertension[29–31]. Those conflicting evidences indicates that multiple underlying mechanisms might play a role at the risk for PTB in women with different BMI.

According to our analyses, the effects of gestational weight gain in the risk for PTB depended on the initial BMI and on the preterm subtype. In general, excessive RWG was

associated with a higher probability for preterm birth, especially for pi-PTB in overweight and obese women. Carnero and colleagues conducted a retrospective cohort study in Peru and found that women with overweight and normal BMI have a "U-shaped" curve of association in which both low and high RWG were associated with an increased risk for preterm birth, especially pi-PTB[10]. The U-shaped form of association is in agreement with the literature, which shows that extremes of RWG are important risk factors for PTB[22,32–34]. Therefore, pregnant women should be in the middle of this curve to minimize the risk. Although we did not observe such association in our study, our findings demonstrate that RWG might determine different effects on the risk for PTB depending on the initial BMI.

Insufficient weight gain during pregnancy was related to more severe prematurity and higher admission to NICU in our analyses. Insufficient weight gain is associated with adverse perinatal outcomes as small for gestational age and spontaneous preterm birth, but it seems to depend on the initial BMI category[35]. Spontaneous preterm birth was associated with insufficient weight gain, but it is usually related with lower prevalence of very and extreme premature than pi-PTB[36]. Therefore, the higher prevalence of sPTB in women with insufficient RWG do not explain higher proportion of very and extreme prematurity. A separate analysis of insufficient weight gain considering the different initial BMI could be useful to determine underlying motivations for this association.

Although our analyses were performed according to international recommendations for RWG, for BMI categories and for preterm subtypes, we identified some potential limitations In order to concretize this analysis, the formula of RWG we used assumes the same weight gain in the first trimester for all women. Although the weight gain recommended by the IOM in the first trimester is the same for all women regardless the initial BMI, having 0.5kg or 2kg of weight gain do could represent substantial difference on the RWG mainly for underweight women. Additionally, we were not able to address another important information as weight loss in the beginning of pregnancy, that happens with a portion of women and affect the first trimester and total weight gain. Another critical point was the fact that only 8.5% of participating women in our study were underweight. The small number of underweight women weakens the analyses, especially when divided in subgroups of PTB or RWG categories. Additionally, the likelihood of underweight women to have excessive weight gain seems to depend on the ethnicity and environmental aspects[10,35,37]. In Brazil, underweight women are more likely to have insufficient weight gain and unlikely to have excessive gain[38]. An excessive weight gain for underweight and obese women might result in different effects[38]. A recent systematic review and meta-

analysis gathering information of women from USA, Europe and Asia showed that ethnicity might play a major role on the association of maternal BMI and gestational weight gain with the risk for preterm birth [39]. Gestational weight gain below IOM recommendation was associated with higher risk for preterm birth in North American and European (OR 1.35 [CI 95% 1.17 - 1.56]), but not in Asian pregnant women (OR 1.06 [CI 95% 0.78 – 1.44]). Authors acknowledge the fact that IOM guidelines may not apply for all populations, as for Asian pregnant women. Specific BMI and weight gain normality and recommended parameters should be established for each population.

Although we have used standard clinical proxies for nutritional status in pregnancy, maternal BMI and weight gain during pregnancy are related to other several aspects of biological, food intake and lifestyle conditions that were not assessed at all. The earliest idea that underweight would be exclusively related with undernutrition and obesity with overconsumption, lately high calories but poor-nutrient food are linked with obesity and low socio-economic status[40].

Initial BMI and weight gain during pregnancy have already been reported as risk factors for neonatal adverse outcomes[41]. We performed a logistic regression analyses to identify whether initial BMI or weight gain during pregnancy have association with any adverse perinatal outcome (APO) in preterm newborns. Nevertheless, no consistent association was observed except know risk factors as multiple pregnancy, fetal anomaly, maternal morbidity and vaginal bleeding.

Despite the limitations of our study, there are great lessons that might be considered in future studies evaluating the burden of initial BMI and weight gain on the risk for PTB: 1) To use the rate of weight gain during pregnancy (per week) and not total weight gain, which does not account for the length of pregnancy; 2) To consider the preterm birth subtypes in separate instead preterm birth as a unique syndrome, as the underlying conditions, motivators and outcomes are not the same; and 3) To use the adequacy of weight gain during pregnancy per initial BMI, once the weight gain might play different effects depending on the initial BMI category. The EMIP study findings highlight the need for further studies and for standardizations on preterm birth assessment of risk considering nutritional status.

MATERIAL AND METHODS

Study design. This is a secondary analysis of data from a multicenter cross-sectional study plus a nested case-control study called EMIP that involved 20 low and high-risk healthcare

facilities in three different geographical regions of Brazil, the Northeast, Southeast and South. The methodological details of EMIP study were already published elsewhere[15,42]. In brief, the data was collected from April 2011 to March 2012, using a form with 306 variables especially developed for this study. All women with preterm birth were identified and invited to participate, including those with multiple pregnancies and stillbirths. The very next woman with term birth after the preterm delivery was invited to participate in the control group, until reaching estimated sample size. In case of non-acceptance, the next was invited. The data collection procedure included an interview with participants until discharge, and a review of the maternal and newborn medical records and prenatal chart. After data was collected for each individual case and the form was completed and checked, information was included in the online database system that used a special platform for clinical studies, the OpenClinica®.

The sample size was calculated using the official prevalence of preterm births in Brazil in 2009 of around 6.5% at the time of the research proposal. Considering an acceptable absolute difference of about 0.25% between the sample and the population prevalence, as well as a type I error of 5%, 37,000 deliveries were necessary to cover for obtaining the sample size. For the case-control component, each group (preterm subtypes and controls) had an estimated sample size of 1,054 women. The samples size was calculated based on the primary objectives of EMIP study as previously published, the current analyses is performed in function of the data collected.

Full ethical approval has been obtained by the National Council for Ethics in Research (CONEP) and by the Institutional Review Board of each participating center. Before enrolment, all individual signed an Informed Consent Form. Several procedures were adopted to ensure high quality of data including preparatory meetings for training assistants and collaborators, development of detailed standard operating procedures manuals (SOP's) explaining how to manage the questionnaire and the database, monitoring site visits, sustained monitoring of data entry by the coordinating center and fast identification and correction of errors. All methods were performed in accordance with the principles stated in the Brazilian National Heath Council (Resolution CNS 196/96) and with local/institutional guidelines and regulations in every stage of this study.

Outcomes and variables. The main outcomes for this analysis are the occurrence of preterm birth, defined as delivery below 37 weeks, due to spontaneous onset of labour (sPTB), pre-labour rupture of membranes (PROM-PTB) or medically indicated because of

maternal or fetal compromise or both (pi-PTB); and term birth, defined as childbirth at or after 37 weeks. The secondary outcomes were the categories of gestational age (<28 weeks, 28-33 weeks and 34-36 weeks of pregnancy)[1], fetal death, and the neonatal outcomes Apgar score <7 at five minutes, admission to neonatal intensive care unit (NICU), neonatal death before discharge and any adverse perinatal outcome (APO: a composite variable defined as the occurrence of any previous neonatal adverse outcomes).

The maternal independent variables were: pre- or early pregnancy BMI, categorized as underweight (<18.5 kg/m^2), normal (18.5-24.99 kg/m^2), overweight (25-29.99 kg/m^2) and obese (≥30 kg/m^2)[22]. The early pregnancy BMI was calculated using the first weight recorded at prenatal chart, since up to 20 weeks of gestation, and measured height. Adequacy of weight gain during pregnancy was based on the rate of weight gain (RWG), calculated using the following formula: RWG= (first maternal weight in pregnancy - last maternal weight)/(gestational age at delivery - 12). The adequacy of weight gain was then categorized as insufficient when RWG <0.44 kg/w for underweight, <0.35 kg/w for normal, <0.23 kg/w for overweight or <0.17 kg/w for obese; adequate when RWG 0.44-0.58 kg/w for underweight, 0.35-0.50 kg/w for normal, 0.23-0.33 kg/w for overweight or 0.17-0.27 kg/w for obese; and excessive when RWG ≥0.58 kg/w for underweight, ≥0.50 kg/w for normal, ≥0.33 kg/w for overweight or ≥0.27 kg/w for obese, according to the pre- or early pregnancy BMI as recommended by the Institute of Medicine (IOM)[22]. The current IOM guidelines recommend the same weight gain during the first trimester despites the categories of BMI. The lower and upper limits to categorize the adequacy of estimated weight gain are narrower as compared to other trimesters. Considering these, we subtracted 12 weeks from the gestational age, considering that there is almost no difference on weight gain through women of different BMI in this period. Moreover, great part of Brazilian women initiates prenatal care after the first trimester.

Statistical analyses. Statistical analyses were conducted to estimate risk for all PTB subtypes, using Odds Ratio (OR) with 95% confidence intervals (CI) for BMI and adequacy of weight gain categories, adjusting for cluster effect design. We estimated the probability for each preterm birth subtype according to initial BMI category and RWG during pregnancy using binary logit analyses optimized by Fisher´s scoring. The dotted lines in each figure of predicted probability delimit the lower and upper limits of recommended weekly RWG according to IOM. The area in orange between the lines, then, shows the recommended RWG for each initial BMI and the upper and lower values are, respectively, considered excessive and insufficient RWG. The occurrence of adverse perinatal outcomes

according to adequacy of weight gain during pregnancy, which is controlled by gestational age, was evaluated by χ^2 tests. Statistical significance was considered when p-value <0.05. Stepwise multiple analysis by non-conditional logistic regression was run to identify factors independently associated with APO in women with preterm birth. To estimate the likelihood of each preterm birth subtype according to early BMI, we dismissed outliers of weight gain, ignoring data of women with weight gain above 99th percentile and below the percentile 1. Statistical analyses were performed using SAS System for Windows (Statistical Analysis System), version 9.4. SAS Institute Inc, 2002-2008, Cary, NC, USA. Institute Inc, 2002-2008, Cary, NC, USA. This manuscript follows STROBE statement[43].

REFERENCES

1. Blencowe, H. *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* **379,** 2162–72 (2012).
2. Torloni, M. R. *et al.* Maternal BMI and preterm birth: a systematic review of the literature with meta-analysis. *J. Matern. Fetal. Neonatal Med.* **22,** 957–70 (2009).
3. World Health Organization (WHO). Make every mother and child count. In: Annual number of deaths by cause for neonates in WHO regions, estimates for 2000-2003. (2005). Available at: http://www.who.int/whr/2005/annex/en/.
4. Ng, M. *et al.* Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **384,** 766–81 (2014).
5. Rahman, M. M. *et al.* Maternal body mass index and risk of birth and maternal health outcomes in low- and middle-income countries: a systematic review and meta-analysis. *Obes. Rev.* **16,** 758–70 (2015).
6. Faucher, M. A., Hastings-Tolsma, M., Song, J. J., Willoughby, D. S. & Bader, S. G. Gestational weight gain and preterm birth in obese women: a systematic review and meta-analysis. *BJOG* **123,** 199–206 (2016).
7. Salihu, H. M., Mbah, A. K., Alio, A. P., Clayton, H. B. & Lynch, O. Low pre-pregnancy body mass index and risk of medically indicated versus spontaneous preterm singleton birth. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **144,** 119–23 (2009).
8. Dietz, P. M. *et al.* Combined effects of prepregnancy body mass index and weight gain during pregnancy on the risk of preterm delivery. *Epidemiology* **17,** 170–7 (2006).
9. Hendler, I. *et al.* The Preterm Prediction Study: association between maternal body mass index and spontaneous and indicated preterm birth. *Am. J. Obstet. Gynecol.* **192,** 882–6 (2005).
10. Carnero, A. M., Mejía, C. R. & García, P. J. Rate of gestational weight gain, pre-pregnancy body mass index and preterm birth subtypes: a retrospective cohort study from Peru. *BJOG* **119,** 924–35 (2012).
11. Nohr, E. A. *et al.* Obesity, gestational weight gain and preterm birth: a study within the Danish National Birth Cohort. *Paediatr. Perinat. Epidemiol.* **21,** 5–14 (2007).
12. Oken, E., Kleinman, K. P., Belfort, M. B., Hammitt, J. K. & Gillman, M. W.

Associations of gestational weight gain with short- and longer-term maternal and child health outcomes. *Am. J. Epidemiol.* **170,** 173–80 (2009).
13. El Rafei, R. *et al.* Association of Pre-Pregnancy Body Mass Index and Gestational Weight Gain with Preterm Births and Fetal Size: an Observational Study from Lebanon. *Paediatr. Perinat. Epidemiol.* **30,** 38–45 (2016).
14. Brazilian Ministry of Health. *SINASC – National Information System on Live Births* (2011). Available at: http://tabnet.datasus.gov.br/cgi/tabcgi.exe?sinasc/cnv/nvuf.def.
15. Passini, R. *et al.* Brazilian Multicentre Study on Preterm Birth (EMIP): Prevalence and Factors Associated with Spontaneous Preterm Birth. *PLoS One* **9,** e109069 (2014).
16. Souza, R. T. *et al.* The Burden of Provider-Initiated Preterm Birth and Associated Factors: Evidence from the Brazilian Multicenter Study on Preterm Birth (EMIP). *PLoS One* **11,** e0148244 (2016).
17. Bailey, K. V & Ferro-Luzzi, A. Use of body mass index of adults in assessing individual and community nutritional status. *Bull. World Health Organ.* **73,** 673–80 (1995).
18. Honest, H. *et al.* The accuracy of maternal anthropometry measurements as predictor for spontaneous preterm birth--a systematic review. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **119,** 11–20 (2005).
19. Parker, M. G. *et al.* Prepregnancy body mass index and risk of preterm birth: association heterogeneity by preterm subgroups. *BMC Pregnancy Childbirth* **14,** 153 (2014).
20. Goldstein, R. F. *et al.* Association of Gestational Weight Gain With Maternal and Infant Outcomes: A Systematic Review and Meta-analysis. *JAMA* **317,** 2207 (2017).
21. Kominiarek, M. A. *et al.* Association Between Gestational Weight Gain and Perinatal Outcomes. *Obstet. Gynecol.* **132,** 875–881 (2018).
22. The National Academies Press. *Institute of Medicine (IOM), National Research Council (NRC). Weight Gain During Pregnancy: Reexamining the Guidelines.* (2009).
23. Salihu, H. M., Luke, S., Alio, A. P., Deutsch, A. & Marty, P. J. The impact of obesity on spontaneous and medically indicated preterm birth among adolescent mothers. *Arch. Gynecol. Obstet.* **282,** 127–34 (2010).
24. Carlson, N. S. *et al.* Parturition dysfunction in obesity: time to target the pathobiology. *Reprod. Biol. Endocrinol.* **13,** 135 (2015).
25. Vahratian, A., Zhang, J., Troendle, J. F., Savitz, D. A. & Siega-Riz, A. M. Maternal prepregnancy overweight and obesity and the pattern of labor progression in term nulliparous women. *Obstet. Gynecol.* **104,** 943–51 (2004).
26. AlSaif, S., Mumtaz, S. & Wray, S. A short review of adipokines, smooth muscle and uterine contractility. *Life Sci.* **125,** 2–8 (2015).
27. Denison, F. C., Price, J., Graham, C., Wild, S. & Liston, W. A. Maternal obesity, length of gestation, risk of postdates pregnancy and spontaneous onset of labour at term. *BJOG* **115,** 720–5 (2008).
28. Blevins, J. E. & Ho, J. M. Role of oxytocin signaling in the regulation of body weight. *Rev. Endocr. Metab. Disord.* **14,** 311–329 (2013).
29. Masho, S. W. *et al.* Pre-pregnancy BMI and weight gain: where is the tipping point for preterm birth? *BMC Pregnancy Childbirth* **13,** 120 (2013).
30. Reilly, J. J. *et al.* Early life risk factors for obesity in childhood: cohort study. *BMJ* **330,** 1357 (2005).

31. Goldenberg, R. L., Culhane, J. F., Iams, J. D. & Romero, R. Epidemiology and causes of preterm birth. *Lancet* **371,** 75–84 (2008).
32. Bloomfield, F. H. How Is Maternal Nutrition Related to Preterm Birth? *Annu. Rev. Nutr.* **31,** 235–261 (2011).
33. Neggers, Y. & Goldenberg, R. L. Some thoughts on body mass index, micronutrient intakes and pregnancy outcome. *J. Nutr.* **133,** 1737S–1740S (2003).
34. Luke, B. The evidence linking maternal nutrition and prematurity. *J. Perinat. Med.* **33,** 500–5 (2005).
35. Johnson, J. *et al.* Pregnancy outcomes with weight gain above or below the 2009 Institute of Medicine guidelines. *Obstet. Gynecol.* **121,** 969–75 (2013).
36. Delorme, P. *et al.* Cause of Preterm Birth as a Prognostic Factor for Mortality. *Obstet. Gynecol.* **127,** 40–8 (2016).
37. Haugen, M. *et al.* Associations of pre-pregnancy body mass index and gestational weight gain with pregnancy outcome and postpartum weight retention: a prospective observational cohort study. *BMC Pregnancy Childbirth* **14,** 201 (2014).
38. Godoy, A. C., Nascimento, S. L. do & Surita, F. G. A systematic review and meta-analysis of gestational weight gain recommendations and related outcomes in Brazil. *Clinics (Sao Paulo).* **70,** 758–64 (2015).
39. Goldstein, R. F. *et al.* Gestational weight gain across continents and ethnicity: systematic review and meta-analysis of maternal and infant outcomes in more than one million women. *BMC Med.* **16,** 153 (2018).
40. Dekker, G. A. *et al.* Risk factors for preterm birth in an international prospective cohort of nulliparous women. *PLoS One* **7,** e39154 (2012).
41. Crane, J. M. G., White, J., Murphy, P., Burrage, L. & Hutchens, D. The effect of gestational weight gain by body mass index on maternal and neonatal outcomes. *J. Obstet. Gynaecol. Canada* **31,** 28–35 (2009).
42. Lajos, G. J. *et al.* Methodological issues on planning and running the brazilian multicenter study on preterm birth. *ScientificWorldJournal.* **2015,** 719104 (2015).
43. von Elm, E. *et al.* The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *PLoS Med.* **4,** e296 (2007).

Funding

This study was supported by Grant from FAPESP (Foundation for Support to Research of the State of Sao Paulo) process number 2009/53245-5, and by CNPq (Brazilian National Research Council) Brazil through the call AP.PPSUS-1. The funders played no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript. The current secondary analyses of EMIP data was designed and developed by part of the Preterm SAMBA study Group (RTS, RPJ and JGC), a collaborative study group on Preterm Birth Research jointly issued by the Brazilian National Research Council (CNPq) and the Bill and Melinda Gates Foundation (Award 401636/2013-5 - Grand Challenges Brazil: Reducing the burden of preterm birth, number 05/2013). The current findings are, then, part of the scope of analyses in prematurity of the Preterm SAMBA study group.

Acknowledgments

The other members of the Brazilian Multicenter Study on Preterm Birth study group: Maria L Costa, Rodolfo C Pacagnella, Samira M Haddad, Sergio T Marba, Ruth Guinsburg, Francisco E Martinez, Vilma Zotarelli, Lucio T Gurgel, Francisco E Feitosa, George N Chaves, Ana M Porto, Isabela C Coutinho, Antonio C Barbosa Lima, Elias F Melo Jr, Débora F Leite, Melania M Amorim, Adriana SO Melo, Fabiana O Melo, Marília G Martins, Marynea V Nunes, Cláudio S Paiva, Moises D Lima, Djacyr M Freire, Edson G Tristão, Denis J Nascimento, Carlos A Menezes, Marcelo Aquino, Janete Vettorazzi, Cintia E Senger, Augusta MB Assumpção, Marcela AF Guedes, Maria EL Moreira, Vera T Borges, Nelson L Maia Filho, Jacinta P Mathias, Eduardo Souza, Ana CP Zamarian, Silvana M Quintana, Patrícia PS Melli, Fátima A Lotufo, Kaliane Uzilin, Elvira A Zanette, Carla B Andreucci, Tenilson A Oliveira, Laércio R Oliveira, Marcos A N Santos, Nelson Sass, Mirian RF Silveira, Pedro R Coutinho, Luciana Siqueira.

Competing Financial Interests

All the authors report no conflict of interest at all.

Author Contributions

Conceived and designed the experiments: FPS RTS RPJ JGC RPT GJL MLN PMR TZD. Performed the experiments: RTS RPJ JGC RPT GJL MLN PMR TZD. Analyzed the data: FPS RTS RPJ JGC RPT PFO CMS. Contributed reagents/materials/analysis tools: FPS RTS RPJ JGC RPT GJL MLN PMR TZD PFO CMS. Wrote the paper: FPS RTS RPJ JGC RPT. Read and approved the final version of the manuscript: all the authors of the Brazilian Multicenter Study on Preterm Birth study group Consortium.

Data Availability Statement: All relevant data are within the paper, and the authors can make available materials, data and associated protocols if requested.

Brazilian Multicenter Study on Preterm Birth study group Consortium:

Maria L Costa[1], Rodolfo C Pacagnella[1], Samira M Haddad[1], Vilma Zotarelli[1], Lucio T Gurgel[1], Nelson L Maia Filho[2], Jacinta P Mathias[2], Sergio T Marba[3], Ruth Guinsburg[4], Francisco E Martinez[5], Silvana M Quintana[5], Patrícia PS Melli[5], Francisco E Feitosa[6], George N Chaves[6], Ana M Porto[7], Isabela C Coutinho[7], Antonio C Barbosa Lima[8], Elias F Melo Jr[8], Débora F Leite[8], Melania M Amorim[9], Adriana SO Melo[9], Fabiana O Melo[9], Marília G Martins[10], Marinea V Nunes[10], Cláudio S Paiva[11], Moises D Lima[11], Djacyr M Freire[11], Edson G Tristão[12], Denis J Nascimento[12], Carlos A Menezes[13], Marcelo Aquino[13], Janete Vettorazzi[14], Cintia E Senger[14], Augusta MB Assumpção[15], Marcela AF Guedes[15], Maria EL Moreira[15], Vera T Borges[16], Eduardo de Souza[17], Ana CP Zamarian[17], Fátima A Lotufo[18], Kaliane Uzilin[18], Elvira A Zanette[19], Carla B Andreucci[19], Tenilson A Oliveira[20], Laércio R Oliveira[20], Marcos A N Santos, Nelson Sass[21], Mirian RF Silveira[21], Pedro R Coutinho[22], Luciana Siqueira[22].

[1] Department of Obstetrics and Gynecology, School of Medical Sciences, University of Campinas (UNICAMP), Campinas, SP, Brazil.

[2] Department of Obstetrics and Gynecology, Jundiaí Medical School, Jundiaí, SP, Brazil.

[3] Department of Pediatrics, School of Medical Sciences, University of Campinas (UNICAMP), Campinas, SP, Brazil.

[4] Division of Neonatal Medicine, Escola Paulista de Medicina (EPM), Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brazil.

[5] Faculty of Medicine Ribeirão Preto, Universidade de São Paulo, São Paulo, Brazil.

[6] Maternity School, Assis Chateaubriand, Fortaleza, Ceará, Brazil.

[7] Instituto de Medicina Integral Prof. Fernando Figueira, Recife, Pernambuco, Brazil.

[8] Department of Mother and Child Health, School of Medicine, Federal University of Pernambuco, Recife, Brazil.

[9] Instituto de Saúde Elpidio de Almeida, Campina Grande, Paraíba, Brazil.

[10] Departmanet of Gynecology and Obstetrics, Federal University of Maranhão.(UFMA), São Luiz, MA, Brazil.

[11] Federal University of Paraíba, João Pessoa, Paraíba, Brazil.

[12] Federal University of Paraná, Curitiba, Paraná, Brazil.

[13] Federal University of Bahia, Salvador, Bahia, Brazil.

[14] Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

[15] Instituto Fernandes Figueira, Rio de Janeiro, RJ, Brazil.

[16] Department of Gynecology and Obstetrics, São Paulo State University, Botucatu, São Paulo, Brazil.

[17] Department of Obstetrics, Paulista School of Medicine, Federal University of São Paulo (EPM-UNIFESP), São Paulo - SP , Brazil.

[18] Santa Casa de Limeira Hospital, Limeira, SP, Brazil.

[19] Santa Casa de São Carlos Hospital, São Carlos, SP, Brazil.

[20] Maternity Casa Maternal Leonor Mendes de Barros, São Paulo, SP, Brazil.

[21] Maternity Escola de Vila Nova Cachoeirinha, São Paulo, SP, Brazil.

[22] Hospital Estadual de Sumaré, Sumaré, SP, Brazil.

Figure Legends:

Figure 1. Flow chart of participants in the study according to the adequacy of weight gain (WG)

Figure 2. Probability of different types of preterm birth for underweighted women according to weight gain rate

Figure 3. Probability of different types of preterm birth for women with normal weight according to weight gain rate

Figure 4. Probability of different types of preterm birth for overweighed women according to weight gain rate

Figure 5. Probability of different types of preterm birth for obese women according to weight gain rate

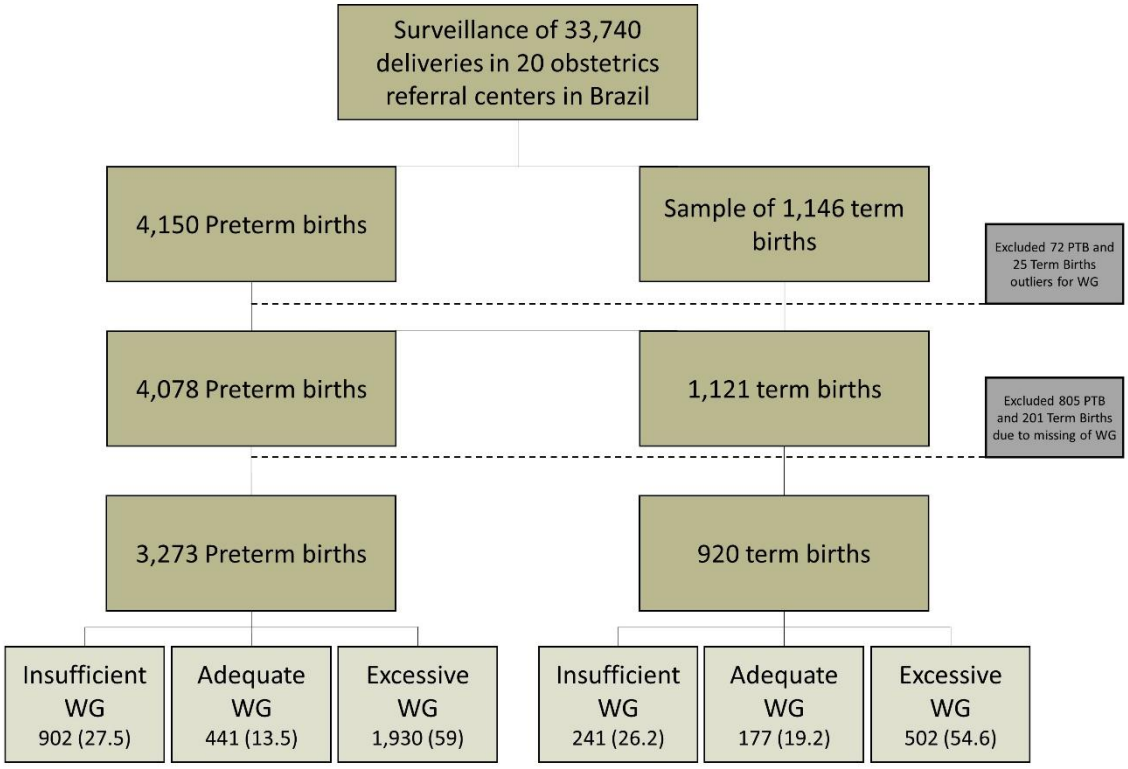


Figure 1. Flow chart of participants in the study according to the adequacy of weight gain (WG)

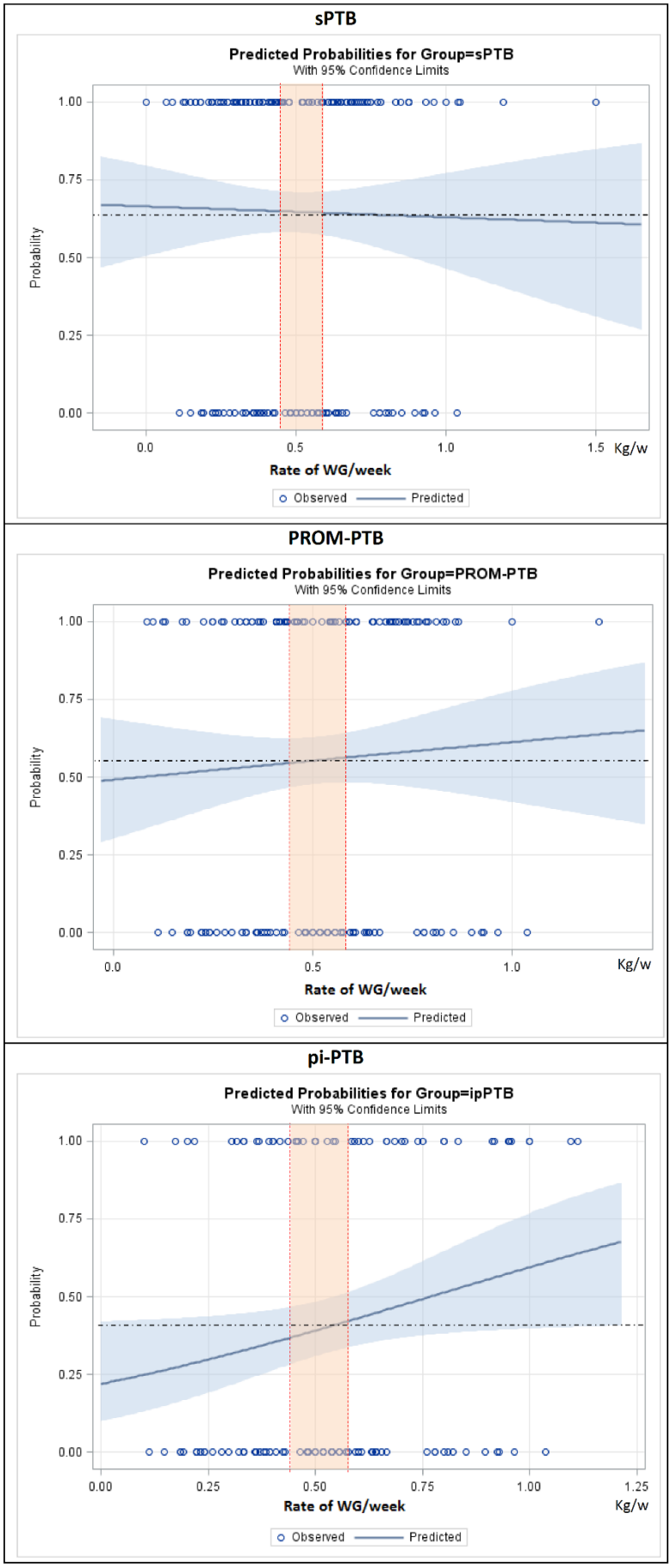


Figure 2. Probability of different types of preterm birth for underweighted women according to weight gain rate

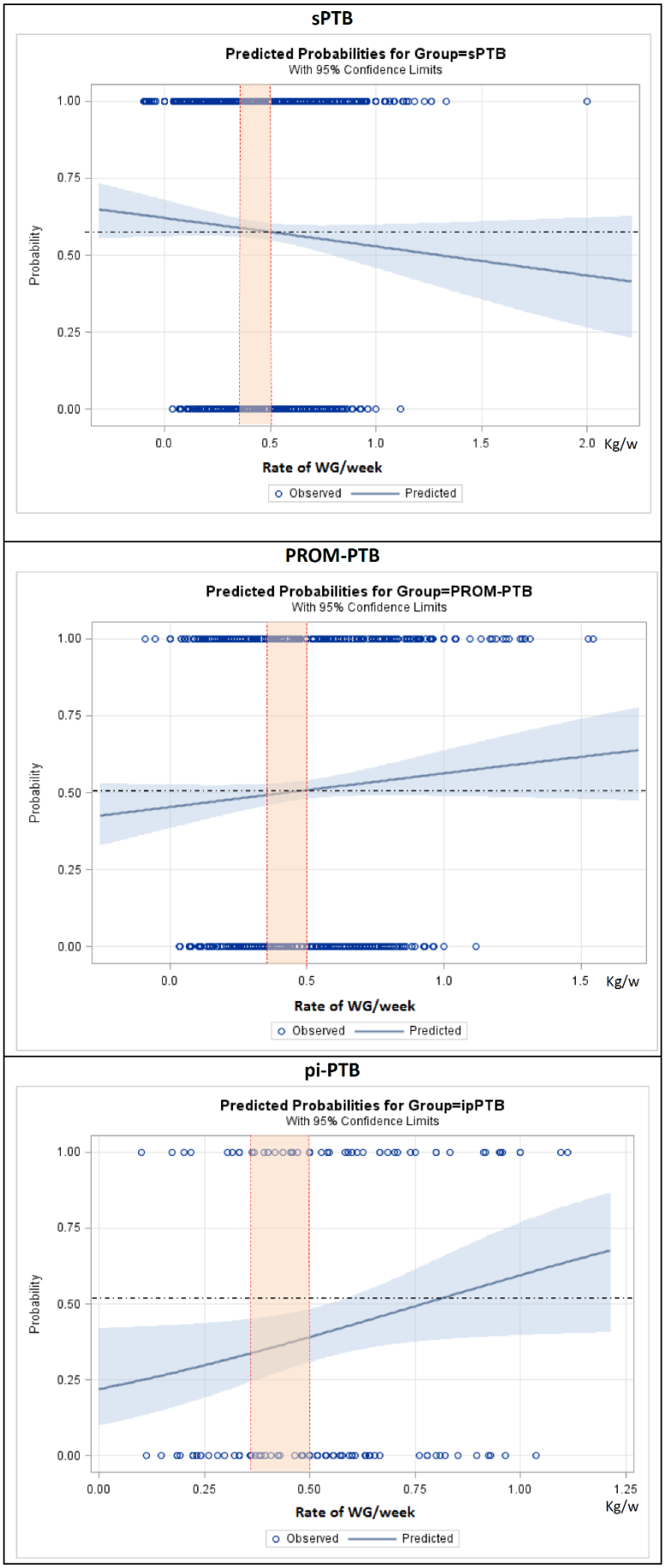


Figure 3. Probability of different types of preterm birth for women with normal weight according to weight gain rate

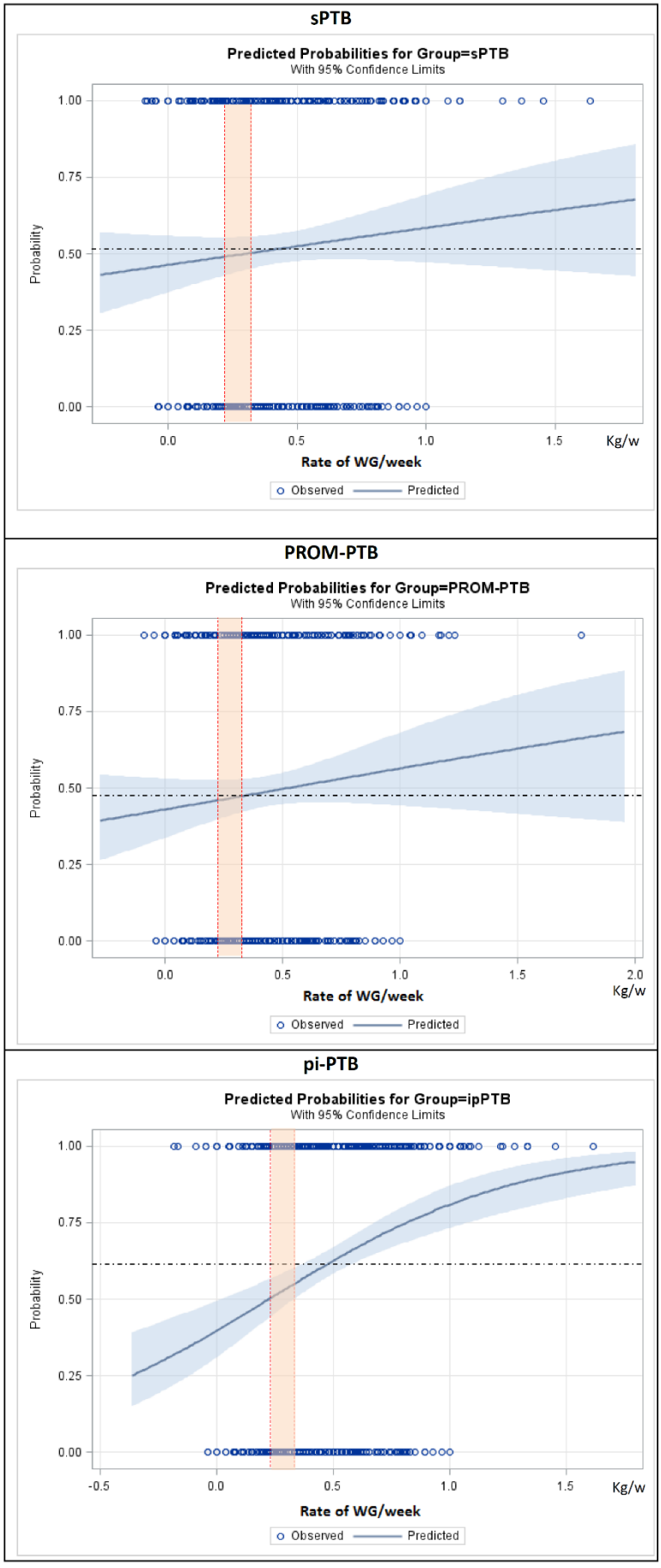


Figure 4. Probability of different types of preterm birth for overweighed women according to weight gain rate

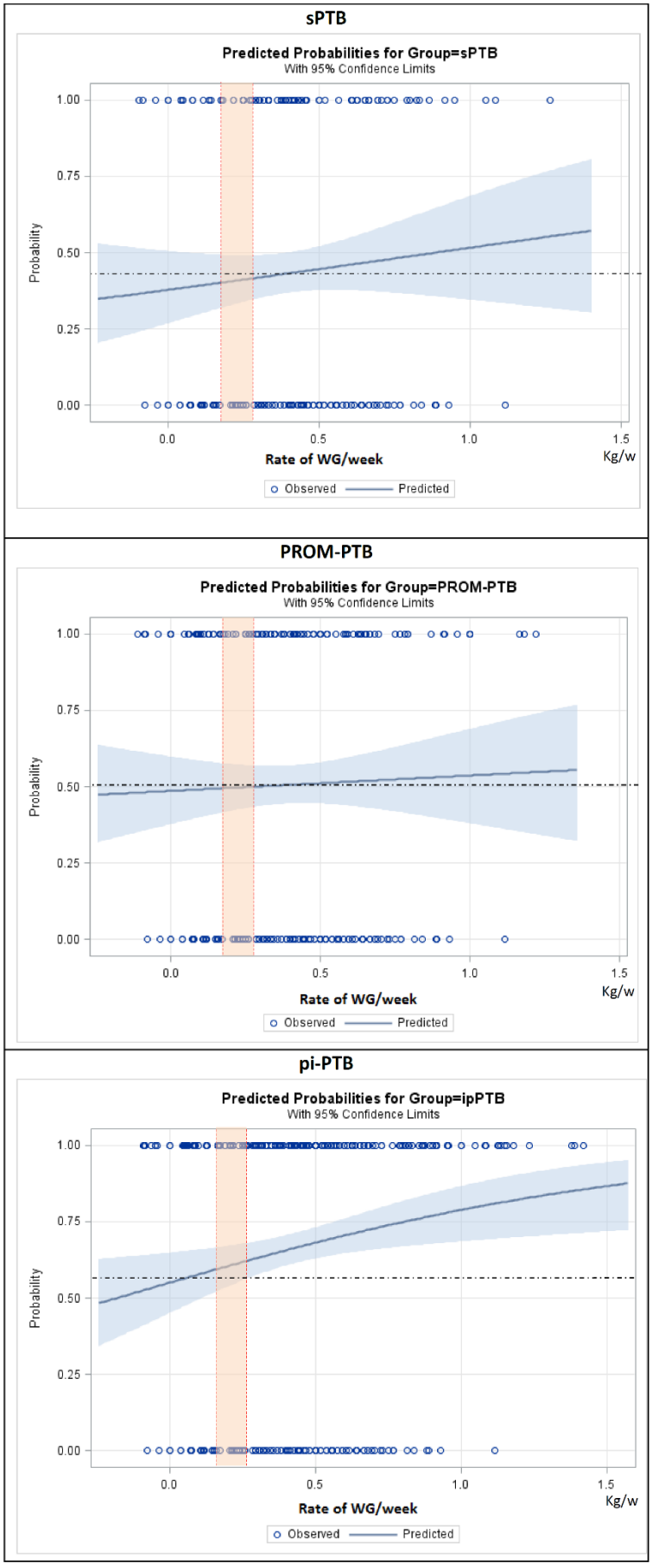


Figure 5. Probability of different types of preterm birth for obese women according to weight gain rate

Table 1. Risk estimates of different subtypes of preterm birth according to maternal initial body mass index (BMI) and adequacy of weight gain

	sPTB	OR* (95% CI)	PROM-PTB	OR* (95% CI)	pi-PTB	OR* (95% CI)	Term birth
Initial Body Mass Index [a]							
Underweight	144 (11.5)	1.26 [0.93-1.70]	100 (9.8)	1.16 [0.84-1.60]	56 (4.5)	**0.62 [0.43-0.89]**	82 (8.3)
Normal	782 (62.6)	1	577 (56.6)	1	604 (48.2)	1	565 (57.4)
Overweight	226 (18.1)	**0.74 [0.59-0.92]**	210 (20.6)	0.92 [0.73-1.16]	338 (27.0)	**1.43 [1.16-1.77]**	209 (21.2)
Obese	98 (7.8)	**0.54 [0.40-0.72]**	132 (12.9)	0.99 [0.75-1.31]	254 (20.3)	**1.76 [1.37-2.26]**	129 (13.1)
Adequacy of Weight Gain [b]							
Insufficient	401(34.5)	**1.76 [1.34-2.31]**	271 (28.6)	**1.54 [1.15-2.06]**	230 (19.8)	1.28 [0.95-1.71]	241 (26.2)
Adequate	172 (14.8)	1	133 (14.0)	1	136 (11.7)	1	177 (19.2)
Excessive	590 (50.7)	1.19 [0.93-1.53]	543 (57.3)	**1.45[1.11-1.88]**	797 (68.5)	**2.01 [1.56-2.59]**	502 (54.6)
Total	**1,470**		**1,173**		**1,435**		**1,121**

OR*: Odds ratio adjusted for the cluster effect design in comparison with term birth group. **CI:** Confidence interval.
sPTB: Spontaneous preterm birth. **PROM-PTB:** preterm premature rupture of membranes. **pi-PTB:** provider-initiated preterm birth.
Missing information for: a) 693 b) 1006 cases.
Values in bold mean they are statistically significant.

Table 2. Perinatal outcomes according to the adequacy of weight gain during pregnancy in preterm births

Perinatal outcomes	Adequacy of Weight Gain			
	Insufficient	**Adequate**	**Excessive**	**p-value**
Gestational age [n=3273]				**<.0001**
<28 weeks	65 (7.2)	23 (5.2)	134 (3.2)	
28-33 weeks	261 (28.9)	108 (24.5)	562 (23.9)	
34-36 weeks	576 (63.9)	310 (70.3)	1234 (72.9)	
Apgar score <7 at 5 minutes [n=3234]	96 (10.8)	30 (6.9)	179 (9.4)	0.0511
NICU admission[n=3060]	653 (64.3)	602 (57.2)	598 (60.3)	**0.0045**
Fetal death [n=3273]	41 (6.4)	11 (2.9)	71 (2.1)	0.1714
Neonatal death before discharge [n=3127]	71 (8.3)	25 (5.9)	139 (7.5)	0.2996
Any adverse perinatal outcome (APO)* [n=3273]	522 (57.9)	233 (52.9)	1133 (58.7)	0.0786

*Any adverse perinatal outcome (APO): Apgar score <7 at 5 minutes **or** NICU admission **or** neonatal death before discharge.
P-values in bold mean they are statistically significant.

Table 3. Variables independently associated with any adverse perinatal outcome (APO) in women with all types of preterm births: stepwise multiple analyses by non-conditional logistic regression

Any preterm birth [n=3040]	**OR_{adj}**	**95% CI**	**p-value**
Fetal malformation	6.73	4.85 - 9.34	<.0001
Vaginal bleeding	1.42	1.19 – 1.71	0.0001
Maternal morbidity	1.31	1.12 – 1.55	0.0010
Multiple pregnancy	1.42	1.09 – 1.84	0.0079
Spontaneous preterm birth [n=1093]			
Fetal malformation	8.35	4.75 – 14.67	<.0001
Multiple pregnancy	1.88	1.28 – 2.77	0.0013
Vaginal bleeding	1.61	1.20 – 2.17	0.0016
Number of C-section	0.77	0.61 – 0.97	0.0281
Preterm birth due to PROM [n=898]			
Fetal malformation	7.72	4.13 – 14.46	<.0001
Vaginal bleeding	1.65	1.16 – 2.28	0.0047
Number of C-section	1.47	1.11 – 1.95	0.0068
Provider-initiated preterm birth [n=1049]			
Fetal malformation	5.01	2.87 – 8.74	<.0001
Initial BMI	0.97	0.95 – 0.99	0.0178

OR_{adj}: Odds ratio adjusted the cluster effect design and for all predictors in this final model; **CI:** confidence interval of OR; **p:** p-value. **Predictors entering the model:** maternal age, parity, number of previous vaginal birth, number of previous cesarean sections, number of abortion (nulliparous: 0/ ≥1: 1); schooling (≥12:0, <12:1), adequacy of weight gain during pregnancy (Adequate:0/Insufficient or Excessive:1); initial BMI (kg/m2); maternal morbidity* (no: 0/ yes: 1); vaginal bleeding (yes: 1/ no: 0) multiple pregnancy (yes: 1/ no: 0); fetal malformation (yes: 1/no: 0).

*Defined as having any of the following: anemia, chronic hypertension, pre-pregnancy diabetes, gestational diabetes, gestational hypertension, preeclampsia/eclampsia/HELLP, hypo/hyperthyroidism, HIV, cardiac disease, renal disease, lung diseases, epilepsy, systemic lupus erythematosus or thrombophilia/thrombosis.

4.2. Artigo *Cluster Analysis to Identify Clinical Phenotypes of Preterm Birth: Maternal and Neonatal outcomes from the Brazilian Multicentre Study on Preterm Birth*

cecatti@unicamp.br

De: em.ijg.0.5dd414.a40dfbc7@editorialmanager.com em nome de International Journal of Gynecology & Obstetrics <em@editorialmanager.com>
Enviado em: segunda-feira, 10 de setembro de 2018 16:10
Para: Jose Guilherme Cecatti
Assunto: Submission Confirmation

09-10-2018

Jose Guilherme Cecatti, PhD
Full Professor of Obstetrics
Department of Gynecology and Obstetrics
University of Campinas
Department of Gynecology and Obstetrics
R. Alexander Fleming, 101
Cidade Universitaria Zeferino Vaz
Campinas, SP 13083881
BRAZIL

cecatti@unicamp.br

Dear Prof. Cecatti:

We are pleased to acknowledge receipt of the following manuscript:

Cluster Analysis IdentifyING Clinical Phenotypes of Preterm Birth: Maternal and Neonatal outcomes from the Brazilian Multicentre Study on Preterm Birth Clinical Article

It has been forwarded for early review and consideration for publication in the International Journal of Gynecology and Obstetrics. When the editorial review is completed we shall inform you of our decision.

You will be able to check on the progress of your paper by logging in to Editorial Manager as an author. The URL is https://ijg.editorialmanager.com/.

Your manuscript will be given a reference number shortly.

We thank you for your patience and we appreciate the opportunity to review this manuscript.

Please proceed to the following link to update your personal classifications and keywords, if necessary:
https://ijg.editorialmanager.com/l.asp?i=164208&l=AWY3NID1

Sincerely,

IJGO Editorial Office
International Journal of Gynecology and Obstetrics Follow on Twitter: @IJGOLive

If you would like your personal information to be removed from the database, please contact the publication office.

CLINICAL ARTICLE

Cluster Analysis to Identify Clinical Phenotypes of Preterm Birth: Maternal and Neonatal outcomes from the Brazilian Multicentre Study on Preterm Birth

Authors

Renato T Souza[1], Jose G Cecatti[1], Renato Passini Jr[1], Rodolfo C Pacagnella[1], Paulo F Oliveira[2], Cleide M Silva[2], for the Brazilian Multicentre Study on Preterm Birth study group[3]

Affiliations

[1] Department of Obstetrics and Gynaecology, University of Campinas (UNICAMP) School of Medicine, Campinas, SP, Brazil

[2] Unit of Statistics, School of Medical Sciences, University of Campinas (UNICAMP), Campinas, Brazil

[3] Membership of the Brazilian Multicentre Study on Preterm Birth study group is provided in the Acknowledgments.

Corresponding Author

JG Cecatti

DO&G

University of Campinas

R. Alexander Fleming, 101

13083-881 Campinas, SP, Brazil

E-mail: cecatti@unicamp.br

Keywords: preterm birth, phenotypes, cluster, k-means

Synopsis: Clustering analysis showed that different subtypes of preterm delivery has different maternal and pregnancy characteristics, and different clusters had similar maternal or perinatal outcomes.

Word count: 2,455 words (main text).

Abstract

Objective: Exploring a conceptual framework of clinical conditions associated with preterm birth by cluster analysis, assessing determinants for different subtypes of preterm birth and related maternal and neonatal outcomes.

Methods: Secondary analyses of the Brazilian Multicentre Study on Preterm Birth study. A surveillance of 33,740 births was held from April 2011 to July 2012 in 20 maternities, identifying 4,150 preterm deliveries. An adapted k-means model and Fuzzy algorithm was used to identify the clusters, using predefined conditions. The mains outcomes were clusters and maternal and neonatal outcomes.

Results: Three clusters of preterm birth phenotypes were identified: Cluster 1 with women who had preterm delivery but had not had any of the predefined conditions, Cluster 2 characterized by mixed conditions, and Cluster 3 that is mostly comprised of women who had preeclampsia/eclampsia/HELLP syndrome and fetal growth restriction. The prevalence of the different preterm subtypes was statistically different through the 3 clusters, 80% of provider-initiated preterm birth in Cluster 3 to 6.62% in Cluster 1. Although some maternal characteristics were different between clusters, maternal and neonatal outcomes did not differ.

Conclusions: The analysis showed three clusters with distinct phenotypes. Women from the different clusters had different subtypes of preterm delivery and maternal and pregnancy characteristics.

Introduction

The limitations of current predictive algorithms reflect the multifactorial nature of spontaneous preterm birth (sPTB), and the need to apply new strategies that can identify specific groups at risk. Known risk factors can play different role in distinct subgroups of women [1]. There might have different pathways and complex interactions of conditions related with sPTB occurrence. A group of specialists proposed a new conceptual framework for preterm birth, selecting conditions presented in the index pregnancy based on maternal, fetal and placental conditions that are not necessarily risk factors for preterm birth, but that are reasonably part of its pathways [2, 3]. Possibly, there is not only one clinical phenotype related to preterm birth and the identification of those phenotypes might contribute to bring to light the complex interactions of underlying conditions related its occurrence.

A recent clustered designed analysis using an multi-ethnic international multicentre study showed that 30% of all spontaneous preterm births do not have any maternal, fetal or placental conditions identified that could be related to the occurrence [4]. On the other hand, there were clusters such as pre-eclampsia and preterm antepartum stillbirth that showed high correlation with women who had pre-eclampsia and severe maternal conditions, respectively. Also, it was possible to specify the most frequent clinical conditions related to its occurrence. Furthermore, not only the predisposing causes were shown to be different in the different clusters, but the maternal and neonatal outcomes were also distinct [4]. A secondary analysis using a database of spontaneous preterm birth cases performed a different clustering approach, establishing 9 clinical phenotypes divided in 3 levels of evidence for each [5]. After a hierarchical cluster analyses,

preterm birth cases were grouped into 5 clusters characterized by different conditions as maternal stress, premature rupture of membranes, familial factors, maternal morbidities and multifactorial. According to the authors, women from the same cluster are more likely to share common causes and common genetic predispositions [5].

In the current analyses, we aim to perform a secondary analysis of The Brazilian Multicenter Study on Preterm Birth (EMIP) to identify if there is a clustering correlation of clinical, maternal and fetal conditions with preterm birth subtypes and demonstrate maternal and neonatal outcomes related to the final clusters. Clustering analysis applied to preterm birth determinants is an innovative approach to identify groups of women that would require special attention, interventions and surveillance depending on the conditions associated with the different subtypes of preterm birth and also the maternal and perinatal outcomes. This is expected to be helpful for the identification of clinical phenotypes related to specific subtypes of preterm birth and, therefore, better study its determinants and associated outcomes as those maternal clinical conditions can be identified by clinicians and health care providers during antenatal care.

Methods

We performed a secondary cluster analysis using data of EMIP study, a multicentre cross-sectional study with a nested case-control component of preterm birth conducted between April 2011 and September 2012 that collected comprehensive data related to the 3 subtypes of preterm births in 20 referral maternities in 3 regions of Brazil [6–8]. Briefly, EMIP study was a comprehensive observational study that identified all preterm births that occurred in the referral

facilities among more than 33 thousand births, as showed in Figure 1, collecting more than 300 variables related to potential associated factors and maternal and neonatal outcomes. Information about medical history, sociodemographic status and pregnancy, delivery and postpartum details were retrospectively collected after childbirth through an interview with the participating women and review of the medical record that included prenatal chart and hospital medical record. Maternal and neonatal data were collected until discharge or 40 days after birth.

All participating women signed an informed consent form. The ethical principles stated in the Brazilian National Heath Council (Resolution CNS 196/96) were respected. The study also complies with the Declaration of Helsinki amended in Hong Kong in 1989. The study was previously approved by the local IRB of the coordinating centre, by each local IRB of all participating centres and by the National Ethics Committee for Research (CONEP).

We adapted the concept framework and maternal, fetal and placental conditions used by Barros *et al*, defining them as potential conditions direct or indirectly related with the occurrence of preterm birth (Table 1) [3, 4]. Those conditions were used to establish different preterm phenotypes.

Preterm birth was classified as spontaneous preterm birth (sPTB), due to spontaneous onset of labour, premature rupture of membranes preterm birth (PROM-PTB) or provider-initiated preterm birth (pi-PTB), due to maternal and/or fetal conditions motivating preterm delivery.

Maternal and neonatal outcomes as mode of delivery, gestational age category (extreme preterm, moderate preterm and late preterm), Apgar score <7 at 5 minutes, admission to neonatal intensive care unit (NICU), neonatal near miss

(using the pragmatic criteria defined as having birth weight below 1700 g or Apgar score below 7 at 5 minutes of life or gestational age below 33 weeks) and neonatal death before discharge were distributed according to the clusters. Some maternal and pregnancy characteristics were also addressed according to preterm birth clusters. Adequacy of weight gain was categorized as insufficient, adequate and excessive according to Institute of Medicine definition for weekly rate of weight gain [9].

Statistical Analysis

A cluster analysis was conducted to identify the clusters, according to the predefined maternal, fetal and placental conditions showed on Table 1. A k-modes model, variation of k-means model for categorical variables, was applied to identify the clusters from the predefined conditions using a Fuzzy algorithm. The number of final clusters was determined by automatized methods (no predefined number of clusters was set). $\chi 2$ tests were used to evaluate significant difference of maternal and neonatal outcomes between clusters. All analysis were conducted using SAS software, version 9.4.

Results

Preterm birth cases were clustered into 3 clusters according to the 12 maternal, fetal and placental predefined conditions (Table 1). The prevalence of the main condition and the subsequent other most frequent conditions in the 3 clusters are presented in Table 2. Cluster 1 represents 15.7% of all women who had preterm birth in EMIP study and is characterized by women who did not had any maternal, fetal and/or placental defined conditions. Cluster 2 comprises 55.9% of all preterm births, being characterized by a set of conditions: 42.5% of women

of this cluster had extra uterine infection, 34.9% had maternal chronic disease and approximately 20% had mid-late pregnancy bleeding. All women who had clinical chorioamnionitis, almost 90% who had antepartum stillbirth and more than 80% who had multiple pregnancy were in the cluster 2 (Table 2 and Table 3). Cluster 3 comprises 28.4% of preterm cases of which 85% had preeclampsia/eclampsia/HELLP syndrome and 32% had fetal growth restriction.

Table 3 shows the distribution of the 11 predefined conditions in the 3 clusters, detailing the prevalence and concentration of a given condition in the clusters. Although only 7.46% of women in cluster 2 had clinical chorioamnionitis, all women with clinical chorioamnionitis were clustered in cluster 2.

The preterm birth subtype was statistically different according to the cluster as demonstrated in Table 4. More than 90% of women of Cluster 1 had sPTB or pPROM-PTB. The proportion of women with pi-PTB was slightly higher in women of cluster 2 (20%) and much higher in cluster 3 (80.9%).

The maternal and neonatal outcomes did not differ between clusters as shown in Table 5. Caesarean section was the most prevalent mode of delivery, ranging from 52.7% to 55% of preterm births.

Table 6 shows maternal and pregnancy characteristics according to the different clusters. White women, obesity (BMI >25), excessive weight gain during pregnancy and previous C-section were more prevalent in Cluster 3 than in 2, and more prevalent in Cluster 2 than in 1. All the other characteristics were not statistically different between clusters.

Discussion

Main findings

The 4,150 preterm births of EMIP study were clustered into only 3 clusters, which are presented with very different clinical conditions (phenotypes): the first one with no conditions associated, the second with mixed conditions and the third related with preeclampsia and fetal growth restriction. No differences in maternal and perinatal outcomes were observed between clusters, except for subtype of preterm birth showing a prevalence of pi-PTB significantly higher in cluster 3.

Strengths and Limitations

We performed an unsupervised data-driven cluster analysis, which means that we neither predefined pre-clusters nor established the initial number of clusters. This methodological approach enables a more genuine clustering of the cases according to the predefined clinical conditions. The reproducibility of cluster analysis might depend on the dataset, and also on the availability definition of clinical conditions. Nevertheless, we consider that the selected clinical conditions are reproducible and common conditions addressed in preterm birth studies, potentially available regardless the setting or population. The EMIP study followed standardized data collection protocols and several procedures to assure data quality [10]. Nevertheless, the study presents some limitations: 1) the absence of data regarding cervical length, a maternal condition highly associated with the occurrence of spontaneous preterm birth [11]; 2) it was an observational study with retrospective data collection after childbirth for variables related with pregnancy. Therefore, definition for some conditions was based only on the report of participating women or on medical record/prenatal chart, limiting the standardization and audit. 3) the definition of maternal chronic disease was based on different diseases with potentially distinct effects for maternal and fetal health during pregnancy.

Interpretation

We have adapted the conceptual framework used by Barros *et al*. to determine the predefined conditions potentially associated with PTB [4]. We have identified a much smaller number of clusters, indicating that the final number of clusters might depend on the criteria for predefined conditions and on the clustering method. In our analyses, the number of clusters was set by the model, avoiding external adjustments, while Barros *et al*. preferred to use a 2-step cluster analysis, which enabled the development of preclusters and the adjustment of the final clusters. We consider that the different methodological approaches might play a significant role on the different findings.

The new conceptual framework still requires validation and possibly new conditions need to be added in the model. For instance, information on cervical length was unavailable for Barros *et al*. analysis, and less than 5% of women in our study had cervical length at 20-24w recorded (data not shown). We consider this is an important condition to be addressed, as also maternal anthropometric status at the beginning of pregnancy.

The proportion of women in Cluster 1 (without any predefined condition) is meaningful. Exactly 15% of all women with preterm birth did not have any of the twelve conditions potentially associated with PTB. The prevalence of women without any conditions was even higher (30%) in the multicountry population-based study of Barros *et al* [4]. Clinical and epidemiological data seems to have limited performance in recognizing conditions related to the occurrence of preterm birth [12, 13].

The mechanisms of preterm and term labour are not completely understood. The proportion of women that had pi-PTB or sPTB/pPROM-PTB in the different clusters was statistically different. The analysis of clinical characteristics of women from different clusters was an effort to explain why. Women in cluster 1 had low rate of pi-PTB. The absence of maternal morbidity or any other related conditions in this cluster could indicate that spontaneous preterm birth due to spontaneous onset of labour or pPROM often are presented without any maternal obstetric condition as a background, confirming the great challenge of recognizing the mechanisms of the determinism of preterm labour or pPROM. Esplin *et al*. performed a cluster analysis of 1,028 women with preterm birth, and showed one cluster with strong familial history of PTB that might have genetic contribution based on insulin gene analysis [5]. The identification of specific groups of women sharing common genetic and clinical conditions might provide better understanding of complex interaction of different biological systems related with preterm birth (maternal, fetal and placental) [2, 3, 14].

Cluster 2 is characterized by women with mixed conditions (extrauterine infection, maternal morbidity, clinical chorioamnionitis, vaginal bleeding during pregnancy and multiple pregnancy). Although all the conditions are known risk factors for preterm birth, it is difficult to determine the role of each condition in the occurrence of preterm birth in this cluster. Almost 80% of women in the mixed conditions cluster (cluster 2) had sPTB or pPROM-PTB, confirming that women with this subtype of preterm birth may have a multiplicity of conditions which cluster analysis resulted into inseparable group – in contrast with Barros *et al* findings.

Not surprisingly women clustered with preeclampsia/eclampsia/HELLP syndrome also presented fetal growth restriction as a secondary most frequent

condition (Cluster 3). Both conditions are “Great Obstetrical Syndromes” that are directly linked with ischemic placental disease, sharing common altered placentation mechanisms [15, 16]. Hypertensive disorders and fetal growth restriction are the most important causes motivating pi-PTB due to maternal or fetal conditions [8, 17], what explains the high rates of pi-PTB in cluster 3. The prevalence of obesity and excessive weigh gain during pregnancy were higher in cluster 3 than in the other clusters. Both conditions are considered risk factors for hypertensive disorders, but not for fetal growth restriction [18]. It is estimated that only around 12% of ischemic placental disease in preterm births are presented with PE and fetal growth restriction [19]. Although there is a concurrence of PE and fetal growth restriction, which is followed by poorer outcomes, the risk factors and conditions associated with each condition are not invariably overlapped [19, 20].

Although we have identified 3 different clusters with very distinct clinical phenotypes, we consider that a better definition of predefined conditions can provide better description of the clusters, as we had only three clusters and one of them was presented with multiple mixed conditions. Esplin *et al*. proposed a score discriminating clinical conditions according the different level of evidence of the association with preterm birth (possible, moderate and strong) [5]. The idea seems to be to refine the presentation of the clinical phenotype of each cluster. Data mining could be another helpful clustering technique to determine clinical conditions and the correspondent clusters. This approach is used to interpret big data in complex syndromes with multiple interaction systems as genome data [21–24]. The combination of phenotype clusters with biological markers could be an innovative initiative to study and predict preterm birth.

In conclusion, the three clusters showed different phenotypes of women: women without any predefined conditions, women with mixed conditions and women with hypertensive disorders in pregnancy and fetal growth restriction. Although the maternal and neonatal outcomes were not different, women of different clusters had different subtypes of preterm delivery. Standardized methods and larger datasets might provide more reliable and helpful findings that could contribute with the study of preterm birth phenotypes.

Acknowledgements
The other members of the Brazilian Multicenter Study on Preterm Birth study group: Ricardo P Tedesco, Giuliane J Lajos, Marcelo L Nomura, Patricia M Rehder, Tabata Z Dias, Maria L Costa, Samira M Haddad, Sergio T Marba, Ruth Guinsburg, Francisco E Martinez, Vilma Zotarelli, Lucio T Gurgel, Francisco E Feitosa, George N Chaves, Ana M Porto, Isabela C Coutinho, Antonio C Barbosa Lima, Elias F Melo Jr, Débora F Leite, Melania M Amorim, Adriana SO Melo, Fabiana O Melo, Marília G Martins, Marynea V Nunes, Cláudio S Paiva, Moises D Lima, Djacyr M Freire, Edson G Tristão, Denis J Nascimento, Carlos A Menezes, Marcelo Aquino, Janete Vettorazzi, Cintia E Senger, Augusta MB Assumpção, Marcela AF Guedes, Maria EL Moreira, Vera T Borges, Nelson L Maia Filho, Jacinta P Mathias, Eduardo Souza, Ana CP Zamarian, Silvana M Quintana, Patrícia PS Melli, Fátima A Lotufo, Kaliane Uzilin, Elvira A Zanette, Carla B Andreucci, Tenilson A Oliveira, Laércio R Oliveira, Marcos A N Santos, Nelson Sass, Mirian RF Silveira, Pedro R Coutinho, and Luciana Siqueira.

Competing Interest

All the authors report no conflict of interest at all.

Author Contributions

RTS: Data collection, conceived and designed secondary data analysis, read, reviewed and approved the final version of the manuscript.

JGC: Protocol/project development, data collection, conceived and designed secondary data analysis, read, reviewed and approved the final version of the manuscript.

RPJ: Protocol/project development, data collection, conceived and designed secondary data analysis, read, reviewed and approved the final version of the manuscript.

RCP: Protocol/project development, data collection, conceived and designed secondary data analysis, read, reviewed and approved the final version of the manuscript.

PFO: Data analysis, read, reviewed and approved the final version of the manuscript.

CMS: Data analysis, read, reviewed and approved the final version of the manuscript.

Brazilian Multicenter Study on Preterm Birth study group: protocol/project development, data collection.

Funding

This study was supported by a Grant from the CNPq (Brazilian National Research Council) through the call AP.PPSUS-1and by FAPESP (Foundation for Support to Research of the State of Sao Paulo) process number 2009/53245-5. The funders played no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript. The current secondary analyses of EMIP data was designed, sponsored and developed as part of the Preterm SAMBA study Group (RTS, JGC and RPJ), a collaborative study group on Preterm Birth Research jointly issued by the Brazilian National Research Council (CNPq) and the Bill and Melinda Gates Foundation (Award 401636/2013-5 - Grand Challenges Brazil: Reducing the burden of preterm birth, number 05/2013). The current findings are, then, part of the scope of analyses in preterm birth of the Preterm SAMBA study group.

Data Sharing Statement

EMIP study database is not available in data repositories, but any data required can be provided under EMIP study group approval.

References

1. Morisaki N, Ganchimeg T, Ota E, et al. Maternal and institutional characteristics associated with the administration of prophylactic antibiotics for caesarean section: a secondary analysis of the World Health Organization Multicountry Survey on Maternal and Newborn Health. *BJOG*. 2014;121:66–75 . doi: 10.1111/1471-0528.12632

2. Goldenberg RL, Gravett MG, Iams J, et al. The preterm birth syndrome: issues to consider in creating a classification system. *Am J Obstet Gynecol*. 2012;206:113–8 . doi: 10.1016/j.ajog.2011.10.865

3. Villar J, Papageorghiou AT, Knight HE, et al. The preterm birth syndrome: a prototype phenotypic classification. *Am J Obstet Gynecol*. 2012;206:119–23 . doi: 10.1016/j.ajog.2011.10.866

4. Barros FC, Papageorghiou AT, Victora CG, et al. The Distribution of Clinical Phenotypes of Preterm Birth Syndrome: Implications for Prevention. *JAMA Pediatr*. 2015;169(3):220-9. doi: 10.1001/jamapediatrics.2014.3040

5. Esplin MS, Manuck TA, Varner MW, et al. Cluster analysis of spontaneous preterm birth phenotypes identifies potential associations among preterm birth mechanisms. *Am J Obstet Gynecol*. 2015;213:429.e1-9 . doi: 10.1016/j.ajog.2015.06.011

6. Passini R, Tedesco RP, Marba ST, et al. Brazilian multicenter study on prevalence of preterm birth and associated factors. *BMC Pregnancy Childbirth.* 2010;10(1):22 . doi: 10.1186/1471-2393-10-22

7. Passini R, Cecatti JG, Lajos GJ, et al. Brazilian Multicentre Study on Preterm Birth (EMIP): Prevalence and Factors Associated with Spontaneous Preterm Birth. *PLoS One*. 2014;9:e109069 . doi: 10.1371/journal.pone.0109069

8. Souza RT, Cecatti JG, Passini R, et al. The Burden of Provider-Initiated Preterm Birth and Associated Factors: Evidence from the Brazilian Multicenter Study on Preterm Birth (EMIP). *PLoS One*. 2016;11:e0148244 . doi: 10.1371/journal.pone.0148244

9. The National Academies Press. Institute of Medicine (IOM), National Research Council (NRC). Weight Gain During Pregnancy: Reexamining the Guidelines. 2009. Washington

DC

10. Lajos GJ, Tedesco RP, Passini R, et al. Methodological issues on planning and running the brazilian multicenter study on preterm birth. *ScientificWorldJournal*. 2015;2015:719104 . doi: 10.1155/2015/719104

11. Berghella V, Palacio M, Ness A, et al. Cervical length screening for prevention of preterm birth in singleton pregnancy with threatened preterm labor: systematic review and meta-analysis of randomized controlled trials using individual patient-level data. *Ultrasound Obstet Gynecol.* 2017;49:322–329 . doi: 10.1002/uog.17388

12. Koullali B, Oudijk MA, Nijman TAJ, et al. Risk assessment and management to prevent preterm birth. *Semin Fetal Neonatal Med.* 2016;21:80–88 . doi: 10.1016/j.siny.2016.01.005

13. Honest H, Hyde CJ, Khan KS. Prediction of spontaneous preterm birth: no good test for predicting a spontaneous preterm birth. *Curr Opin Obstet Gynecol.* 2012;24:422–33 . doi: 10.1097/GCO.0b013e328359823a

14. Goldenberg RL, Goepfert AR, Ramsey PS. Biochemical markers for the prediction of preterm birth. *Am J Obstet Gynecol.* 2005;192:S36-46 . doi: 10.1016/j.ajog.2005.02.015

15. Di Renzo GC. The great obstetrical syndromes. *J Matern neonatal Med.* 2009;22:633–5 . doi: 10.1080/14767050902866804

16. Brosens I, Pijnenborg R, Vercruysse L, Romero R. The “Great Obstetrical Syndromes” are associated with disorders of deep placentation. *Am J Obstet Gynecol.* 2011;204:193–201 . doi: 10.1016/j.ajog.2010.08.009

17. Morisaki N, Togoobaatar G, Vogel J, et al. Risk factors for spontaneous and provider-initiated preterm delivery in high and low Human Development Index countries: a secondary analysis of the World Health Organization Multicountry Survey on Maternal and Newborn Health. *BJOG*. 2014;121 Suppl:101–9 . doi: 10.1111/1471-0528.12631

18. Bartsch E, Medcalf KE, Park AL, et al. Clinical risk factors for pre-eclampsia determined in early pregnancy: systematic review and meta-analysis of large cohort studies. *BMJ.* 2016;353:i1753

19. Parker SE, Werler MM. Epidemiology of ischemic placental disease: a focus on preterm

gestations. *Semin Perinatol*. *2014;*38:133–8 . doi: 10.1053/j.semperi.2014.03.004

20. Ananth C V., Vintzileos AM. Ischemic placental disease: epidemiology and risk factors. *Eur J Obstet Gynecol Reprod Biol.* 2011;159:77–82 . doi: 10.1016/j.ejogrb.2011.07.025

21. Beckmann JS, Lew D. Reconciling evidence-based medicine and precision medicine in the era of big data: challenges and opportunities. *Genome Med*. 2016;8:134 . doi: 10.1186/s13073-016-0388-7

22. Oyelade J, Isewon I, Oladipupo F, et al. Clustering Algorithms: Their Application to Gene Expression Data. *Bioinform Biol Insights*. 2016;10:237–253 . doi: 10.4137/BBI.S38316

23. Wang L, Wang Y, Chang Q. Feature selection methods for big data bioinformatics: A survey from the search perspective. *Methods.* 2016;111:21–31 . doi: 10.1016/j.ymeth.2016.08.014

24. Peek N, Holmes JH, Sun J. Technical Challenges for Big Data in Biomedicine and Health: Data Sources, Infrastructure, and Analytics. I*MIA Yearb*. 2014;9:42–47 . doi: 10.15265/IY-2014-0018

Figure Legend

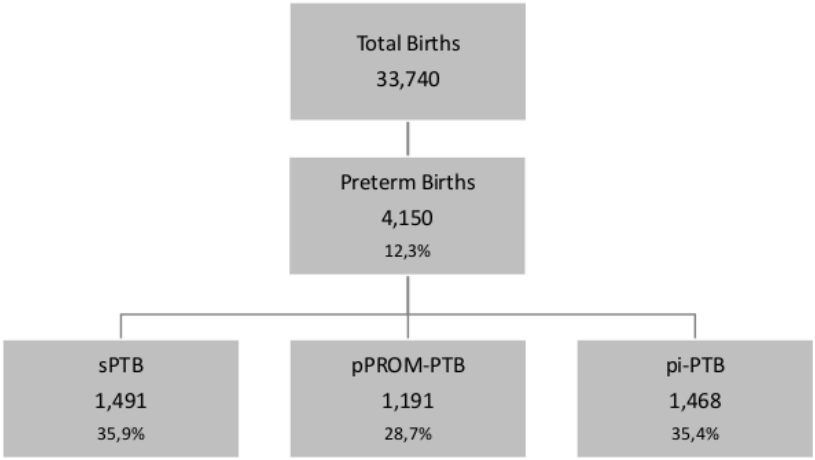


Figure 1. Flowchart of EMIP study – Preterm Birth component (sPTB: spontaneous preterm birth; pPROM-PTB: preterm birth due to preterm premature rupture of membranes; pi-PTB: provider initiated preterm birth).

Table 1. Definition of maternal, fetal and placental conditions possibly associated with preterm birth

Condition	Definition
Maternal	
Extrauterine infection during pregnancy	Prenatal care chart or medical record detailing occurrence during pregnancy of syphilis, tuberculosis, HIV, HPV, hepatitis, febrile diarrhoea, pneumonia, sinusitis, toxoplasmosis, genital herpes, asymptomatic bacteriuria, cystitis, pyelonephritis or sepsis.
Clinical chorioamnionitis	Medical record showing the occurrence of clinical chorioamnionitis.
Maternal chronic disease	Medical record showing history of diabetes, HIV, chronic hypertension, hypo/hyperthyroidism, nephropathy, sickle cell disease or other chronic anaemia, cardiopathy, pneumopathy, epilepsy, lupus, other collagenosis, chronic gastrointestinal, psychiatric, neurologic or orthopaedic diseases, neoplasms, thrombosis, thrombophilia or bariatric surgery.
Preeclampsia/Eclampsia/HELLP syndrome	Medical record of having preeclampsia, eclampsia and/or HELLP syndrome.
Fetal	
Antepartum Stillbirth	Antepartum stillbirth (after 22weeks of gestation) before or after hospital admission.
Fetal Growth Restriction (suspicious or confirmed)	Cases suspicious or confirmed of fetal growth restriction including cases whose ultrasound scan showed fetal estimated weight under the 10^{th} percentile during prenatal care or birthweight was considered small for gestational age.
Perinatal sepsis	Medical record describing clinical or laboratory diagnosis of neonatal sepsis.
Multiple pregnancy	Pregnancy with more than one live fetus after 12 weeks of gestation.
Fetal anomaly	Suspicious (ultrasound findings of fetal anomaly) or confirmed minor or major fetal anomaly (after childbirth).
Placental	
Early bleeding	Women who reported bleeding before 13 weeks of gestation.
Mid-/late-pregnancy bleeding	Women who reported vaginal bleeding after 14 weeks of gestation.
None	Cases in which none of the previous conditions are present.

Table 2. Distribution of clusters of phenotypes of preterm birth according to maternal, fetal and neonatal conditions

Cluster	No (%)	Main Condition (%)	Other most frequent Conditions (%)[a]
Cluster 1	650 (15.7)	None	
Cluster 2	2,319 (55.9)	Extrauterine infection (42.5)	Maternal chronic disease (34.9) Mid—late pregnancy bleeding (20.2) Multiple pregnancy (15.6) Clinical chorioamnionitis (7.5) and Antepartum stillbirth (6.3)
Cluster 3	1,181 (28.4)	Preeclampsia/Eclampsia/HELLP syndrome (85.8)	Fetal growth restriction (32.2in)
All	4,150 (100)		

[a]Only conditions with prevalence above 30% or almost exclusively of the cluster (80%)

Table 3. Distribution of maternal, fetal and placental conditions according to clusters of preterm birth phenotype

Condition	Cluster 1	Cluster 2	Cluster 3
Extrauterine infection *n*	**0**	**986**	**343**
Row %	0	74.19	25.81
Column %	0	42.52	29.04
Clinical Chorioamnionitis *n*	**0**	**173**	**0**
Row %	0	100.0	0
Column %	0	7.46	0
Maternal chronic disease *n*	**0**	**809**	**222**
Row %	0	78.47	21.53
Column %	0	34.89	18.80
Preeclampsia/Eclampsia/HELLP syndrome *n*	**0**	**51**	**1,013**
Row %	0	4.79	95.21
Column %	0	2.20	85.77
Antepartum Stillbirth *n*	**0**	**147**	**17**
Row %	0	89.63	10.37
Column %	0	6.34	1.44
Fetal Growth Restriction *n*	**0**	**49**	**380**
Row %	0	11.42	88.58
Column %	0	2.11	32.18
Perinatal sepsis *n*	**0**	**564**	**212**
Row %	0	72.68	27.32
Column %	0	24.32	17.95
Multiple pregnancy *n*	**0**	**362**	**75**
Row %	0	82.84	17.16
Column %	0	15.61	6.35
Fetal anomaly *n*	**0**	**383**	**112**
Row %	0	77.37	22.63
Column %	0	16.52	9.48
Early bleeding *n*	**0**	**431**	**134**
Row %	0	76.28	23.72
Column %	0	18.59	11.35
Mid-/late- pregnancy bleeding *n*	**0**	**468**	**86**
Row %	0	84.48	15.52
Column %	0	20.18	7.28
None *n*	**650**	**0**	**0**
Row %	100.00	0	0
Column %	100.00	0	0
Total *n*	**650**	**2,319**	**1,181**

Table 4. Preterm birth subtypes according to preterm birth phenotype clusters

Cluster	Cluster 1 (%)	Cluster 2 (%)	Cluster 3 (%)	p-value
sPTB	52.77	43.90	11.01	**<0.0001**
pPROM-PTB	40.62	35.88	8.04	
pi-PTB	6.62	20.22	80.95	
All cases	100	100	100	

P-values in bold mean they are statistically significant. **sPTB:** Spontaneous preterm birth, **pPROM-PTB:** preterm birth due to preterm premature rupture of membranes, **pi-PTB:** provider-initiated preterm birth.

Table 5. Maternal and neonatal outcomes according to preterm birth phenotype clusters

Maternal and neonatal outcomes	Cluster 1 N (%)	Cluster 2 N (%)	Cluster 3 N (%)	p-value
Caesarean section	55.0	52.7	53.9	0.528
Preterm birth <28wks	7.6	7.3	7.3	0.959
Preterm birth <32wks	20.3	21.3	21.5	0.822
Preterm birth 34-36wks	62.4	62.6	62.9	0.977
Apgar score <7 at 5 minutes [a]	10.0	10.0	11.8	0.227
NICU >7 days [b]	34.1	34.2	32.0	0.492
Neonatal near miss [c]	35.5	33.3	33.0	0.510
Neonatal death before discharge [d]	9.5	8.5	7.3	0.257

NICU: neonatal intensive care unit. Missing information for: a (69), b (597), c (13), d (198) cases.

Table 6. Maternal and pregnancy characteristics according to preterm birth phenotype clusters

Characteristics	Cluster 1 N (%)	Cluster 2 N (%)	Cluster 3 N (%)	p-value
Age [a]				0.8503
<19y	126 (19.41)	496 (21.39)	242 (20.49)	
19-35y	428 (65.95)	1,488 (64.17)	764 (64.69)	
>35y	95 (14.64)	335 (14.45)	175 (14.82)	
Ethnicity				**0.0424**
White	262 (40.31)	1,023 (44.11)	548 (46.40)	
Non-white	388 (59.69)	1296 (55.89)	633 (53.60)	
Schooling [b]				0.1261
<12y	125 (80.53)	1,792 (78.67)	948 (81.51)	
≥12y	125 (19.47)	486 (21.33)	215 (18.49)	
Family Income [c]				0.1829
<400 USD	354 (60.62)	1,307 (61.65)	636 (58.30)	
≥400 USD	230 (39.38)	813 (38.35)	455 (41.70)	
Initial BMI [d]				**<0.0001**
<18,5	58 (10.74)	193 (9.53)	51 (4.99)	
18,5-25	343 (63.52)	1161 (57.31)	486 (47.51)	
>25	139 (25.74)	672 (33.17)	486 (47.51)	
Adequacy of weight gain [e]				**<0.0001**
Insufficient	191 (38.20)	575 (30.49)	172 (18.01)	
Adequate	83 (16.60)	246 (13.04)	112 (11.73)	
Excessive	226 (45.20)	1065 (56.47)	671 (70.26)	
Nulliparity	306 (47.08)	1130 (48.73)	539 (45.64)	0.2150
Parity>2	66 (10.15)	253 (10.91)	147 (12.45)	0.2530
Previous PTB [f]	124 (19.14)	433 (18.72)	255 (21.67)	0.1105
Previous SGA [g]	107 (16.72)	365 (15.90)	220 (18.66)	0.1191
Previous C-section [h]	131 (20.15)	475 (20.49)	296 (25.06)	**0.0046**
Smoking, alcohol or other drugs [i]	97 (14.99)	386 (16.79)	185 (15.74)	0.4812
Stress [j]	276 (42.86)	937 (40.85)	507 (43.19)	0.3536

P-values in bold mean they are statistically significant. Missing information for: a) 1, b) 67, c) 355, d) 561, e) 809, f) 12, g) 35, h) 1, i) 29, j) 38.

4.3. Artigo *Metabolomics applied to maternal and perinatal health: a review of new frontiers with a translation potential*

UNICAMP

José Guilherme Cecatti <cecatti@unicamp.br>

CLINICS - Decision on Manuscript ID CLINICS-2018-0894.R1

2 mensagens

CLINICS Editorial Office <onbehalfof@manuscriptcentral.com> 22 de novembro de 2018 10:57
Responder a: clinics@hc.fm.usp.br
Para: cecatti@unicamp.br, jgcecatti@gmail.com
Cc: clinics@hc.fm.usp.br

22-Nov-2018

Dear Dr. Cecatti:

It is a pleasure to accept your manuscript entitled "Metabolomics applied to maternal and perinatal health: a review of new frontiers with a translation potential" for publication in CLINICS.

The publication fee for this article is R$ 1.500,00. Please print this letter, complete and sign the DECLARAÇÃO below and return it attached to an email to our Editorial Office (clinics@hc.fm.usp.br).

As stated in our Instructions to Authors, please send the current version of your manuscript to the company American Journal Experts (AJE) for revision. We have been working with AJE for many years and are very satisfied with the quality of their work. Premium Editing is mandatory and you are entitled to a 20% discount. Please make sure you send all sections related to the manuscript for revision: title, running title, abstract, authors contributions, text, references, figures, and legends. All manuscripts should use American English spelling.

Website: https://www.aje.com
Discount code: CLINICS2018

If you have any doubts, please contact Flavia Jaszczak (support@aje.com).

The final version and AJE certificate must be sent to clinics@hc.fm.usp.br as soon as possible so that we can proceed with the publication of your article.

Final version = After AJE revision, please review/accept modifications and answer all queries. The final version must not contain AJE modifications or notes.

Deadline: 20 calendar days

Thank you for your fine contribution. On behalf of the Editorial Board of CLINICS, we look forward to your continued contributions to the Journal.

Sincerely,
Edmund Baracat
Editor, CLINICS
http://www.scielo.br/clinics / https://www.ncbi.nlm.nih.gov/pmc/journals/795

REVIEW ARTICLE

Metabolomics applied to maternal and perinatal health: a review of new frontiers with a translation potential

Running title: Metabolomics for maternal and perinatal health

Renato Teixeira Souza [1], Jussara Mayrink [1], Débora Leite [1,2], Maria Laura Costa [1], Iracema Mattos Calderon [3], Edilberto Alves Rocha Filho [2], Janete Vettorazzi [4], Francisco Edson Feitosa [5], and José Guilherme Cecatti [1]*, Preterm SAMBA Study Group[#]

(1) Department of Obstetrics and Gynecology, School of Medical Sciences, University of Campinas.
(2) Department of Mother and Child Health, School of Medicine, Federal University of Pernambuco.
(3) Department of Obstetrics and Gynecology, Botucatu Medical School, Sao Paulo State University - UNESP.
(4) Department of Obstetrics and Gynecology, School of Medicine, Federal University of Rio Grande do Sul.
(5) Department of Obstetrics and Gynecology, School of Medicine, Federal University of Ceará.

[#]Membership of the Preterm SAMBA Study Group is provided in the Acknowledgments
*Corresponding author. *José G Cecatti: cecatti@unicamp.br

Abstract

The prediction or early diagnosis of maternal complications is challenging mostly because the main conditions, such as preeclampsia, preterm birth, fetal growth restriction, and gestational diabetes mellitus, are complex syndromes with multiple underlying mechanisms related to their occurrence. Limited advances in maternal and perinatal health in recent decades with respect to preventing these disorders have led to new approaches, and "omics" sciences have emerged as a potential field to be explored. Metabolomics is the study of a set of metabolites in a given sample and can represent the metabolic functioning of a cell, tissue or organism. Metabolomics has some advantages over genomics, transcriptomics, and proteomics, as metabolites are the final result of the interactions of genes, RNAs and proteins. Considering the recent "boom" in metabolomic studies and their importance in the research agenda, we here review the topic, explaining the rationale and theory of the metabolomic approach in different areas of maternal and perinatal health research for clinical practitioners. We also demonstrate the main exploratory studies of these maternal complications, commenting on their promising findings. The potential translational application of metabolomic studies, especially for the identification of predictive biomarkers, is supported by the current findings, although they require external validation in larger datasets and with alternative methodologies.

Keywords: Maternal health (MeSH), Metabolomics (MeSH), Translational Medical Research (MeSH), Prediction

Introduction

In addition to the vast knowledge already available in physiology, pathology and therapeutics, there are still some key areas lacking the global and equally spread advantages of health sciences. One of these areas is undoubtedly maternal and perinatal health. Although a significant improvement has been achieved worldwide during the last two decades with the focus provided by the United Nations' Millennium Development Goals (1) and currently by the Sustainable Development Goals (2), the capacity for predicting the most prevalent and hazardous conditions affecting pregnancies, mothers and babies is still very limited. Without prediction, there is no prevention, the pillar for providing good public health. Therefore, generally speaking, there are no available options other than trying to perform an early diagnosis of those potentially harmful conditions, including preterm birth (PTB), preeclampsia (PE), gestational diabetes mellitus (GDM), fetal growth restriction (FGR), maternal and fetal infections and other conditions possibly associated with severe maternal morbidities. However, early diagnosis is almost always more expensive and not truly available for a significant proportion of populations, especially those in low- and middle-income settings.

Considering all these aspects, the purpose of the current review is to summarize the already existing knowledge on technologies that possibly represent new frontiers and future tools for predicting maternal and perinatal conditions responsible for a significant burden of disease in women and children in the world. Metabolomic biomarkers are one of these new promising tools and deserve special attention due to their potentially important role in the management approach for such conditions in the near future.

Metabolomics as part of omics technologies

The study of the biological systems of an organism leads to the understanding of complex interactions between the genes, their products (RNAs, proteins, and metabolites) and many environmental factors that determine their functioning (3, 4). The recent development of what is called systems biology enables the recognition of these integrative pathways. The field of systems biology is related to the capacity to identify thousands of biological molecules and to establish their interactions using advances in bioinformatics, statistics, and high-throughput techniques of sample analysis. The “omics” sciences emerged from this concept of integrative analysis in the field of systems biology and includes genomics, for the study of a set of genes (genome); transcriptomics, for a set of RNAs (transcriptome); proteomics, for a set of proteins (proteome); and metabolomics, for a set of metabolites (metabolome), as shown in Figure 1.

Figure 1. **Omics science components of biological systems**

The clinical importance of omics sciences arises from the innovative approach of the dynamic regulations of biological systems, which can be explored by both holistic and reductionist strategies (5). A reductionist strategy is the use of only one part of a complex system or only one hypothesis to build conclusions or to understand the determinants of the whole process. On the other hand, a holistic approach considers multiple interactions and complexities to elucidate the whole system. Prevalent maternal complications, such as PE, PTB, GDM or FGR, are commonly addressed using a reductionist approach. For instance, a woman with a short cervix is considered to have a higher risk for PTB, or a fetus with an estimated weight below the 10^{th} percentile is considered growth restricted. The omics sciences enable discovery-driven studies with a holistic and hypothesis-free

approach and may provide the key step forward in addressing complex research problems in maternal and perinatal health, decreasing bias and confounders (3, 6-10).

Genomics

Genomics is the study of genes and their functions and demonstrates the gene codification for proteins that modulate biological systems. Therefore, genomics may be useful to determine the genetic predisposition for a particular disease, identifying the genes involved in its pathophysiology. Genomics studies are more commonly performed using the candidate gene approach, through which preselected genes are sequenced. The complex interactions of genes in the development of a pathologic phenotype and the presence of nongenetic factors limit the results of this approach. Regularly, there is not only one gene involved in the development of a disease but also groups of genes. Moreover, parts of a gene (locus) can be related to the development of the disease, while other parts might be related to its prevention, and the polymorphisms and mutations of genes can determine a wide variance in gene expression. The genome-wide sequencing techniques of the exome can demonstrate a more complete view of the genetic predisposition and establish individuals' prognostics and treatment resistance (11, 12).

The main potential limitations of genomics are high cost and uncertainty between gene codification and gene expression/phenotype. Only a small portion of the whole genome will be translated, and there are several mechanisms and processes that control the expression of genes (methylation, acetylation, epigenetic mechanisms, imprinting, etc.). Therefore, having the gene codification for a disease does not mean it will develop.

Transcriptomics

The next step to understand the development of a disease would be the study of gene expression. Transcriptomics is a step-forward technique compared to genomics in terms of downscaling genetic predispositions. Transcriptomics determines mRNA expression using many techniques, such as microarray-based methods, which are used to measure mRNA transcript levels, and sequence-based methods, such as serial analysis of gene expression (SAGE), cap analysis of gene expression (CAGE), massively parallel signature sequencing (MPSS) and RNA-Seq technology (13). RNA-Seq appears to be the most advantageous transcriptomics approach since it requires a small amount of RNA and is a more precise, “clean” (less background signal) and much lower-cost method considering the high-throughput technology (13). The expression of mRNA in a particular tissue can differ according to the timeframe observed. For instance, the expression of proinflammatory proteins during labor is different from that during other pregnancy periods (4, 14). Therefore, it is possible to compare the expression of mRNA in a tissue using arrays with tens or hundreds of genes and determine down- or upregulation of the gene expression according to the level of mRNA (4). The overexpression of a gene at a given phase of the biological process related to the development of a particular pathologic condition can uncover key points of the physiopathology of the disease and potential targets for prevention and treatment.

Despite these benefits, transcriptomics faces similar limitations to genomics. There is a recent conceptual discussion against the traditional idea that the final products of our genes are mostly proteins: gene (DNA) → mRNA → protein (15). The main genome-wide sequencing projects have targeted only long protein-coding mRNAs, given this classical interpretation. However, more recent studies have identified many other gene-encoded contributors as long noncoding RNAs that play different roles in biological systems and

metabolic pathways but do not encode proteins (16, 17). The various mechanisms that take part in the regulation of gene expression, such as chromatin remodeling, adenylation, elongation, splicing, editing, nuclear export, and degradation, may limit the unbiased recognition of all mechanisms between gene expression and the clinical phenotype (15). These mechanisms seem to be extremely complex, requiring more developed research techniques and new approaches for the study of biological systems (5, 18, 19).

Proteomics

Proteins are key instruments of biological systems, and their abnormalities can cause or be a consequence of organism dysfunctions. The study of the proteins contained in a sample is called proteomics, which basically includes identification and quantification (3). Proteomics is a promising approach that reflects genetic and environmental effects in the development of pathological conditions. According to an evolutionary hypothesis regarding protein function, there are some proteins involved in the various metabolic pathways of an organism and others related to gene regulation and expression, including intra- and extracellular signaling and the mechanisms of gene expression (20). The abundance of proteins required to perform such roles is different depending on the complexity of their functions. Contrary to what would be obvious, highly specialized biological processes do not require high protein availability. Moreover, protein activity can vary depending on many factors, such as the concentration of substrates and the presence of other coenzymes. Therefore, the abundance of proteins seems not to be a reliable parameter to address their function. In addition to proteomics limitations, isolating proteins from blood remains a difficult task, and there is no single reliable, accurate and reproducible method capable of obtaining all proteomes (20).

Metabolomics

The metabolic pathway comprises different biochemical reactions occurring in the intracellular or extracellular compartment. Metabolites are the substrates and products of these metabolic reactions, which require enzymes (proteins), minerals, vitamins, and other cofactors. Metabolomics is the study of the set of metabolites of an organism, identifying and quantifying them with higher sensitivity and more reliable reproducibility (21). The set of these small low-weight molecules is called the metabolome, which is a fingerprint of the metabolism at a given time (3, 4, 7). Therefore, it is possible to understand the metabolism at that moment or, depending on the sample, to understand the metabolism of days to months ago. The hairs, for example, are stable, contain endogenous compounds, and reflect environmental exposure for many months prior (22). Human metabolomic research emerged from previous experience with plants, microbes, and other less complex mammals after years of bioinformatics/statistics advances to address massive output data (3, 4, 7). Metabolomics in maternal and perinatal health may enable the identification of biomarkers related to maternal and perinatal complications and the understanding of the physiopathology of the most complex and prevalent diseases, such as PE, PTB, GDM, FGR, maternal/fetal infections and other severe maternal morbidities (10, 23-25).

Advantages of metabolomics include its hypothesis-free and unbiased approach and the downstream result of gene expression, being the closest to the phenotype of the omics sciences. Different technologies have to be employed to identify and quantify fatty acids, bile acids, ketone bodies, amino acids, peptides, carnitines, carbohydrates, vitamins, xenobiotics, steroids, etc. The modalities generally used are gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry

(LC-MS) and nuclear magnetic resonance (NMR) spectroscopy. GC-MS and LC-MS can identify and quantify tens of thousands of metabolites at one time, creating substantial output data in mass spectrum format, as shown in Figure 2. Mass spectrometry consists of the following components: 1) Sample inlet, a system that differs between GC-MS and LC-MS; 2) Ion source that ionizes molecules of the sample; 3) Analyzer that separates molecules through a long tube under vacuum according to their mass and charge (using the mass-to-charge ratio – m/z or the time-of-flight method to discriminate different metabolites); 4) Ion detector that detects different metabolites with a sensitivity capable of differing isomeric molecules and measuring the quantity of ions converted into electrical signals. The more molecules that are present, the greater is the electrical signal; and 5) Data analysis system matched to a computer that interprets the signal as a mass spectra data (3, 5, 9, 25). The next step is to identify metabolites using their m/z values and to compare them with their corresponding molecules in a previously known library, namely, the Human Metabolome Database - www.hmdb.ca (26).

Figure 2. **Mass spectrometry spectrum – scheme of metabolite output data**

There are many different customizations when using metabolomic analytical methods, allowing better identification of a specific range of mass of molecules or metabolites with different solubilities (5, 21). The advantages and disadvantages of each method should be considered according to the experimental objectives. NMR methods require minimal sample preparation, preserving the samples in their natural form and can identify metabolites in intact tissues (3-D tissues). However, NMR-based metabolomics has lower sensitivity and requires a greater sample volume. Mass spectrometry-based methods (GC or LC-MS) are usually used as complementary methods, depending on the molecular

polarity (polar or nonpolar), sample solubility, and choice for targeted or untargeted metabolomic analyses (3, 5, 27). The untargeted approach means the study of the whole set of metabolites in a given sample, while the targeted approach refers to the identification of a specific group. The untargeted approach is normally employed to identify new potential predictor biomarkers, and a targeted approach is commonly used for validation analysis to measure previously known metabolites in a given sample (3, 9). Especially in untargeted studies, there is a high possibility of returning to the biochemical reactions and to the metabolic pathways with which the identified metabolites are involved. This approach would present an opportunity to address complex diseases that are related to different biological systems (inflammatory, metabolic, energetic, immunologic, etc.).

Tissues and samples for exploring metabolomics

The use of appropriate biological samples is a key issue in defining the experimental design for metabolomic experiments. Considering pregnancy and childbirth, the available biological tissues can come from the mother (plasma, urine, vaginal fluids, milk, and hair), the fetus/newborn (amniotic fluid, umbilical cord blood, plasma, urine, meconium, saliva and other fluids from the infant), or the placenta. The choice will depend upon each study and the investigating aim defined (3, 10).

The most commonly used biological sample is plasma, which is easy to obtain and rich in proteins and metabolites. In biomarker discovery, the final goal is to characterize markers that could enable the early identification of high-risk conditions, and the obvious and most simple screening test would be a blood sample (3, 10, 23). However, many biomarkers are present in low concentrations in plasma, and its study can be optimized

by choosing a biological sample closer to the disease process, especially in the discovery phase, where a great number of identified biomarkers can suggest relevant conditions and pathways involved in the studied diseases (10).

Urine is the second most studied biological sample for metabolomic assays; it is easy to obtain, noninvasive and, like blood, an integrative biofluid. However, the profiling of urine is challenging overall with a wide-ranging variation in metabolite concentrations and fluctuating dilutions due to urine volume alterations. Implementing appropriate statistical analysis methods are pivotal (28).

There is an opportunity for specific metabolomic approaches to study changes in samples closer to diseases or samples clearly influenced by or specific to pregnancy. Vaginal secretions have been studied to understand markers of labor and preterm labor, considering that they can represent changes occurring in the vagina, cervix and adjacent overlying fetal membranes (10, 29). There are still challenges in choosing the best way to collect samples and in considering the influences of individual characteristics, protein variations and contamination from previous intercourse or bleeding.

Amniotic fluid (AF) is another potential target for studying metabolomics during pregnancy. Collection of AF depends on an invasive and specialized procedure early during gestation, with potential risks of miscarriage, infection, preterm labor, or bleeding; however, AF is considered to have the best predictive value of metabolic profiling for malformed fetuses (10, 30-32). It can also be obtained during labor or prior to delivery, and the discoveries arising could justify and overcome potential risks (33). The use of cord blood has mostly been addressed in neonatal research, with great potential in perinatal asphyxia as a noninvasive method to investigate and understand different conditions in the neonate after delivery (34).

A very important limitation on the use of plasma and most other biofluids is that the metabolites found are greatly dynamic and influenced by diet and immune status; furthermore, one isolated sample may not reflect the actual profile. There are standard procedures for sample collection and storage that need to be respected to guarantee accurate results, and immediate processing and freezing are mandatory (3, 35, 36).

An additional source for metabolomic studies, considering pregnancy and childbirth, is the placenta. There are, however, important considerations regarding how to sample and peculiar difficulties in managing and controlling for confounding factors such as route of delivery, duration of labor, sex of the baby, and the time between delivery and sampling and storage for diverse assays (37). The study of the placenta may play a relevant role in the discovery phase and might help elucidate the involved mechanism and pathophysiology of conditions complicating pregnancy.

The search for biomarkers that can possibly be identified in an easy source of tissue, with no risks during pregnancy, that are noninvasive to obtain and store and, most importantly, that are able to represent long-term metabolite profiles has motivated researchers over the years. Hair metabolomics can provide these advantages and has great potential, with recent interesting results for predicting FGR (22).

Preterm birth

PTB is defined as childbirth before 37 weeks of gestation, and the real mechanisms of the spontaneous onset of preterm labor/rupture of membranes involved in its occurrence are still unknown. Uterine distension, decidual hemorrhage, stress, autoimmune, infection, inflammation, environmental, behavioral and socioeconomic factors are some of the hypothetical remarkable conditions linked with PTB (38). The identification of risk factors

related to PTB has been studied for decades, but the complexity and dynamism of PTB development seem to require a multifactorial approach (4). The attempt to identify biological and sociodemographic markers capable of identifying high-risk pregnant women still has not achieved reasonable results. The only new screening and intervention-based recommendation for PTB prevention discovered in the last two decades is the second-trimester transvaginal measurement of cervical length and vaginal progesterone for women with a short cervix (39, 40). Further studies on cervical remodeling and associated biomechanical mechanisms must be performed (41).

Compared to isolated biological markers, the metabolomic approach seems to be superior for demonstrating the organism function (3, 4, 24, 25, 38). In 2010, Romero and colleagues published a study evaluating metabolites from processed and stored AF (42). They identified women who had spontaneous preterm labor (PTL) with intact membranes and had been submitted to transabdominal amniocentesis to assess the microbial state of the amniotic cavity and/or fetal lung maturity in three facilities. According to their pregnancy outcomes, women were divided into three groups: 1) with PTL and delivery at term; 2) with PTL, with preterm delivery and without intra-amniotic inflammation/infection (IAI); 3) with PTL, preterm delivery and IAI. Using phase 1 to identify potential metabolites that could differ from subgroups and phase 2 to validate the results of discrimination, the authors demonstrated that the metabolic profile from AF achieved 96.3% and 88.5% accuracy for phase 1 and 2, respectively, to predict the subgroups. Moreover, they also showed differences in the presence of amino acids (AAs) and carbohydrates in the AF samples in the subgroups. The results can contribute to developing AF tests for PTB, to new interventions for high-risk populations or to understanding PTB syndrome.

Menon et al. selected 50 African American women who participated in the Nashville Birth Cohort and collected AF samples during labor: 25 with PTB (before 34 weeks, excluding pPROM) and 25 with full-term births (33). Approximately 350 metabolites were identified in the AF using LC/MS and GC/MS mass analyzers. The metabolites were categorized following their participation in different biochemical pathways, such as histidine, steroids, xanthine, acetaminophen, bile acids, fatty acids, detoxification of xenobiotics and cosmetic-formulation chemical metabolism. The mean predictive accuracy to discriminate PTB from full term was 90%, with 14.2% false negatives. Although the predictive performance of this set of metabolites deserves additional validation, valuable information regarding the possible relationship between maternal liver function and PTB was reported.

In general, the collection of AF is an invasive procedure, and its use for metabolomic analysis is usually reserved when amniocentesis is indicated for another purpose, especially in women with higher risk for spontaneous onset of preterm labor. The reasons must be taken into account to avoid sampling bias, such as risk for fetal malformation or intra-amniotic cavity infection. Graça et al. and Diaz et al. conducted metabolomic studies related to maternal and fetal outcomes, such as GDM, fetal malformations/chromosomal disorders, PTB and pPROM (32, 43). Urine, blood and AF samples were collected from women at high risk for fetal malformations/chromosomal disorders. Therefore, the different concentrations of allantoin, myo-inositol, alanine, citrate and 2-hydroxyisobutyrate identified in the PTB group might not be generalized.

More recently, Ghartey et al. conducted a nested case-control study inside a prospective cohort to identify cervicovaginal (CV) biomarkers related to spontaneous PTB (29). CV samples were collected at 20 weeks – 23 weeks + 6 days (first visit - V1) and 24

weeks – 27 weeks + 6 days (second visit – V2) gestational age intervals. The CV metabolome was analyzed from 10 women who had spontaneous PTB and 10 women who had term births using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC/MS) and GC/MS. More than 300 metabolites were identified in the CV samples, and women with PTB had a distinct CV metabolome compared with that in samples of women with term birth. Considering V1 samples, more than half of the identified AAs were decreased in PTB samples, and methyl-4-hydroxybenzoate, an antimicrobial agent, was increased in the CV samples of women with term birth. Thus, an increased presence of sialic acid in CV samples of PTB women in V2 samples and downregulation of carbohydrates were identified. Sialic acid plays a role in immune function, specifically on viral entry into the cell. Therefore, there were remarkable differences in CV metabolic markers in women with PTB, enabling the study of some metabolic pathways related to PTB occurrences, such as cervicovaginal protein hydrolysis, inflammation, carbohydrate metabolism and viral/bacterial infection modulation. Metabolomic analyses can be performed using different technical approaches depending on the spectrum of metabolites to be identified. These differences can modify the sensitivity of metabolite identification. Thomas and colleagues conducted a metabolomic analysis to identify discriminatory metabolites in CV fluid in women with PTB and term birth (44). In this study, the identification of AAs, organic acids and fatty acids was prioritized, but the number of metabolites identified was much less than that in Ghartey's study. No significant difference was noticed between the CV metabolites of women with PTB and term birth (44).

Nonetheless, the collection of AF during preterm and term labor brings up a discussion of what benefits each period of sample collection during pregnancy may provide and the

possible confounders. There are insufficient data to clarify if different metabolomic profiles during preterm labor are related to the mechanisms of preterm labor determinism or to the proper maternal and/or fetal metabolism that is characteristic of each gestational age period (45, 46). A longitudinal targeted metabolomic profile conducted by Lindsay and colleagues showed AAs, nonesterified fatty acids, polar lipids, tricarboxylic acid cycle intermediate metabolic changes in healthy pregnancies (47). HPLC-MS, LC-MS and flow-injection mass spectrometry analyses of plasma samples demonstrated the variance in amino acid concentrations across three set points of pregnancy (at the first, second and third trimester). The sum of the nonbranched chain essential AAs and tricarboxylic acid cycle intermediates increases from the first to the third trimester, whereas free carnitine and acetylcarnitine decrease. The findings seem to be consistent with placental and fetal mediation of AAs during pregnancy, with energetic synthesis and nitrogen cycle changes due to anabolic and catabolic phenomena.

There are complex metabolic cycles mediating fetal development, pregnancy homeostasis, autoimmune regulation, and placental multiple functions. There are many options of samples, a period of collection and metabolomic analyzers to study preterm syndrome, and each one might result in innovative contributions (3, 4). The collection of samples during the preclinical phase (asymptomatic women) may provide early identification of higher-risk women as well as lightening trigger mechanisms of preterm labor. Another way to elucidate those triggering mechanisms is collecting samples during labor or from the neonates, demonstrating the resulting PTB metabolic profile (48-50). Multiethnic validation of current findings is required to move forward on preterm prediction and prevention. Therefore, international collaborative studies are essential, shortening the time and financial resources (38).

Fetal growth restriction

Intrauterine growth restriction is an obstetric disorder characterized by the failure of a fetus to achieve its growth potential (51). It has a multivariate etiology, which includes genetic factors, infections, or uteroplacental insufficiency (52, 53). This pathological condition is part of a broad spectrum, composed of “Small for Gestational Age” (SGA) fetuses, which includes all fetuses whose weight is below the 10^{th} percentile for gestational age from the reference ranges applied to the specific population or based on customized charts (51, 54). Some of them are physiologically small (constitutional) and therefore are not associated with adverse outcomes (52, 53). The incidence of FGR is approximately 4 - 8% in industrialized countries and 6 - 30% in low- and middle-income countries (55).

FGR is associated with perinatal complications such as prematurity, fetal death and chronic metabolic disease in adulthood, such as diabetes mellitus type 2, hypertension and metabolic syndrome (56). The prenatal detection of fetuses with FGR is still a challenge in daily obstetric practice, and among low-risk pregnancies, the detection rate is approximately 15% (56). Whereas birth weight is a determinant of mortality and neonatal morbidity, much interest has been raised by research on new and effective means for the early diagnosis and/or prediction of FGR and the improvement of the clinical management (53).

Some single parameters have been tested or, when combined, compose a multifactorial model. The isolated analysis of serum adiponectin in pregnant women between 11 and 13 weeks, performed by Nanda and colleagues in 2011, revealed the inability of that factor to predict FGR because the concentration of adiponectin was the

same in both the group with and the group without FGR (57). In an analysis of a multifactorial model, a group in the United Kingdom tested the association of mean maternal blood pressure, nuchal translucency, chorionic gonadotropin, serum pregnancy-associated plasma protein (PAPP-A), pulsatility index of the uterine arteries, placental growth factor (PlGF), placental protein 13, and disintegrin and metalloprotease 12 (ADAM12). This model showed a 73% detection rate for FGR, with a false positive rate of 10% (58). In a second analysis, associating the pulsatility index of the uterine arteries, average maternal blood pressure, PAPP-A, and PlGF, the group achieved a 52.3% detection rate of FGR, with 10% false positives (59). However, the main challenge of this analysis is the great influence imposed by the frequent coexistence between preeclampsia and FGR in their results (60).

More recently, a meta-analysis evaluating the predictive ability of uterine artery Doppler performed in the first trimester achieved a sensitivity of 39.2% for cases of FGR established early (61). In this search for effective screening tools to accurately identify women at the highest risk of FGR, metabolomics has emerged as a new science, seeking biomarkers that can compose a predictive model, which could provide early diagnosis of FGR, with a reduction in morbidity and neonatal mortality. These studies have evaluated the metabolomes of biofluids (urine and blood) or hair for comparison between fetuses with FGR and fetuses with adequate weight for gestational age.

In 2010, Horgan and colleagues studied venous cord blood from women exhibiting the delivery of a healthy singleton fetus and from women with a suspected diagnosis of SGA (birth weight below the 10^{th} percentile for gestational age) (62). Parallel to this analysis, women at 15 weeks of gestation underwent the analysis of plasma samples. Forty women who delivered SGA babies were matched to 40 controls who had uncomplicated

pregnancies. The metabolomics of both analyses showed 29 metabolites in very different concentrations between the comparison groups (adequate for gestational age vs SGA). Of these, 19 metabolites were identified as predictors of the model and applied to analyses of cord blood and maternal peripheral blood. In the latter case, the predictive model showed an area under the curve (AUC) of 0.9, which represents a robust predictive model of presymptomatic SGA (62).

Dessi and colleagues studied the urine metabolic profiles of neonates with FGR and compared them with controls to define the metabolic patterns associated with this pathological condition. They observed a higher concentration of metabolites such as myo-inositol (also found by Barberini et al. (63)), sarcosine, creatine, and creatinine in FGR neonates. An increase in these metabolites in the urine is observed in states of hypercatabolism, as is the case with fasting, and there is a lower level of protein synthesis (64). On the other hand, Maitre and colleagues found decreased levels of tyrosine, acetate, trimethylamine, and formate in maternal urine samples of the late first trimester (65). These molecules can play a role in carbohydrate and fat metabolism (acetate), function as precursors of neurotransmitters (tyrosine), act as mediators of cell death due to enhanced levels of reactive oxygen species (formate), or simply be markers of vegetable intake (trimethylamine). These findings highlight the complexity of FGR pathogenesis and provide some clues that metabolic changes can take place as soon as 11 weeks of gestation. Recently, Sulek and colleagues evaluated the metabolome of hair collected between 26 and 28 weeks from 41 healthy pregnant women whose fetuses developed FGR (birth weight below the 10^{th} percentile for gestational age) and 42 women whose fetuses had adequate birthweight for gestational age. Thirty-two discriminatory metabolites were found, mostly AAs and fatty acids. Five of these metabolites composed

a predictive model, which showed an extremely high AUC of 0.998 (22). However, the most severe cases of FGR can be diagnosed as early at 26 weeks of pregnancy, and perhaps 26-28 weeks would not be a suitable interval to screen for FGR in some women.

FGR has a multifactorial etiology, apparently resulting from the interaction between a complex biochemical profile and impaired placental perfusion, which negatively impacts the function of transporting nutrients. The knowledge of metabolism for this condition is still scarce. Therefore, metabolomics could contribute to a better understanding of the pathophysiology of FGR and to a more precise definition of this syndrome. An early detection of fetuses at a higher risk for truly pathological growth restriction or a diagnosis of FGR among pregnant women could improve perinatal outcomes if appropriate interventions can be implemented during prenatal care.

Pregnancy hypertensive disorders

Preeclampsia (PE) is still a challenging condition in obstetrics practice, involving different clinical manifestations (i.e., early- and late-onset PE) that share the physiopathological aspect of inadequate trophoblast invasion of the maternal vasculature early in pregnancy. There are already known clinical (66) and ultrasonographic risk factors (67): the former can be identified at booking, and the latter can be better screened at the 2nd trimester (uterine artery waveform). Although easily and almost universally accessible, the strongest clinical risk factor history of preeclampsia cannot be applied to nulliparous women, which is a target group for PE (68). In addition, the good performance of uterine artery Doppler velocimetry requires availability of equipment and operator expertise (69). Therefore, great effort has been made to identify biomarkers that could be applied to diagnostic, prediction, or prognostic factors (i.e., soluble endoglin, PIGF and pregnancy-

associated plasma protein-A PAPP-A) (70). However, these biomarkers still are not fully and easily available for clinical practice and some of them can only be evaluated at the 2nd or 3rd trimester when there are few therapeutic alternatives for these women.

Considering poor placentation and systemic endothelial activation in PE (71), some authors have been searching for metabolites that could explain the pathogenesis of the disease. Kenny et al. found increased levels of uric acid, which is related to ischemic conditions, and 2-oxoglutarate, or α-ketoglutaric acid, an intermediate of the citric acid cycle (or tricarboxylic acid cycle), which is increased with limited oxidative capacity (72). These metabolites could reinforce the hypothesis of placental hypoxia in preeclampsia, and low levels of taurine could be a marker of defective trophoblast invasion in early-onset PE (71, 73).

It is interesting to note that alanine and glutamic acid levels are increased in women already diagnosed with PE (72) and early in pregnancy (74). Both alanine and glutamic acid are nonessential AAs and neurotransmitters. It seems that α-glutamate excitatory effects play an important role in membrane depolarization observed in epileptic seizures (75), which sheds light on the understanding of eclampsia. Alanine represents the main muscle energy source and has an inhibitory action in the brain, as does taurine (75). Renal function may also be affected in PE (76), and augmented levels of creatinine have been observed, either in the 1st (77) or in the 3rd trimester (72).

Omics studies have found many metabolic disturbances in preeclamptic women, such as disorders in carnitine, AAs, carbohydrate or fatty acid pathways, by diverse platforms. However, the available studies have used small sample sizes and do not control for pregnancy complications, such as SGA infants (78), fetal malformations or PTB (31), which

can also show metabolic profile alterations. Additionally, it would be enlightening if longitudinal studies could perform data collection at different gestational ages.

On the other hand, it is notable that the AUC, sensitivity, and specificity of predicting early or late PE (71, 77) can be improved when regression models mix metabolites with clinical factors, including weight, ethnicity, and mean arterial blood pressure. A first-trimester predictive model that showed promising results, with an AUC of 0.835 in a validation study, enrolled 50 women with early preeclampsia and 108 matched controls (79), which is still a modest number of participants. Therefore, it is fundamental, in the near future, to perform a study involving a heterogeneous group of nulliparous low-risk pregnant women, taking into account that this is the set with the highest risk of preeclampsia. The understanding of the metabolome profile of preeclampsia may contribute not only to prediction but also to improving the knowledge regarding cellular and molecular pathophysiology, providing better management of preeclampsia and, ultimately, better maternal and perinatal outcomes.

Gestational diabetes mellitus

Gestational diabetes mellitus (GDM) is diabetes that is first diagnosed in the second or third trimester of pregnancy that is not clearly either preexisting type 1 or type 2 diabetes, according to the American Diabetes Association (80). It is related to important maternal, fetal, and neonatal outcomes and is a high-risk factor for diabetes mellitus later in life (80). There are still controversies regarding diagnostic criteria, treatment and monitoring of GDM, but recent advances in omics studies can provide clues about the maternal metabolic profile in normal and diabetic affected pregnancies and may be helpful in understanding and predicting the disease.

Normal pregnancy is characterized by progressive insulin resistance (81) and increased levels of lipoproteins and lipoprotein-cholesterol (82), and both phenomena increase glucose levels. Fat stores can be used as an energy source by the mother, so glucose can reach the fetus, which is the main energy source of the fetal unit and is easily transported through the placenta by facilitated diffusion (81, 82). GDM, however, seems to promote a shift from gluconeogenesis to ketone body production, which is probably why fasting acylcarnitine ester levels are lower and 3-hydroxybutyrate are higher in diabetic compared to normal pregnancies (83).

In urine samples of 2nd-trimester diabetic pregnancies, Diaz et al. (2011) found increased levels of 2-hydroxyisobutyrate (43). This observation reinforces the association between GDM and type 2 diabetes (81), since this biomarker has also been identified in diabetic patients, reflecting disturbances in free fatty acid metabolism (84). Urinary excretion of 3-hydroxyisovalerate also appears to be increased in GDM (43) and reflects the reduced activity of β-methylcrotonyl-CoA carboxylase, a biotin-dependent enzyme (85). It is important to consider the (1) relationship between previous diabetes and congenital malformation (80) and (2) the possible teratogenic effect of biotin deficiency (85). This finding requires further investigation and may be helpful to improve the diagnostic criteria and follow-up of GDM.

Using proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy to study urine samples of 1st and 2nd-trimester pregnancies, Sachse et al. (2012) have shown that urine citrate increases with higher degrees of hyperglycemia (86). However, they did not find any difference in the metabolomic profile between normal and affected women, regardless of the diagnostic criteria of GDM, even after normalizing the results to the creatinine level or considering that the urine metabolomic profile is influenced by the

immediate lifestyle and diet and less by the genetic background. On the other hand, UPLC-MS identified slightly increased levels of choline in urine in the 2nd trimester of prediagnostic GDM women (31). However, as choline was also altered in fetal malformations and plays a role in fetal brain development, it seems to be nonspecific for GDM (43).

Hair metabolomics was also investigated as a potential marker for GDM. Baker and colleagues analyzed hair metabolites in two different cohorts using samples of women who developed GDM matched by BMI to controls with uncomplicated pregnancies. Analyzing hair samples collected at the time of oral glucose tolerance testing (24-28 weeks of gestation) of 47 Chinese women in each group, the authors found one metabolite (adipic acid) that differs significantly between groups and might be potential discriminatory (87). Using hair samples collected earlier in pregnancy (at 20 weeks) of women who participated in the SCOPE study, two metabolites (itaconic acid and cis-aconitate) had significantly different levels between groups (20 women with GDM and 26 controls) (88). Although the results are from pilot studies, new potential approaches demand investigations in larger datasets.

Studies have used various metabolomic technologies, in either AF, maternal plasma, serum, hair, or urine samples, at various times during pregnancy. Each technique and biological sample has distinct properties of sensitivity, specificity, and power to detect metabolic changes that affect GDM, and findings are still inconclusive (89). A comprehensive overview of this condition could be approached by studies involving several biological fluids in different gestational ages and taking into account dietary intake and treatment options (89). GDM is a worldwide problem, and its assessment during pregnancy can improve gestational and perinatal outcomes. More research is needed to

identify metabolites that could be used as biomarkers of disease and to better define this condition.

Maternal and fetal infections

Metabolomics has also been used for translational research in infectious diseases during pregnancy. The possibility of identifying a pattern of metabolites related to the more severe condition or to the development of fetal/neonatal sequelae, confirming a maternal-fetal transmission and recognizing potential pathological conditions of the fetus is a worthy approach, especially when current diagnostics tests are not sufficiently sensitive and specific. The current spread of Zika virus infection is a very recent example of how challenging the identification of pathophysiological mechanisms and the creation of reliable serological tests in some infectious diseases can be. The laboratory testing for Zika virus infection guidelines of the WHO demonstrates the difficulty in confirming maternal infection for suspected cases with more than one week after onset of symptoms or with a fetal diagnosis of neurological impairment in asymptomatic women (90). The CDC diagnostic testing recommendations highlight the common cross-reaction with other related flaviviruses, such as dengue, chikungunya, and yellow fever viruses (91), calling attention to the importance of more reliable tests.

Recently, Zhou and colleagues demonstrated dynamic changes in the metabolomic profile of mice during *Toxoplasma gondii* infection (92). The authors showed that a set of metabolites could discriminate infected samples from controls, showing an AUC of 0.996. Fanos and colleagues conducted a metabolomic approach using urinary samples of newborns infected by cytomegalovirus and controls (10). The preliminary investigation showed that the abundance of a set of metabolites was significantly different between

the groups. Various studies related to nonpregnancy infections, such as *Haemophilus influenzae*, dengue, malaria, tuberculosis, and *Clostridium difficile,* have already demonstrated the potential contribution of metabolomics to the pathogenesis, mechanisms of adaptation, severity, host response and impairment (93-96).

Another potential applicability for metabolomic studies is chorioamnionitis, an infection that can present not only as a subclinical condition with minimum consequences for women or fetuses/newborns but also as a severe infection leading to sepsis and severe maternal and neonatal morbidity and mortality (97-101). Chorioamnionitis is a major cause of mortality in many countries and lacks reliable markers for early diagnosis (97, 102, 103). For instance, women with PROM, especially if preterm, are at higher risk for infection, and currently available markers collected from blood, vaginal secretions or AF have not proven to be highly accurate in the prediction or early diagnosis of chorioamnionitis (97, 102, 104-108). In this case, a false positive test for infection can lead to unnecessary interventions, such as prematurity, and a false negative test might determine the development of neonatal sepsis. Therefore, not only a timely identification of infection but a more accurate diagnosis is crucial to prevent severe adverse outcomes.

A few metabolomic studies have been conducted to address the early identification of women with amniotic infection. A metabolomic analysis of AF from 40 women with rupture of membranes or preterm labor was performed, and the results were compared according to the microbiological/histological status of infection and neonatal outcomes (infection and perinatal brain injury) (109). Several metabolites of more than 8 metabolic pathways were identified as potential markers of chorioamnionitis. The sphingolipid metabolic pathway was the most important group of metabolites, showing the strongest

discriminant power with an AUC of >0.99% and >0.95% to discriminate cases with and without brain injuries in chorioamnionitis cases, respectively. The authors presented a set of metabolites as potential best biomarker candidates, discussing many possible correlations and underlying mechanisms related to chorioamnionitis. However, they also clarified that the external validation of findings is limited due to the use of specific methodology, excluding some phenotypes of women and newborns to create a more homogenous comparison analysis. According to the authors, this homogeneity of outcomes is not what is found “in real life”.

A study from Romero and colleagues also demonstrated that metabolomic profiling is useful for discriminating cases of preterm delivery with and without intra-amniotic infection (42). Another case-control study, this time using lipidomic analysis of the AF, also identified potential markers for amniotic infection (110, 111). Both findings still require further validation.

Metabolomic studies assessing potential makers for chorioamnionitis usually use AF samples collected after the rupturing of membranes through amniocentesis in sites where this invasive procedure is generally standard to address amniotic infection. The AF is a key tissue for maternal and perinatal research, considering its interactions with the placenta, mother, and fetal tissues, providing RNA, DNA and metabolites of all three components (24, 112). Nevertheless, more studies using blood, urine, hair, or vaginal secretion markers might be important to develop a more reproducible and less invasive method to investigate chorioamnionitis.

Severe maternal morbidity and other conditions

Maternal complications during pregnancy, childbirth and postpartum periods are part of a continuum or spectrum of morbidity classified from mild to severe. Depending on the severity, the morbidity can be potentially life-threatening and put women at risk for “maternal near miss” or maternal death. Pragmatically, maternal near miss is defined as an experience of near death that can be identified considering aspects of clinical, laboratory and management-based criteria, according to the World Health Organization (113).

Delays in preventing, diagnosing, or treating maternal complications are related to poorer maternal outcomes (114). Therefore, the early identification of severe maternal morbidity might provide a window of opportunity to save mothers from short- and long-term adverse outcomes. The main causes of maternal mortality are related to hemorrhage and hypertensive complications (101), followed by sepsis, abortion, embolism, and other indirect causes. Clinical tools are being studied to identify, measure, and monitor maternal morbidity (113, 115, 116). Ordinarily, the clinical tools are composed of maternal symptoms and signs and clinical support characteristics. This means that the diagnosis of maternal morbidity occurs in an advanced stage of severity. Despite being a great advance, the use of the definition of potentially life-threatening conditions and maternal near miss would be even more useful to prevent maternal death and short- and long-term adverse consequences, with earlier diagnoses.

The clinical tool called the Sequential Organ Failure Assessment (SOFA) can be used to identify severe maternal morbidity. However, even this well-known and widely used tool demands a certain degree of organ dysfunction as a proxy for prediction. Metabolomic analyses could be an interesting approach to early identify severe maternal morbidity

(life-threatening conditions and maternal near miss). The identification of common pathways of preclinical disorders related to maternal morbidity could be useful, widening the window of opportunity to act before severe organ dysfunction. Some studies have already described altered metabolomic profiles related to acute heart failure induced by shock, severe sepsis, and septic and hemorrhagic shock (117-119). The results show that metabolomic profiles can discriminate degrees of severity and may be used for early diagnosis. Therefore, such profiles might be useful to predict organ failure or systemic inflammatory response syndrome in pregnant women as well, independent of the cause of morbidity.

Based on the same idea, the identification of a metabolomic pattern directly related to the main causes of severe maternal morbidity, hypertensive and hemorrhage disorders seems to be tangible. Aberrant placentation, for instance, is related to hypertensive and hemorrhagic complications during pregnancy. The abnormal remodeling of spiral arteries leads to ischemic placental disease mediated by many inflammatory, immunologic, and oxidative stress pathways (120, 121). A discovery of metabolomic analyses using samples from women with potentially life-threatening conditions, maternal near miss, and non-complicated pregnancies might demonstrate whether it is possible to identify common metabolites related to maternal morbidity.

Discussion

The 21st century has had remarkable innovations in technology, especially in service industries, such as communication and informatics involving smartphones and high-quality wireless networks, changing the way people live and interact. Modification is usually required by the need for new ways to deal with a competitive routine and a large

amount of information in different parts of the globe, all of which is part of the new era of globalization phenomena. There is a classic theory about innovations and economic growth based on creative destruction (122). Briefly, one of the bases of this theory is the stationary state, which is the gap period between the development of new research and, consequently, new innovations. This state can lead to innovation mostly depending on the rate of interest and the number and impact of available innovations. Translating this concept to our area of interest, we could say that research on maternal and perinatal health urges innovation.

Maternal and child health are priority topics of the third sustainable development goal (2). Obstetric complications, such as PE, PTB, FGR, and GDM, have a major impact on maternal and perinatal health because they can lead to short- and long-term consequences for women or newborns, from childhood to adulthood. Biological biomarkers seem to be essential for the development of predictive models because they play an important role in the pathophysiology, whether in cause or in consequence mechanisms. The integrated study of the biological interactions in an organism (systems biology) has led to omics-based research, which has been demonstrated to be a promising and useful approach to assess such complex syndromes (3, 38). To yield reliable and reproducible information, samples must follow the specific and detailed methodology for processing and storage, and data on these details are key to allow comparisons between studies and advances in metabolomic research in maternal-fetal medicine.

Metabolomics seems to be the most reasonable approach to studying the majority of obstetrical syndromes, considering the costs, confounders, analytical techniques, output data generation and the possibility of addressing final pathways for the underlying

mechanisms involved in the occurrence of each complication. Metabolomic studies have generated optimistic results so far, widening the opportunity to explore new methodologies for sample collection, preparation, and analysis. Apart from that, the technique requires validation of the initial findings of pilot studies in larger datasets, using numerous multiethnic population cohorts. Thus, metabolomics might be a sensible way to move forward in the prediction and prevention of maternal complications, achieving a significant impact on people's lives as the 21st-century technology revolution may have done.

The current review aimed to present how metabolomics has become a promising approach for maternal and perinatal health research in recent years, showing (especially for a nonexpert audience) the rationale behind this high-technology method in the omics science perspective and its potential translational application. The main maternal complications during pregnancy, such as preeclampsia, PTB, FGR and GDM, have multifactor complex etiologies, and certainly, an expressive number of unknown underlying factors, but the pathways related to the occurrence of these complications are not yet clarified. Such complexity makes their prediction extremely challenging also considering that the pathophysiology might involve genetic and environmental adaptive mechanisms from maternal and fetal components. Metabolomics is the study of the metabolites in a given sample, and it is the closest correspondent to the cell, tissue or organism function compared to other omics sciences, such as genomics, transcriptomics or proteomics. Metabolomic studies of maternal complications during pregnancy can be a valuable method to explore biomarkers in different sources of samples, although

advances in reproducible analytical procedures and external validations in larger datasets are still necessary.

Acknowledgments

The other members of the Preterm SAMBA Study Group that supported this review as an initiative for describing new approaches in maternal and perinatal health research are as follows: Philip N. Baker, University of Leicester, United Kingdom; Louise Kenny, University of Cork, Cork, Ireland; Karolina Sulek, Gravida: National Center for Growth & Development, Liggins Institute, University of Auckland, Auckland, New Zealand; Mary A. Parpinelli, School of Medical Sciences, University of Campinas, Campinas, Brazil; Kleber G. Franchini, LNBio, Campinas, Brazil; Elias Melo, Jr., and Danilo Anacleto, Department of Mother and Child Health, School of Medicine, Federal University of Pernambuco, Recife, Brazil; Bianca F. Cassettari and Joice Vernini, School of Medicine of Botucatu, UNESP, Brazil; Lucia Pfitscher, Hospital de Clínicas de Porto Alegre and School of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil; Daisy Lucena, School of Medicine, Federal University of Ceará, Fortaleza, Brazil.

Funding: This review is part of the scope of the Preterm SAMBA Study Group, which is an initiative to investigate metabolomic approaches in maternal and perinatal health. The Preterm SAMBA Study Group was jointly funded by The Brazilian National Research Council (CNPq) and the Bill and Melinda Gates Foundation (Award 401636/2013-5, Grand Challenges Brazil: Reducing the burden of PTB). RTS and DFL have been awarded Ph.D. scholarships from the CAPES Foundation, an agency under the Ministry of Education of Brazil, process 88881.134095/2016-01 and 8881.134512/2016-01, respectively.

Authors' contributions

RTS and JGC conceived the concept and design of this review. RTS, JM, DFL, and MLC performed the literature review. RTS, JM, DFL, MLC, and JGC participated in the drafting of the manuscript. All the authors read, agreed and contributed to writing the final version.

References

1. United Nations Development Programme (UNDP). The Millennium Development Goals. 2002. http://www.un.cv/files/MDGReport.pdf. Accessed 01 Jul 2017.
2. United Nations Development Programme (UNDP). Sustainable Development Goals. 2015. http://www.un.org/sustainabledevelopment/health/. Accessed 20 June 2017. Contract No.: 20 August 2017.
3. Horgan RP, Clancy OH, Myers JE, Baker PN. An overview of proteomic and metabolomic technologies and their application to pregnancy research. BJOG. 2009;116(2):173-81.
4. Romero R, Espinoza J, Gotsch F, Kusanovic JP, Friel LA, Erez O, et al. The use of high-dimensional biology (genomics, transcriptomics, proteomics, and metabolomics) to understand the preterm parturition syndrome. BJOG: An International Journal of Obstetrics & Gynaecology. 2006;113:118-35.
5. Kell DB. Metabolomics and systems biology: making sense of the soup. Current opinion in microbiology. 2004;7(3):296-307.
6. Romero R, Kuivaniemi H, Tromp G. Functional Genomics and Proteomics in Term and Preterm Parturition. The Journal of Clinical Endocrinology & Metabolism. 2013;87(6):2431-4.
7. Putri SP, Nakayama Y, Matsuda F, Uchikata T, Kobayashi S, Matsubara A, et al. Current metabolomics: practical applications. Journal of bioscience and bioengineering. 2013;115(6):579-89.
8. Dettmer K, Hammock BD. Metabolomics--a new exciting field within the omics sciences. Environmental health perspectives. 2004;112(7):A396-7.
9. Alonso A, Marsal S, Julià A. Analytical methods in untargeted metabolomics: state of the art in 2015. Frontiers in bioengineering and biotechnology. 2015;3:23-.
10. Fanos V, Atzori L, Makarenko K, Melis GB, Ferrazzi E. Metabolomics application in maternal-fetal medicine. BioMed research international. 2013;2013:720514-.
11. Dharuri H, Demirkan A, van Klinken JB, Mook-Kanamori DO, van Duijn CM, t Hoen PAC, et al. Genetics of the human metabolome, what is next? Biochimica et biophysica acta. 2014;1842(10):1923-31.
12. Zhang H, Baldwin DA, Bukowski RK, Parry S, Xu Y, Song C, et al. A Genome-Wide Association Study of Early Spontaneous Preterm Delivery. Genetic epidemiology. 2015.
13. Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nature reviews Genetics. 2009;10(1):57-63.
14. Hassan SS, Romero R, Haddad R, Hendler I, Khalek N, Tromp G, et al. The transcriptome of the uterine cervix before and after spontaneous term parturition. American journal of obstetrics and gynecology. 2006;195(3):778-86.
15. Wirta V. Mining the transcriptome – methods and applications. 2006.
16. Carninci P. RNA dust: where are the genes? DNA research : an international journal for rapid publication of reports on genes and genomes. 2010;17(2):51-9.
17. Saxena A, Carninci P. Whole transcriptome analysis: what are we still missing? Wiley interdisciplinary reviews Systems biology and medicine. 2011;3(5):527-43.
18. Mousavian Z, Kavousi K, Masoudi-Nejad A. Information Theory in Systems Biology. Part I: Gene Regulatory and Metabolic Networks. Seminars in Cell & Developmental Biology. 2015;51:3-13.
19. Engelhardt B, Frőhlich H, Kschischo M. Learning (from) the errors of a systems biology model. Scientific reports. 2016;6:20772-.
20. Zhong F, Yang D, Hao Y, Lin C, Jiang Y, Ying W, et al. Regular patterns for proteome-wide distribution of protein abundance across species. PloS one. 2012;7(3):e32423-e.
21. Dettmer K, Aronov PA, Hammock BD. Mass spectrometry-based metabolomics. Mass spectrometry reviews. 2007;26(1):51-78.

22. Sulek K, Han TL, Villas-Boas SG, Wishart DS, Soh SE, Kwek K, et al. Hair metabolomics: identification of fetal compromise provides proof of concept for biomarker discovery. Theranostics. 2014;4(9):953-9.
23. Dessi A, Marincola FC, Fanos V. Metabolomics and the great obstetrical syndromes--GDM, PET, and IUGR. Best practice & research Clinical obstetrics & gynaecology. 2015;29(2):156-64.
24. Kamath-Rayne BD, Smith HC, Muglia LJ, Morrow AL. Amniotic fluid: the use of high-dimensional biology to understand fetal well-being. Reproductive sciences (Thousand Oaks, Calif). 2014;21(1):6-19.
25. Li S, Dunlop AL, Jones DP, Corwin EJ. High-Resolution Metabolomics: Review of the Field and Implications for Nursing Science and the Study of Preterm Birth. Biological research for nursing. 2016;18(1):12-22.
26. Wishart DS, Jewison T, Guo AC, Wilson M, Knox C, Liu Y, et al. HMDB 3.0--The Human Metabolome Database in 2013. Nucleic acids research. 2013;41:D801-7.
27. Patti GJ. Separation strategies for untargeted metabolomics. Journal of separation science. 2011;34(24):3460-9.
28. Zhang S, Zheng C, Lanza IR, Nair KS, Raftery D, Vitek O. Interdependence of signal processing and analysis of urine 1H NMR spectra for metabolic profiling. Anal Chem. 2009;81(15):6080-8.
29. Ghartey J, Bastek JA, Brown AG, Anglim L, Elovitz MA. Women with preterm birth have a distinct cervicovaginal metabolome. Am J Obstet Gynecol. 2015;212(6):776.e1-.e12.
30. Graca G, Duarte IF, B JG, Carreira IM, Couceiro AB, Domingues Mdo R, et al. Metabolite profiling of human amniotic fluid by hyphenated nuclear magnetic resonance spectroscopy. Anal Chem. 2008;80(15):6085-92.
31. Graca G, Goodfellow BJ, Barros AS, Diaz S, Duarte IF, Spagou K, et al. UPLC-MS metabolic profiling of second trimester amniotic fluid and maternal urine and comparison with NMR spectral profiling for the identification of pregnancy disorder biomarkers. Mol Biosyst. 2012;8(4):1243-54.
32. Graça G, Duarte IF, Barros AS, Goodfellow BJ, Diaz SO, Pinto J, et al. Impact of prenatal disorders on the metabolic profile of second trimester amniotic fluid: a nuclear magnetic resonance metabonomic study. Journal of proteome research. 2010;9(11):6016-24.
33. Menon R, Jones J, Gunst PR, Kacerovsky M, Fortunato SJ, Saade GR, et al. Amniotic Fluid Metabolomic Analysis in Spontaneous Preterm Birth. Reproductive Sciences. 2014;21(6):791-803.
34. Denihan NM, Boylan GB, Murray DM. Metabolomic profiling in perinatal asphyxia: a promising new field. BioMed research international. 2015;2015:254076.
35. Dunn WB, Broadhurst D, Begley P, Zelena E, Francis-McIntyre S, Anderson N, et al. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. Nat Protoc. 2011;6(7):1060-83.
36. Zhou B, Xiao JF, Tuli L, Ressom HW. LC-MS-based metabolomics. Mol Biosyst. 2012;8(2):470-81.
37. Burton GJ, Sebire NJ, Myatt L, Tannetta D, Wang YL, Sadovsky Y, et al. Optimising sample collection for placental research. Placenta. 2014;35(1):9-22.
38. Gracie S, Pennell C, Ekman-Ordeberg G, Lye S, McManaman J, Williams S, et al. An integrated systems biology approach to the study of preterm birth using "-omic" technology--a guideline for research. BMC pregnancy and childbirth. 2011;11:71-.
39. Fonseca EB, Celik E, Parra M, Singh M, Nicolaides KH. Progesterone and the risk of preterm birth among women with a short cervix. The New England journal of medicine. 2007;357(5):462-9.
40. Hassan SS, Romero R, Vidyadhari D, Fusey S, Baxter JK, Khandelwal M, et al. Vaginal progesterone reduces the rate of preterm birth in women with a sonographic short cervix: a

multicenter, randomized, double-blind, placebo-controlled trial. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2011;38(1):18-31.
41. Jain J, Gyamfi-Bannerman C. Future directions in preterm birth research. Seminars in fetal & neonatal medicine. 2015.
42. Romero R, Mazaki-Tovi S, Vaisbuch E, Kusanovic JP, Chaiworapongsa T, Gomez R, et al. Metabolomics in premature labor: a novel approach to identify patients at risk for preterm delivery. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians. 2010;23(12):1344-59.
43. Diaz SO, Pinto J, Graça G, Duarte IF, Barros AS, Galhano E, et al. Metabolic biomarkers of prenatal disorders: an exploratory NMR metabonomics study of second trimester maternal urine and blood plasma. Journal of proteome research. 2011;10(8):3732-42.
44. Thomas MM, Sulek K, McKenzie EJ, Jones B, Han T-L, Villas-Boas SG, et al. Metabolite Profile of Cervicovaginal Fluids from Early Pregnancy Is Not Predictive of Spontaneous Preterm Birth. International journal of molecular sciences. 2015;16(11):27741-8.
45. Hadden DR, McLaughlin C. Normal and abnormal maternal metabolism during pregnancy. Seminars in fetal & neonatal medicine. 2009;14(2):66-71.
46. Lain KY, Catalano PM. Metabolic Changes in Pregnancy. Clinical Obstetrics and Gynecology. 2007;50(4):938-48.
47. Lindsay KL, Hellmuth C, Uhl O, Buss C, Wadhwa PD, Koletzko B, et al. Longitudinal Metabolomic Profiling of Amino Acids and Lipids across Healthy Pregnancy. PloS one. 2015;10(12):e0145794-e.
48. Clark RH, Kelleher AS, Chace DH, Spitzer AR. Gestational age and age at sampling influence metabolic profiles in premature infants. Pediatrics. 2014;134(1):e37-46.
49. Hill M, Pařízek A, Kancheva R, Dušková M, Velíková M, Kříž L, et al. Steroid metabolome in plasma from the umbilical artery, umbilical vein, maternal cubital vein and in amniotic fluid in normal and preterm labor. The Journal of Steroid Biochemistry and Molecular Biology. 2010;121(3-5):594-610.
50. Wilson K, Hawken S, Ducharme R, Potter BK, Little J, Thébaud B, et al. Metabolomics of prematurity: analysis of patterns of amino acids, enzymes, and endocrine markers by categories of gestational age. Pediatric research. 2014;75(2):367-73.
51. American College of Obstetricians and Gynecologists - ACOG. Practice bulletin no. 134: fetal growth restriction. Clinical management guidelines for obstetrician-gynecologists. Washington, USA

Obstet Gynecol. 2013;121(5):1122-33.
52. Bamberg C, Kalache KD. Prenatal diagnosis of fetal growth restriction. Seminars in Fetal and Neonatal Medicine. 2004;9(5):387-94.
53. Albu AR, Anca AF, Horhoianu VV, Horhoianu IA. Predictive factors for intrauterine growth restriction. Journal of medicine and life. 2014;7(2):165-71.
54. Gardosi J. Customised assessment of fetal growth potential: implications for perinatal care. Arch Dis Child Fetal Neonatal Ed. 2012;97(5):F314-7.
55. Diderholm B. Perinatal energy metabolism with reference to IUGR and SGA: studies in pregnant women and newborn infants. The Indian journal of medical research. 2009;130(5):612-7.
56. Figueras F, Gardosi J. Intrauterine growth restriction: new concepts in antenatal surveillance, diagnosis, and management. American journal of obstetrics and gynecology. 2011;204(4):288-300.

57. Nanda S, Akolekar R, Sodre D, Vaikousi E, Nicolaides KH. Maternal serum adiponectin at 11-13 weeks of gestation in pregnancies delivering small for gestation neonates. Fetal diagnosis and therapy. 2011;29(4):274-9.
58. Karagiannis G, Akolekar R, Sarquis R, Wright D, Nicolaides KH. Prediction of small-for-gestation neonates from biophysical and biochemical markers at 11-13 weeks. Fetal diagnosis and therapy. 2011;29(2):148-54.
59. Poon LCY, Syngelaki A, Akolekar R, Lai J, Nicolaides KH. Combined screening for preeclampsia and small for gestational age at 11-13 weeks. Fetal diagnosis and therapy. 2013;33(1):16-27.
60. Crovetto F, Crispi F, Scazzocchio E, Mercade I, Meler E, Figueras F, et al. First-trimester screening for early and late small-for-gestational-age neonates using maternal serum biochemistry, blood pressure and uterine artery Doppler. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2014;43(1):34-40.
61. Velauthar L, Plana MN, Kalidindi M, Zamora J, Thilaganathan B, Illanes SE, et al. First-trimester uterine artery Doppler and adverse pregnancy outcome: a meta-analysis involving 55,974 women. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2014;43(5):500-7.
62. Horgan RP, Broadhurst DI, Dunn WB, Brown M, Heazell AEP, Kell DB, et al. Changes in the metabolic footprint of placental explant-conditioned medium cultured in different oxygen tensions from placentas of small for gestational age and normal pregnancies. Placenta. 2010;31(10):893-901.
63. Barberini L, Noto A, Fattuoni C, Grapov D, Casanova A, Fenu G, et al. Urinary metabolomics (GC-MS) reveals that low and high birth weight infants share elevated inositol concentrations at birth. The Journal of Maternal-Fetal & Neonatal Medicine. 2014.
64. Dessì A, Atzori L, Noto A, Visser GHA, Gazzolo D, Zanardo V, et al. Metabolomics in newborns with intrauterine growth retardation (IUGR): urine reveals markers of metabolic syndrome. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians. 2011;24 Suppl 2:35-9.
65. Maitre L, Fthenou E, Athersuch T, Coen M, Toledano MB, Holmes E, et al. Urinary metabolic profiles in early pregnancy are associated with preterm birth and fetal growth restriction in the Rhea mother-child cohort study. BMC medicine. 2014;12:110-.
66. Duckitt K, Harrington D. Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies. BMJ (Clinical research ed). 2005;330(7491):565-.
67. Cnossen JS, Morris RK, ter Riet G, Mol BWJ, van der Post JAM, Coomarasamy A, et al. Use of uterine artery Doppler ultrasonography to predict pre-eclampsia and intrauterine growth restriction: a systematic review and bivariable meta-analysis. CMAJ. 2008;178(6):701-11.
68. Myers JE, Kenny LC, McCowan LM, Chan EH, Dekker GA, Poston L, et al. Angiogenic factors combined with clinical risk factors to predict preterm pre-eclampsia in nulliparous women: a predictive test accuracy study. BJOG. 2013;120(10):1215-23.
69. Poon LC, Nicolaides KH. First-trimester maternal factors and biomarker screening for preeclampsia. Prenat Diagn. 2014;34(7):618-27.
70. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. The New England journal of medicine. 2006;355(10):992-1005.
71. Kuc S, Koster MPH, Pennings JLA, Hankemeier T, Berger R, Harms AC, et al. Metabolomics profiling for identification of novel potential markers in early prediction of preeclampsia. PloS one. 2014;9(5):e98540-e.

72. Kenny LC, Broadhurst D, Brown M, Dunn WB, Redman CWG, Kell DB, et al. Detection and identification of novel metabolomic biomarkers in preeclampsia. Reproductive sciences (Thousand Oaks, Calif). 2008;15(6):591-7.
73. Desforges M, Parsons L, Westwood M, Sibley CP, Greenwood SL. Taurine transport in human placental trophoblast is important for regulation of cell differentiation and survival. Cell death & disease. 2013;4(3):e559-e.
74. Odibo AO, Goetzinger KR, Odibo L, Cahill AG, Macones GA, Nelson DM, et al. First-trimester prediction of preeclampsia using metabolomic biomarkers: a discovery phase study. Prenatal diagnosis. 2011;31(10):990-4.
75. Rainesalo S, Keränen T, Palmio J, Peltola J, Oja SS, Saransaari P. Plasma and cerebrospinal fluid amino acids in epileptic patients. Neurochemical research. 2004;29(1):319-24.
76. Magee LA, Pels A, Helewa M, Rey E, von Dadelszen P, Canadian Hypertensive Disorders of Pregnancy Working G. Diagnosis, evaluation, and management of the hypertensive disorders of pregnancy. Pregnancy hypertension. 2014;4(2):105-45.
77. Bahado-Singh RO, Akolekar R, Mandal R, Dong E, Xia J, Kruger M, et al. First-trimester metabolomic detection of late-onset preeclampsia. American journal of obstetrics and gynecology. 2013;208(1):58.e1-7.
78. Horgan RP, Broadhurst DI, Walsh SK, Dunn WB, Brown M, Roberts CT, et al. Metabolic profiling uncovers a phenotypic signature of small for gestational age in early pregnancy. Journal of proteome research. 2011;10(8):3660-73.
79. Bahado-Singh RO, Syngelaki A, Akolekar R, Mandal R, Bjondahl TC, Han B, et al. Validation of metabolomic models for prediction of early-onset preeclampsia. American journal of obstetrics and gynecology. 2015;213(4):530.e1-.e10.
80. American Diabetes Association. Classification and Diagnosis of Diabetes. Diabetes Care. 2017;40(Suppl 1):S11-S24.
81. Lowe WL, Karban J. Genetics, genomics and metabolomics: new insights into maternal metabolism during pregnancy. Diabetic medicine : a journal of the British Diabetic Association. 2014;31(3):254-62.
82. Pinto J, Barros AS, Domingues MRM, Goodfellow BJ, Galhano E, Pita C, et al. Following healthy pregnancy by NMR metabolomics of plasma and correlation to urine. Journal of proteome research. 2015;14(2):1263-74.
83. Pappa KI, Vlachos G, Theodora M, Roubelaki M, Angelidou K, Antsaklis A. Intermediate metabolism in association with the amino acid profile during the third trimester of normal pregnancy and diet-controlled gestational diabetes. American journal of obstetrics and gynecology. 2007;196(1):65.e1-5.
84. Li X, Xu Z, Lu X, Yang X, Yin P, Kong H, et al. Comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry for metabonomics: Biomarker discovery for diabetes mellitus. Analytica chimica acta. 2009;633(2):257-62.
85. Mock DM. Marginal biotin deficiency is common in normal human pregnancy and is highly teratogenic in mice. The Journal of nutrition. 2009;139(1):154-7.
86. Sachse D, Sletner L, Mørkrid K, Jenum AK, Birkeland KI, Rise F, et al. Metabolic changes in urine during and after pregnancy in a large, multiethnic population-based cohort study of gestational diabetes. PloS one. 2012;7(12):e52399-e.
87. He X, de Seymour JV, Sulek K, Qi H, Zhang H, Han T-L, et al. Maternal hair metabolome analysis identifies a potential marker of lipid peroxidation in gestational diabetes mellitus. Acta diabetologica. 2015.
88. de Seymour JV, Conlon CA, Sulek K, Villas Bôas SG, McCowan LME, Kenny LC, et al. Early pregnancy metabolite profiling discovers a potential biomarker for the subsequent development of gestational diabetes mellitus. Acta diabetologica. 2014;51(5):887-90.
89. Huynh J, Xiong G, Bentley-Lewis R. A systematic review of metabolite profiling in gestational diabetes mellitus. Diabetologia. 2014;57(12):2453-64.

90. World Health Organization (WHO). Laboratory testing for Zika virus infection. Interim guidance. 2016. http://www.who.int/emergencies/zika-virus/en/. Accessed 14 Jul 2017.
91. Centers for Disease Control and Prevention (CDC). Diagnostic Testing. 2016. http://www.cdc.gov/zika/hc-providers/diagnostic.html. Accessed 01 June 2017
92. Zhou C-X, Zhou D-H, Elsheikha HM, Liu G-X, Suo X, Zhu X-Q. Global Metabolomic Profiling of Mice Brains following Experimental Infection with the Cyst-Forming Toxoplasma gondii. PloS one. 2015;10(10):e0139635-e.
93. Harrison A, Dubois LG, St John-Williams L, Moseley MA, Hardison RL, Heimlich DR, et al. Comprehensive Proteomic and Metabolomic Signatures of Nontypeable Haemophilus influenzae-Induced Acute Otitis Media Reveal Bacterial Aerobic Respiration in an Immunosuppressed Environment. Molecular & cellular proteomics : MCP. 2016;15(3):1117-38.
94. Haas CT, Roe JK, Pollara G, Mehta M, Noursadeghi M. Diagnostic 'omics' for active tuberculosis. BMC medicine. 2016;14(1):37-.
95. Ghosh S, Sengupta A, Sharma S, Sonawat HM. Early prediction of cerebral malaria by (1)H NMR based metabolomics. Malaria journal. 2016;15(1):198-.
96. Antharam VC, McEwen DC, Garrett TJ, Dossey AT, Li EC, Kozlov AN, et al. An Integrated Metabolomic and Microbiome Analysis Identified Specific Gut Microbiota Associated with Fecal Cholesterol and Coprostanol in Clostridium difficile Infection. PloS one. 2016;11(2):e0148824-e.
97. Menon R, Taylor RN, Fortunato SJ. Chorioamnionitis--a complex pathophysiologic syndrome. Placenta. 2010;31(2):113-20.
98. Garite TJ, Freeman RK. Chorioamnionitis in the preterm gestation. Obstet Gynecol. 1982;59(5):539-45.
99. 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. 2017.
100. Vogel JP, Souza JP, Mori R, Morisaki N, Lumbiganon P, Laopaiboon M, et al. Maternal complications and perinatal mortality: findings of the World Health Organization Multicountry Survey on Maternal and Newborn Health. BJOG : an international journal of obstetrics and gynaecology. 2014;121 Suppl:76-88.
101. Say L, Chou D, Gemmill A, Tunçalp Ö, Moller A-B, Daniels J, et al. Global causes of maternal death: a WHO systematic analysis. The Lancet Global Health. 2014;2(6):e323-e33.
102. Popowski T, Goffinet F, Batteux F, Maillard F, Kayem G. Prediction of materno fetal infection in preterm premature rupture of membranes: serum maternal markers. Gynécologie, obstétrique & fertilité. 2011;39(5):302-8.
103. Yu H, Wang X, Gao H, You Y, Xing A. Perinatal outcomes of pregnancies complicated by preterm premature rupture of the membranes before 34 weeks of gestation in a tertiary center in China: A retrospective review. Biosci Trends. 2015;9(1):35-41.
104. Oludag T, Gode F, Caglayan E, Saatli B, Okyay RE, Altunyurt S. Value of maternal procalcitonin levels for predicting subclinical intra-amniotic infection in preterm premature rupture of membranes. The journal of obstetrics and gynaecology research. 2014;40(4):954-60.
105. Howman RA, Charles AK, Jacques A, Doherty DA, Simmer K, Strunk T, et al. Inflammatory and haematological markers in the maternal, umbilical cord and infant circulation in histological chorioamnionitis. PloS one. 2012;7(12):e51836-e.
106. Chaouachi S, Marrakchi O, Ben Hamida E, Abidi K, Béchir Z, Abdelmoula Jouda J, et al. Usefulness of serum procalcitonin in the early diagnosis of maternal-fetal bacterial infection. A prospective study. Archives de pédiatrie : organe officiel de la Société française de pédiatrie. 2011;18(3):267-71.
107. Tita AT, Andrews WW. Diagnosis and management of clinical chorioamnionitis. Clin Perinatol. 2010;37(2):339-54.

108. Thornburg LL, Queenan R, Brandt-Griffith B, Pressman EK. Procalcitonin for prediction of chorioamnionitis in preterm premature rupture of membranes. J Matern Fetal Neonatal Med. 2016;29(13):2056-61.
109. Dudzik D, Revello R, Barbas C, Bartha JL. LC-MS-based metabolomics identification of novel biomarkers of chorioamnionitis and its associated perinatal neurological damage. J Proteome Res. 2015;14(3):1432-44.
110. Maddipati KR, Romero R, Chaiworapongsa T, Chaemsaithong P, Zhou SL, Xu Z, et al. Lipidomic analysis of patients with microbial invasion of the amniotic cavity reveals up-regulation of leukotriene B4. FASEB J. 2016;30(10):3296-307.
111. Maddipati KR, Romero R, Chaiworapongsa T, Chaemsaithong P, Zhou SL, Xu Z, et al. Clinical chorioamnionitis at term: the amniotic fluid fatty acyl lipidome. J Lipid Res. 2016;57(10):1906-16.
112. Palmas F, Fattuoni C, Noto A, Barberini L, Dessì A, Fanos V. The choice of amniotic fluid in metabolomics for the monitoring of fetus health. Expert Rev Mol Diagn. 2016;16(4):473-86.
113. Say L, Souza JP, Pattinson RC. Maternal near miss--towards a standard tool for monitoring quality of maternal health care. Best practice & research Clinical obstetrics & gynaecology. 2009;23(3):287-96.
114. Pacagnella RC, Cecatti JG, Parpinelli MA, Sousa MH, Haddad SM, Costa ML, et al. Delays in receiving obstetric care and poor maternal outcomes: results from a national multicentre cross-sectional study. BMC pregnancy and childbirth. 2014;14:159-.
115. Chou D, Tunçalp Ö, Firoz T, Barreix M, Filippi V, von Dadelszen P, et al. Constructing maternal morbidity – towards a standard tool to measure and monitor maternal health beyond mortality. BMC Pregnancy and Childbirth. 2016;16(1):45-.
116. Firoz T, Chou D, von Dadelszen P, Agrawal P, Vanderkruik R, Tunçalp O, et al. Measuring maternal health: focus on maternal morbidity. Bulletin of the World Health Organization. 2013;91(10):794-6.
117. Whelan SP, Carchman EH, Kautza B, Nassour I, Mollen K, Escobar D, et al. Polymicrobial sepsis is associated with decreased hepatic oxidative phosphorylation and an altered metabolic profile. The Journal of surgical research. 2014;186(1):297-303.
118. Garcia-Simon M, Morales JM, Modesto-Alapont V, Gonzalez-Marrachelli V, Vento-Rehues R, Jorda-Miñana A, et al. Prognosis Biomarkers of Severe Sepsis and Septic Shock by 1H NMR Urine Metabolomics in the Intensive Care Unit. PloS one. 2015;10(11):e0140993-e.
119. Aletti F, Conti C, Ferrario M, Ribas V, Bollen Pinto B, Herpain A, et al. ShockOmics: multiscale approach to the identification of molecular biomarkers in acute heart failure induced by shock. Scandinavian journal of trauma, resuscitation and emergency medicine. 2016;24(1):9-.
120. Roberts JM. Pathophysiology of ischemic placental disease. Seminars in perinatology. 2014;38(3):139-45.
121. Schatz F, Guzeloglu-Kayisli O, Arlier S, Kayisli UA, Lockwood CJ. The role of decidual cells in uterine hemostasis, menstruation, inflammation, adverse pregnancy outcomes and abnormal uterine bleeding. Human reproduction update. 2016.
122. Aghion P, Howitt P. A model of growth through creative destruction. Econometrica. 1992;60:323-51.

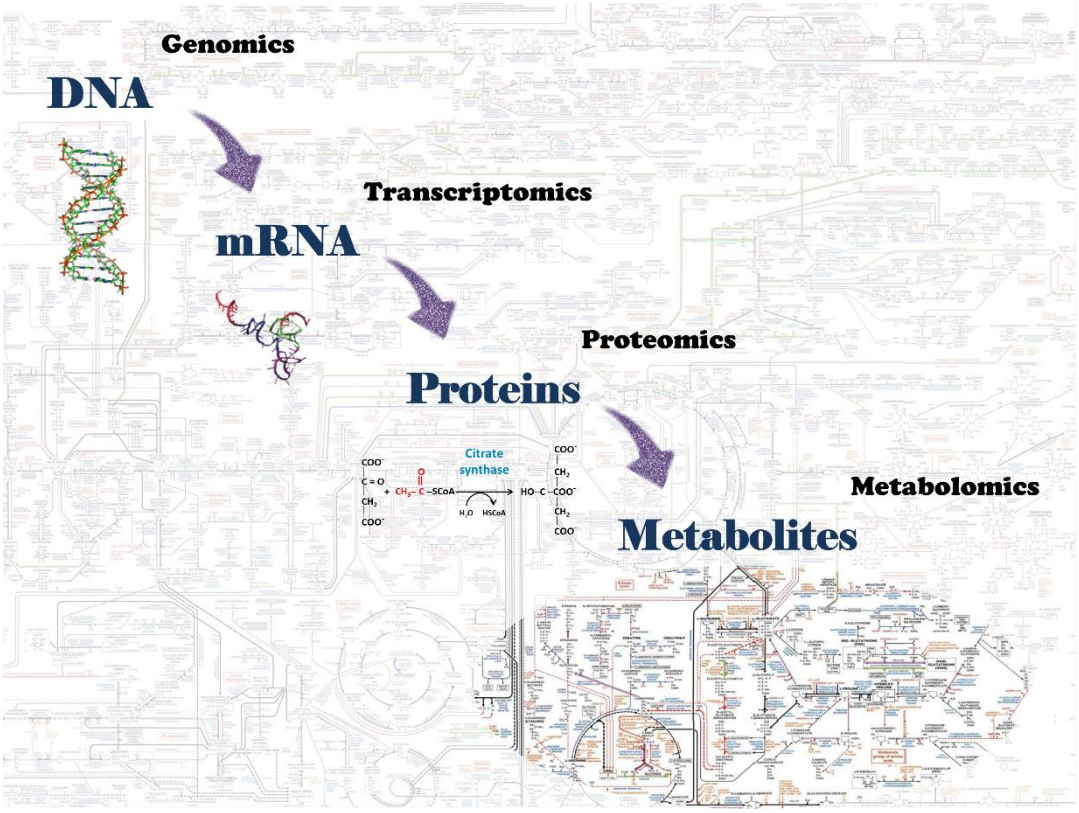


Figure 1. **Omics Science components of biological systems**

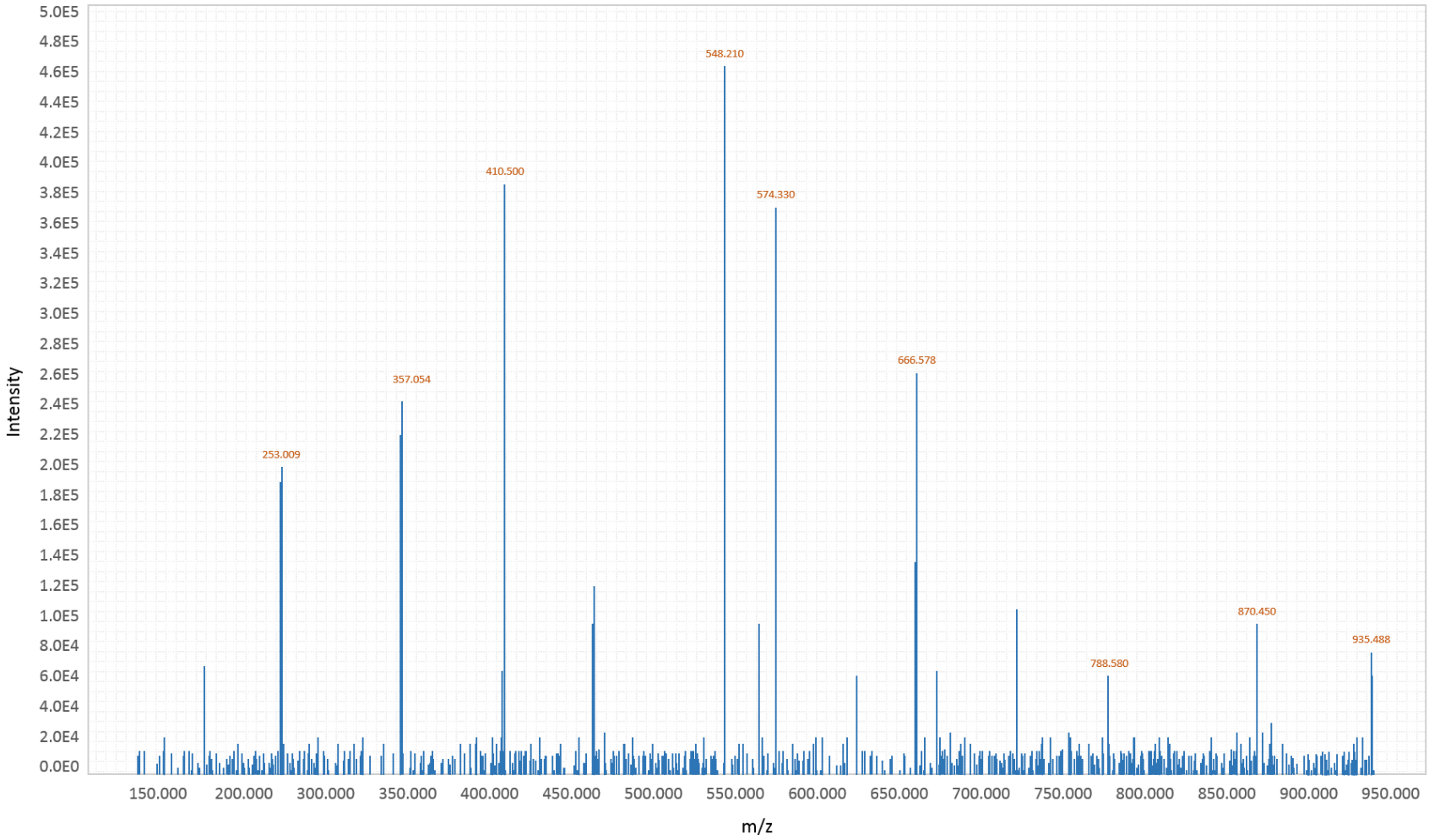


Figure 2. **Mass** spectometry **spectrum-scheme of metabolites output data**

4.4. Artigo *The use of metabolomics for predicting spontaneous preterm birth in asymptomatic pregnant women: protocol for a systematic review and meta-analysis*

UNICAMP

José Guilherme Cecatti <cecatti@unicamp.br>

BMJ Open - Decision on Manuscript ID bmjopen-2018-026033.R3

1 mensagem

BMJ Open <onbehalfof@manuscriptcentral.com> 28 de janeiro de 2019 13:54
Responder a: info.bmjopen@bmj.com
Para: cecatti@unicamp.br

28-Jan-2019

Dear Prof. Cecatti:

It is a pleasure to accept your manuscript entitled "The use of metabolomics for predicting spontaneous preterm birth in asymptomatic pregnant women: protocol for a systematic review and meta-analysis" in its current form for publication in BMJ Open.

In order to support making all research published in BMJ Open fully open access, an article-processing charge is levied. This charge supports the peer review process, production costs (typesetting, copy editing, etc.), and the costs of maintaining the content online and marketing it to readers.

Therefore, your payment of £1350 (excluding VAT) for accepted manuscript bmjopen-2018-026033.R3 is now due.

A separate e-mail will follow shortly with a link to payment options and instructions. This will be from BMJ's partner, Copyright Clearance Center, through their RightsLink system. Please note that it can sometimes take a few days before you receive this email. Using this link you can choose to pay by card or invoice.

Your article will not be processed further until payment is completed.

If you have reviewed for the journal within the 12 months prior to submitting this paper, please contact the editorial office (editorial.bmjopen@bmjgroup.com) about your discount. Information regarding waivers and discounts is included in our instructions for authors; however, we anticipate that most authors will have the resources to pay.

Please note, a number of institutions have taken out open access memberships with BMJ, which either covers the cost of open access publishing for authors at participating institutes, or allows authors to receive a discount on the article-processing charge. Please visit our open access page to see a full list of participating institutions, find out if you are eligible and how to obtain your discount code - http://journals.bmj.com/site/authors/openaccess.xhtml#open-access-institutional-memberships.

All accepted papers are reviewed for their media potential by our PR office within two weeks of acceptance. If your paper is selected for press release by the journal, we will let you know within this timeframe.

Once your article is published online you will be able to keep track of usage. Each article published in BMJ Open has individual usage statistics which are updated daily and can be accessed from the Article Usage Statistics link in the Services section of the right hand column on each page of the article. In this column you can also sign up to be alerted about any e-letter responses to your article.

Thank you for your contribution, and we hope that you will continue to submit to the journal in future.

Sincerely,
Emma Johnson
Assistant Editor, BMJ Open
ejohnson@bmj.com

We are constantly trying to find ways of improving our peer review system and continually monitor processes and methods by including article submissions and reviewers' reports in our research. If you do not wish your paper or review entered into a our peer review research programme, please let us know by emailing The BMJ's editorial office papersadmin@bmj.com as soon as possible.

The use of metabolomics for predicting spontaneous preterm birth in asymptomatic pregnant women: protocol for a systematic review and meta-analysis

Renato T. Souza [1] (renatotsouzasp@gmail.com)

Rafael Bessa Freitas Galvão [1] (rafaelbfg@gmail.com)

Débora Farias Batista Leite [1,2] (deborafariasleite@gmail.com)

Renato Passini Jr [1] (passini@caism.unicamp.br)

Philip N. Baker [3] (philip.baker@leicester.ac.uk)

José Guilherme Cecatti [1] (cecatti@unicamp.br)

[1] Department of Obstetrics and Gynaecology, University of Campinas, Campinas, São Paulo, Brazil.

[2] Clinics Hospital of Federal University of Pernambuco, Recife, Pernambuco, Brazil.

[3] College of Life Sciences, University of Leicester, England, United Kingdom.

This systematic review protocol is registered at PROSPERO platform (CRD42018100172). Available from: http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42018100172.

Corresponding author:

José Guilherme Cecatti

101 Alexander Fleming, Cidade Universitária, campinas, São Paulo, Brazil.

ZIPCODE 13083-881

cecatti@unicamp.br

Word count: 2,233.

ABSTRACT

Introduction: Preterm birth (PTB) is the leading cause of neonatal mortality and short- and long-term morbidity. The aetiology and pathophysiology of spontaneous PTB are still unclear, which makes the identification of reliable and accurate predictor markers more difficult, particularly for unscreened or asymptomatic women. Metabolomics biomarkers have been demonstrated to be potentially accurate biomarkers for many disorders with complex mechanisms such as PTB. Therefore, we aim to perform a systematic review of metabolomics markers associated with spontaneous PTB. Our research question is "What is the performance of metabolomics for predicting spontaneous preterm birth in asymptomatic pregnant women?"

Methods and analysis: We will focus on studies assessing metabolomics techniques for predicting spontaneous preterm birth in asymptomatic pregnant women. We will conduct a comprehensive systematic review of the literature from the last 10 years. Only observational cohort and case-control studies will be included. Our search strategy will be carried out by two independent reviewers, who will scan title and abstract before carrying out a full review of the article. The scientific databases to be explored include PubMed, MedLine, ScieLo, EMBASE, LILACS, Web of Science, Scopus, and others.

Ethics and dissemination: This systematic review protocol does not require ethical approval.

We intend to disseminate our findings in scientific peer-reviewed journal, the Preterm SAMBA study open access website, specialists' conferences, and to our funding agencies.

Registration details: This protocol is registered in PROSPERO platform (code CRD42018100172).

Keywords: preterm birth, spontaneous preterm birth, metabolomics, biomarkers, prediction, metabolome.

Strengths and limitations of this study

- This systematic review protocol takes into account some important aspects regarding conducting a systematic review about spontaneous preterm birth and metabolomics such as the criteria used for defining spontaneous preterm birth, different population risk stratification, method used to estimate gestational age, and metabolomics techniques details.
- Two independent reviewers are responsible for searching and selecting studies, as also extracting data, and a third reviewer will resolve any disagreement.
- If possible, proper statistical methods will be applied to investigate metabolomics accuracy in predicting spontaneous preterm birth.
- Possible limitations to this review include the different criteria applied for defining spontaneous preterm birth, and the diverse population risk stratification.

INTRODUCTION

Spontaneous preterm birth (sPTB) is the leading cause of perinatal mortality and short- and long-term morbidity [1,2]. It is defined as birth that occurs before 37 weeks gestation due to spontaneous onset of labour or preterm premature rupture of membranes (pPROM) [3,4]. Several pathways and mechanisms linked with preterm birth have been proposed including, neuroendocrine, vascular, immune-inflammatory, and behavioural processes [5]. More specifically, several markers associated with uterine distension/contraction, decidual inflammation/infection and activation of hypothalamic–pituitary–adrenal axis had been studied in the past decades [5,6]. However, no single marker or combination of markers has been found to be accurate enough for predicting sPTB [7–10]. History of previous preterm birth, cervical length at second trimester and cervico-vaginal fetal fibronectin are the most promising clinical tests for predicting spontaneous preterm, but they seem not to be clinically useful for asymptomatic women. Sensitivity of short cervical length (<25mm) and high cervico-vaginal fFN (>50ng/ml) are around 33-36% and 46%, respectively [11–13].

Preterm birth is a complex and multifactorial syndrome that possibly has a long pre-clinical phase, maternal and fetal interactions, genetic and environmental influences, and adaptive mechanisms [14,15]. These challenging aspects, and the presence of still unknown underlying mechanisms, are the main limitations for the identification of an accurate predictor for sPTB [16–18]. None of the predictors used in clinical practice, such as previous history of preterm birth, infection (vaginal and urinary contaminants), fibronectin and transvaginal ultrasonography cervical length demonstrated exceptional

accuracy for predicting spontaneous preterm birth [7]. An exploration of innovative approaches is urgently required.

Metabolomics is the study of metabolites, through identification and quantification of low-weight molecular particles, i.e. tens to hundreds thousands of intermediate products and substrates of systems biology chemical reactions [19,20]. This novel approach has been applied for identifying biomarkers and underlying biochemical pathways associated with complex obstetrical syndromes as preeclampsia, fetal growth restriction, gestational diabetes and preterm birth. In contrast to other "*Omics Sciences*" techniques, metabolomics is more closely associated with the phenotype of the disease and might thus identify a more robust and reliable set of predictors [21]. Importantly, implementing an adequate *Omics* experimental design is crucial for metabolomics studies. Using different baseline population (asymptomatic vs symptomatic or low- vs high-risk women for developing sPTB), study designs (prospective cohorts, case-control or cross sectional studies), sources of samples (amniotic fluid, vaginal fluid, blood, urine, hair, etc) and the timing of sample collection each have significant effects on study findings and the consequent interpretation and contribution to the current gap of knowledge [19].

Different reviews collating scientific knowledge regarding preterm birth biomarkers/predictors has been conducted. Different methodology approaches has been applied so far, including narrative, systematic and umbrella reviews, a more comprehensive review that includes not only original studies but also other reviews [7,22–24]. At the best of our knowledge, there is no systematic review on metabolomics markers. Therefore, we aim to conduct a systematic review of original studies investigating the use of metabolomics biomarkers for predicting spontaneous preterm

birth in asymptomatic pregnant women. This protocol describes the methods that will be applied in our systematic review.

METHODS AND ANALYSIS

The current systematic review proposal will be conducted, written and published following the Preferred Reporting Items for Systematics Reviews and Meta-Analyses (PRISMA-P) recommendations [25]. Also, it is properly registered at PROSPERO platform – code CRD42018100172.

Review question

What is the performance of metabolomics for predicting spontaneous preterm birth in asymptomatic pregnant women?

Eligibility Criteria

Original cohort or case-control studies involving asymptomatic pregnant women at the moment of sample collection (exposure) and with samples analysed using metabolomics techniques. Studies will be excluded if (1) they are cross-sectional studies, clinical trials, editorials, letter to editors, case reports, expert opinions, commentaries, or any type of review; (2) they describes only experimental studies with animals; or (3) they are duplicated data (e.g. data published in conferences proceedings and, then, published again in scientific journals). In this case, only the most complete publication will be considered, after comparing and confirming that the same technique and metabolites were explored. Studies published from 2008 to 2018 will be considered, and there will be no language restriction. Before submitting this systematic review for publication, we will rerun the search strategy to identify new studies that have been published after

performing the first round of search.

Participants

The current review is interested in evaluating the performance of metabolomics biomarkers for spontaneous preterm birth in asymptomatic pregnant women, which may contribute to clinical practice, potentially providing information regarding onset of preterm labour. Nevertheless, we aim to identify studies addressing only early predictors collected from women who are in an early preclinical stage, which might contribute to a wider window of opportunity for interventions and also to develop a widely reproducible screening test. Asymptomatic pregnant women should not have regular uterine tightening/contractions or signs of rupture of membranes (i.e. watery discharge). In addition, the study should preferably have a standardized definition of spontaneous preterm birth, the outcome of interest.

Information Sources

The search will be held in the following databases: PubMed, EMBASE, Scopus, CINAHL, and Web of Science, BVS/BIREME, which includes the Latin American and Caribbean Health Sciences Literature (LILACS), Medline and the Scientific Electronic Library Online (Scielo). In addition, secondary sources of original studies will be explored such as Google Scholar, hand-held searching of the reference list of eligible studies, conference proceedings, and contact with authors when necessary.

Search Strategy

The following terms will be used in our search strategy for the different scientific databases: (preterm birth, premature birth, premature infant, premature labor,

extremely premature infant, premature obstetric labor, spontaneous preterm birth, extreme preterm birth, late preterm birth, moderate preterm birth, preterm premature rupture of membranes, preterm delivery, PROM, sPTB, preterm PROM, pPROM, p-PROM) AND (metabolomic*, metabonomic*, metabolit*, lipidomic*, H NMR, proton NMR, proton nuclear magnetic resonance, liquid chromatogra*, gas chromatogra*, UPLC, ultra-performance liquid chromatograph*, ultra-performance liquid chromatograph*, HPLC, high performance liquid chrormatograph*, high-performance liquid chrormatograph*) AND (pregnan*, antenat*, ante nat*, prenat*, pre nat*) (Supplementary Material). Respective adaptations in the syntax of search for each database will be applied accordingly. No filters - such as “research in animal’s models” and “reviews” - will be used in our search strategy, as it will be excluded according to eligibility criteria. The complete search strategy, including Boolean terms, is provided as Supplementary Material.

Data Management

We will export search results to a reference manager (Mendeley®). Then, the following information will be collected from each study using an appropriate form, which will be entered in an Excel® spreadsheet: author’s name, year of publication, country, study design, number of participants with and without spontaneous preterm birth, type of metabolomics analysis technique (liquid or gas chromatography, nuclear resonance), laboratory methods for metabolites data acquiring (targeted or untargeted techniques, etc), subtype of preterm birth (spontaneous preterm labour or pPROM), number of fetuses (singleton vs multiple), gestational age when samples were collected, source of samples (type/site of tissue), low or high-risk for preterm birth (authors criteria used to define the population will be collected) and method applied to estimate gestational age.

If possible, additional variables related to spontaneous preterm birth categories (delivery before 28 weeks and before 34 weeks) will be recorded for secondary analyses. Original authors will be contacted to clarify data, when needed. Finally, we will check the biochemical class of identified metabolites in Human Metabolome Database (HMDB, version 4.0) to explore and synthetize whether there are common biological pathways associated with spontaneous preterm birth [20].

Selection Process

Two independent reviewers (RTS and RBFG) will be responsible for screening and selecting studies initially according to title or abstract. Both researchers will read the full text of non-excluded studies to discriminate eligibility. A third reviewer (DFBL) will consider any disagreement; additional reviewers (RPJ, PNB and JGC) will be responsible for supervising all steps and approving data extraction.

Data Collection Process

We will extract search results to a reference manager where all studies will be stored. Then, included studies will be placed in a new folder. Finally, we will manually extract data of interest from these included studies to an Excel® file. Each reviewer will have their own reference manager account, file and folder and discrepant results will be discussed together with the third reviewer.

Outcomes and Prioritization

The primary outcome is spontaneous preterm birth, defined as any birth occurred before 37 weeks of gestation due to spontaneous onset of labor or preterm premature rupture of membranes. Secondary outcomes are:

1. Spontaneous preterm birth before 28 weeks;
2. Spontaneous preterm birth before 32 weeks;
3. Spontaneous preterm birth before 34 weeks;

The capacity to predict different degrees of sPTB (categories of gestational age) is important as the extreme (<28wks), moderate (<32 weeks) and non-late preterm (<34wks) newborns have different adverse outcomes compared to non-extreme (≥28wks); non-moderate (≥32wks) or late (≥34 wks) preterm newborns.

Ideally, the method of gestational age estimation should be clearly reported. For instance, it can be reported as estimated by last menstrual period (LMP) and confirmed by an early ultrasound or only by an early ultrasound when LMP is unknown/uncertain.

Index test

Metabolomics techniques to predict spontaneous preterm birth is the diagnostic test of interest. Metabolomics is a technique to identify and quantify metabolites from biological samples using different type of platforms/equipment. The most common platforms include gas, liquid chromatography or ultra-performance liquid chromatography coupled to a mass spectrometer or a proton nuclear magnetic resonance [26]. The performance of the different thresholds of each metabolite will be compared and summarized through hierarchical summary receiver operator characteristic curve (HSROC) (meta-analysis) according to the subgroups described above. Considering that the raw data is not available in the majority of the diagnostic test accuracy studies [27] and that metabolites levels are usually reported as continuous variables, we intend to use a meta-analysis model based on ROC curves [28]. Briefly, a two-parameter model, based on the estimation of α and β parameters (using standard errors or maximum likelihood), will be applied as reported by

Kester & Buntinx [28]. Therefore, pooled ROC curves can be converted to an estimated ROC curve with 95% confidence interval. This method can also be applied in categorical-ordinal variables tests.

Risk of Bias in individual Studies

We will apply the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool [29] to assess the risk of bias and applicability of primary diagnostic accuracy studies. Each study will be classified as “low”, “high” or “unclear” regarding risk of bias for each of the four domains of QUADAS tool: Patient Selection, Index Test (metabolomics), Reference Standard (occurrence of preterm birth), and Flow and Timing of participant’s inclusion and follow-up. For example, studies will be labelled as “low” risk of bias for Reference Standard when definition of spontaneous preterm birth and gestational age estimation are clear; “high” risk of bias would be considered when the moment of sample collection is not well described.

Data Synthesis

We will report details of identification, screening, eligibility and included studies using a flow diagram, according to PRISMA recommendations [25]. Data from included studies will be synthetized into tables according to the variables of interest. If possible, we will present data meta-analysis according to study design, metabolomics technique and type of samples analysed. We intend to perform subgroup analysis according to:

- Different metabolomics methods applied: gas or liquid chromatography coupled with mass spectrometry or proton nuclear magnetic resonance;
- Singleton and multiple pregnancies;

- Low-risk and high-risk women for developing preterm birth;
- Subtype of preterm birth: Spontaneous preterm birth exclusively due to spontaneous onset of labour with intact membranes or sPTB due to premature rupture of membranes.
- Gestational age interval when samples were collected: 1st trimester, 2nd trimester and 3rd trimester.

Heterogeneity will be assessed by Cochran's Q, Hotelling's T-squared (τ^2) and I^2 tests. Funnel plots and sensitivity and cumulative analyses will be applied for detection of temporal trends and publication bias.

Potential anticipated limitations to this review

Firstly, although we have not considered any language restriction, we consider that there might be a limitation in studies published entirely in non-English language. However, in the last decade, more than 95% of scientific biomedical literature has been published in English [30], then we consider this a minor selection bias. Secondly, we intend to stratify the groups according to population risk. However, the characterization of low- or high-risk for spontaneous preterm birth is controversial and lacks standardization, which might limit data comparison and subgroup analysis. Finally, categorization of sPTB into spontaneous onset of labour or pPROM is another topic of potential limitation - the recognition of the main initial mechanism for preterm delivery might not always be possible. Even when specified, it might provoke uncertainty and could limit further considerations regarding preterm phenotypes. In addition, another limitation is that individual patient data will not be collected.

Patient and Public Involvement

Patients will not be directly involved in the study and no experience or direct impact from their perspective can be discussed.

ETHICS AND DISSEMINATION

This systematic review does not require ethical approval from the Research Council or Ethics board. We intend to disseminate our findings in scientific peer-reviewed journal, general free access website of Preterm Screening and Metabolomics in Brazil and Auckland (Preterm SAMBA) study, specialists' conferences, and to our funding agencies.

DISCUSSION

This systematic review will comprise current knowledge related with metabolomics in the context of preterm birth prediction. Metabolomics science, a resourceful innovative field that allows better understanding on pathophysiology of complex syndromes, may address the main compounds associated with the spontaneous preterm delivery and, therefore, motivate further researchers to validate early measurable predictors of preterm birth.

Metabolomics performance for predicting sPTB remains unclear and standardized and high-quality studies are needed to clarify the clinical application of metabolites for predicting sPTB. Nevertheless, metabolomics discovery studies commonly requires further validation studies; reproducible methodology is crucial. This systematic review protocol will collate the main potential early biomarkers, subgroup analysis and standardized definition for spontaneous preterm birth to better understand metabolomics performance in predicting sPTB and also to show its heterogeneity in terms of methodology (samples used, metabolomics technique, definition of SPTB phenotype,

etc). High performing predictors of preterm birth will help combat this leading cause of neonatal mortality and morbidity.

References

1 Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: when? Where? Why? *Lancet* 2005;**365**:891–900. doi:10.1016/S0140-6736(05)71048-5

2 Howson CP, Kinney M V, McDougall L, *et al.* Born Too Soon: Preterm birth matters. *Reprod Health* 2013;**10 Suppl 1**:S1. doi:10.1186/1742-4755-10-S1-S1

3 Blencowe H, Cousens S, Chou D, *et al.* Born Too Soon: The global epidemiology of 15 million preterm births. *Reprod Health* 2013;**10**:S2. doi:10.1186/1742-4755-10-S1-S2

4 Ananth C V, Vintzileos AM. Epidemiology of preterm birth and its clinical subtypes. *J Matern Fetal Neonatal Med* 2006;**19**:773–82. doi:10.1080/14767050600965882

5 Behrman R, ButlerAS, editors. *Institute of Medicine (IOM). Preterm Birth: Causes, Consequences, and Prevention.* Washington, D.C.: : National Academies Press 2007. doi:10.17226/11622

6 Manuck TA, Esplin MS, Biggio J, *et al.* The phenotype of spontaneous preterm birth: application of a clinical phenotyping tool. *Am J Obstet Gynecol* Published Online First: February 2015. doi:10.1016/j.ajog.2015.02.010

7 Honest H, Hyde CJ, Khan KS. Prediction of spontaneous preterm birth: no good test for predicting a spontaneous preterm birth. *Curr Opin Obstet Gynecol* 2012;**24**:422–33. doi:10.1097/GCO.0b013e328359823a

8 Conde-Agudelo A, Papageorghiou A, Kennedy S, *et al.* Novel biomarkers for the prediction of the spontaneous preterm birth phenotype: a systematic review and meta-analysis. *BJOG An Int J Obstet Gynaecol* 2011;**118**:1042–54. doi:10.1111/j.1471-0528.2011.02923.x

9 Hee L. Likelihood ratios for the prediction of preterm delivery with biomarkers. *Acta Obstet Gynecol Scand* 2011;**90**:1189–99. doi:10.1111/j.1600-0412.2011.01187.x

10 Goldenberg RL, Iams JD, Mercer BM, *et al.* The Preterm Prediction Study: toward a multiple-marker test for spontaneous preterm birth. *Am J Obstet Gynecol* 2001;**185**:643–51. doi:10.1067/mob.2001.116752

11 Iams JD, Goldenberg RL, Meis PJ, *et al.* The length of the cervix and the risk of spontaneous premature delivery. National Institute of Child Health and Human Development Maternal Fetal Medicine Unit Network. *N Engl J Med* 1996;**334**:567–72. doi:10.1056/NEJM199602293340904

12 Smith V, Devane D, Begley CM, *et al.* A systematic review and quality assessment of systematic reviews of fetal fibronectin and transvaginal length for predicting preterm birth. *Eur J Obstet Gynecol Reprod Biol* 2007;**133**:134–42.

doi:10.1016/j.ejogrb.2007.03.005

13 Abbott DS, Hezelgrave NL, Seed PT, *et al.* Quantitative fetal fibronectin to predict preterm birth in asymptomatic women at high risk. *Obstet Gynecol* 2015;**125**:1168–76. doi:10.1097/AOG.0000000000000754

14 Di Renzo GC. The great obstetrical syndromes. *J Matern neonatal Med* 2009;**22**:633–5. doi:10.1080/14767050902866804

15 Brosens I, Pijnenborg R, Vercruysse L, *et al.* The 'Great Obstetrical Syndromes' are associated with disorders of deep placentation. *Am J Obstet Gynecol* 2011;**204**:193–201. doi:10.1016/j.ajog.2010.08.009

16 Practice bulletin no. 130: prediction and prevention of preterm birth. *Obstet Gynecol* 2012;**120**:964–73. doi:10.1097/AOG.0b013e3182723b1b

17 Goldenberg RL, Culhane JF, Iams JD, *et al.* Epidemiology and causes of preterm birth. *Lancet* 2008;**371**:75–84. doi:10.1016/S0140-6736(08)60074-4

18 Honest H, Hyde CJ, Khan KS. Prediction of spontaneous preterm birth. *Curr Opin Obstet Gynecol* 2012;**24**:422–33. doi:10.1097/GCO.0b013e328359823a

19 Horgan RP, Clancy OH, Myers JE, *et al.* An overview of proteomic and metabolomic technologies and their application to pregnancy research. *BJOG* 2009;**116**:173–81. doi:10.1111/j.1471-0528.2008.01997.x

20 Wishart DS, Feunang YD, Marcu A, *et al.* HMDB 4.0: the human metabolome database for 2018. *Nucleic Acids Res* 2018;**46**:D608–17. doi:10.1093/nar/gkx1089

21 Dettmer K, Hammock BD. Metabolomics--a new exciting field within the omics sciences. *Environ Health Perspect* 2004;**112**:A396-7.http://www.ncbi.nlm.nih.gov/pubmed/15159211 (accessed 5 Sep 2017).

22 Lucaroni F, Morciano L, Rizzo G, *et al.* Biomarkers for predicting spontaneous preterm birth: an umbrella systematic review. *J Matern Neonatal Med* 2018;**31**:726–34. doi:10.1080/14767058.2017.1297404

23 Horgan RP, Clancy OH, Myers JE, *et al.* An overview of proteomic and metabolomic technologies and their application to pregnancy research. *BJOG* 2009;**116**:173–81. doi:10.1111/j.1471-0528.2008.01997.x

24 Romero R, Espinoza J, Gotsch F, *et al.* The use of high-dimensional biology (genomics, transcriptomics, proteomics, and metabolomics) to understand the preterm parturition syndrome. *BJOG An Int J Obstet Gynaecol* 2006;**113**:118–35. doi:10.1111/j.1471-0528.2006.01150.x

25 Shamseer L, Moher D, Clarke M, *et al.* Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ* 2015;**350**:g7647. doi:10.1136/BMJ.G7647

26 Zhang A, Sun H, Wang P, *et al.* Modern analytical techniques in metabolomics analysis. *Analyst* 2012;**137**:293–300. doi:10.1039/c1an15605e

27 McGrath TA, Alabousi M, Skidmore B, *et al.* Recommendations for reporting of systematic reviews and meta-analyses of diagnostic test accuracy: a systematic review. *Syst Rev* 2017;**6**:194. doi:10.1186/s13643-017-0590-8

28 Kester ADM, Buntinx F. Meta-analysis of ROC Curves. *Med Decis Mak* 2000;**20**:430–9. doi:10.1177/0272989X0002000407

29 Whiting PF, Rutjes AWS, Westwood ME, *et al.* QUADAS-2: A Revised Tool for the Quality Assessment of Diagnostic Accuracy Studies. *Ann Intern Med* 2011;**155**:529. doi:10.7326/0003-4819-155-8-201110180-00009

30 Rosselli D. The language of biomedical sciences. *Lancet* 2016;**387**:1720–1. doi:10.1016/S0140-6736(16)30259-8

Author´s Contributions

RTS and RBFG will conduct the systematic review as independent first reviewers. JGC, RPJ and DFBL will decide about conflicting decisions regarding papers selections. PNB, RPJ and JGC participated in the systematic review conception, methodology and framework, together will all the others co-authors.

Funding

This research was supported by Brazilian National Research Council (grant number 401636/2013-5) and Bill and Melinda Gates Foundation (grant number OPP1107597- Grand Challenges Brazil: Reducing the burden of preterm birth, FIOTEC number 05/2013), which provided funding to PRETERM-SAMBA project (www.medscinet.com/samba). RTS and DFL have been awarded PhD scholarships from the CAPES Foundation, an agency under the Ministry of Education of Brazil, process 88881.134095/2016-01 and 8881.134512/2016-01 respectively. The sponsors played no role on the study design or manuscript writing.

Competing interests

All authors are carrying original research about metabolomics and presenting conferences about this topic, including spontaneous preterm birth, preeclampsia, gestational diabetes mellitus and fetal growth restriction. Philip N Baker is principal investigator of Metabolomics Diagnostics Ltd, a company dedicated to develop innovative screening tests using metabolomics technology.

Acknowledgements

Ana Paula de Morais e Oliveira, librarian of University of Campinas – Unicamp, Brazil, for collaborating in developing search strategy and Rachel Hanisch for her suggestions to some sections of the paper.

Ethics approval and consent to participate

This systematic review does not require ethical approval from the Research Council or Ethics board.

Supplementary Material

		Seacrh strategy: #1 AND #2 AND #3						
		1 (OR for each term)	preterm birth					
			premature birth					
			premature infant					
			premature labor					
			extremely premature infant					
			premature obstetric labor					
			spontaneous preterm birth					
			extreme preterm birth					
			late preterm birth					
			moderate preterm birth					
			preterm premature rupture of membranes					
			preterm delivery					
			PROM					
			sPTB					
			preterm PROM					
			pPROM					
			p-PROM					
		2 (OR for each term)	metabolomic*					
			metabonomic*					
			metabolit*					
			lipidomic*					
			H NMR					
			proton NMR					
			proton nuclear magnetic resonance					
			liquid chromatogra*					
			UPLC					
			ultra-performance liquid chromatograph*					
			ultra performance liquid chromatograph*					
			HPLC					
			high performance liquid chromatograph*					
			high-performance liquid chromatograph*					
		3 (OR for each term)	pregnan*					
			antenat*					
			ante nat*					
			prenat*					
			pre nat*					

Supplementary Material

Search strategy according to literature database

PUBMED, CINAHL, SCOPUS

"preterm birth" OR "premature birth" OR "premature infant" OR "premature labor" OR "extremely premature infant" OR "premature obstetric labor" OR "spontaneous preterm birth" OR "extreme preterm birth" OR "late preterm birth" OR "moderate preterm birth" OR "preterm premature rupture of membranes" OR "preterm delivery" OR PROM OR sPTB OR "preterm PROM" OR pPROM OR p-PROM

AND

metabolomic* OR metabonomic* OR metabolit* OR lipidomic* OR "H NMR" OR "proton NMR" OR "proton nuclear magnetic resonance" OR "liquid chromatogra*" OR UPLC OR "ultra-performance liquid chromatograph*" OR "ultra performance liquid chromatograph*" OR HPLC OR "high performance liquid chromatograph*" OR "high-performance liquid chromatograph*"

AND

pregnan* OR antenat* OR "ante nat*" OR prenat* OR "pre nat*"

EMBASE

'preterm birth' OR 'premature birth' OR 'premature infant' OR 'premature labor' OR 'extremely premature infant' OR 'premature obstetric labor' OR 'spontaneous preterm birth' OR 'extreme preterm birth' OR 'late preterm birth' OR 'moderate preterm birth' OR 'preterm premature rupture of membranes' OR 'preterm delivery' OR PROM OR sPTB OR 'preterm PROM' OR pPROM OR p-PROM

AND

metabolomic* OR metabonomic* OR metabolit* OR lipidomic* OR 'H NMR' OR 'proton NMR' OR 'proton nuclear magnetic resonance' OR 'liquid chromatogra*' OR UPLC OR 'ultra-performance liquid chromatograph*' OR 'ultra performance liquid chromatograph*' OR HPLC OR 'high performance liquid chromatograph*' OR 'high-performance liquid chromatograph*'

AND

pregnan* OR antenat* OR 'ante nat*' OR prenat* OR 'pre nat*'

BVS/BIREME

("preterm birth" OR "premature birth" OR "premature infant" OR "premature labor" OR "extremely premature infant" OR "premature obstetric labor" OR "spontaneous preterm birth" OR "extreme preterm birt" OR "late preterm birth" OR "moderate preterm birth" OR "preterm premature rupture of membranes" OR "preterm delivery" OR prom OR sptb OR "preterm PROM" OR pprom OR "p-PROM")

AND

(metabolomic* OR metabonomic* OR metabolit* OR lipidomic* OR h nmr OR "proton NMR" OR "proton nuclear magnetic resonance" OR "liquid chromatogra*" OR uplc OR "ultra-performance liquid chromatograph*" OR "ultra performance liquid chromatograph*" OR hplc OR "high performance liquid chromatograph*" OR "high-performance liquid chromatograph*")

AND

(pregnan* OR antenat* OR "ante nat*" OR prenat* OR "pre nat*") AND (instance:"regional") AND (year_cluster:("2015" OR "2011" OR "2016" OR "2014" OR "2013" OR "2012" OR "2009" OR "2017" OR "2010" OR "2008" OR "2018"))

Web of Science

preterm birth OR premature birth OR premature infant OR premature labor OR extremely premature infant OR premature obstetric labor OR spontaneous preterm birth OR extreme preterm birth OR late preterm birth OR moderate preterm birth OR preterm premature rupture of membranes OR preterm delivery OR PROM OR sPTB OR preterm PROM OR pPROM OR p-PROM

AND

metabolomic* OR metabonomic* OR metabolit* OR lipidomic* OR H NMR OR proton NMR OR proton nuclear magnetic resonance OR liquid chromatogra* OR UPLC OR ultra-performance liquid chromatograph* OR ultra performance liquid chromatograph* OR HPLC OR high performance liquid chromatograph* OR high-performance liquid chromatograph*

AND

pregnan* OR antenat* OR ante nat* OR prenat* OR pre nat*

SCIELO

("preterm birth" OR "premature birth" OR "premature infant" OR "premature labor" OR "extremely premature infant" OR "premature obstetric labor" OR "spontaneous preterm birth" OR "extreme preterm birth" OR "late preterm birth" OR "moderate preterm birth" OR "preterm premature rupture of membranes" OR "preterm delivery" OR PROM OR sPTB OR "preterm PROM" OR pPROM OR "p-PROM")

AND

(metabolomic* OR metabonomic* OR metabolit* OR lipidomic* OR H NMR OR "proton NMR" OR "proton nuclear magnetic resonance" OR "liquid chromatogra*" OR UPLC OR "ultra-performance liquid chromatograph*" OR "ultra performance liquid chromatograph*" OR HPLC OR "high performance liquid chromatograph*" OR "high-performance liquid chromatograph*")

AND

(pregnan* OR antenat* OR "ante nat*" OR prenat* OR "pre nat*")

4.5. Artigo *Use of metabolomics for the identification and validation of clinical biomarkers for preterm birth: Preterm SAMBA*

Cecatti *et al. BMC Pregnancy and Childbirth* (2016) 16:212
DOI 10.1186/s12884-016-1006-9

BMC Pregnancy and Childbirth

STUDY PROTOCOL Open Access

Use of metabolomics for the identification and validation of clinical biomarkers for preterm birth: Preterm SAMBA

Jose G. Cecatti[1*], Renato T. Souza[1], Karolina Sulek[2], Maria L. Costa[1], Louise C. Kenny[3], Lesley M. McCowan[4], Rodolfo C. Pacagnella[1], Silas G. Villas-Boas[5], Jussara Mayrink[1], Renato Passini Jr[1], Kleber G. Franchini[6], Philip N. Baker[2] and for the Preterm SAMBA and SCOPE study groups

Abstract

Background: Spontaneous preterm birth is a complex syndrome with multiple pathways interactions determining its occurrence, including genetic, immunological, physiologic, biochemical and environmental factors. Despite great worldwide efforts in preterm birth prevention, there are no recent effective therapeutic strategies able to decrease spontaneous preterm birth rates or their consequent neonatal morbidity/mortality. The Preterm SAMBA study will associate metabolomics technologies to identify clinical and metabolite predictors for preterm birth. These innovative and unbiased techniques might be a strategic key to advance spontaneous preterm birth prediction.

Methods/design: Preterm SAMBA study consists of a discovery phase to identify biophysical and untargeted metabolomics from blood and hair samples associated with preterm birth, plus a validation phase to evaluate the performance of the predictive modelling. The first phase, a case–control study, will randomly select 100 women who had a spontaneous preterm birth (before 37 weeks) and 100 women who had term birth in the Cork Ireland and Auckland New Zealand cohorts within the SCOPE study, an international consortium aimed to identify potential metabolomic predictors using biophysical data and blood samples collected at 20 weeks of gestation. The validation phase will recruit 1150 Brazilian pregnant women from five participant centres and will collect blood and hair samples at 20 weeks of gestation to evaluate the performance of the algorithm model (sensitivity, specificity, predictive values and likelihood ratios) in predicting spontaneous preterm birth (before 34 weeks, with a secondary analysis of delivery before 37 weeks).

Discussion: The Preterm SAMBA study intends to step forward on preterm birth prediction using metabolomics techniques, and accurate protocols for sample collection among multi-ethnic populations. The use of metabolomics in medical science research is innovative and promises to provide solutions for disorders with multiple complex underlying determinants such as spontaneous preterm birth.

Keywords: Spontaneous preterm birth, Metabolomics, Prediction, Biological biomarker, Mass spectrometry

* Correspondence: cecatti@unicamp.br
[1]Department of Obstetrics and Gynecology, University of Campinas (UNICAMP) School of Medical Sciences, R. Alexander Fleming, 101, Campinas, SP, 13083-881, Brazil
Full list of author information is available at the end of the article

BioMed Central

© 2016 The Author(s). **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.

Background

Despite improvements in antenatal and neonatal care, the number of premature newborns each year has not significantly decreased since the 1960s. Preterm birth (PTB) is the leading cause of neonatal morbidity and mortality and a major contributor to loss of life, long-term disability, and health care costs [1–4]. The associated morbidity, mortality and high health costs have been well documented with premature infants facing life-threatening short and long term complications [5–8].

Despite the enormity of the health economic burden of preterm birth, and many years of focused research, a common aetiology and/or predictive test have not yet been identified. Spontaneous preterm birth (sPTB) is considered one of the "Great Obstetrical Syndromes", which are conditions resulting from complex interactions between the maternal and fetal genome and the environment and which have a long preclinical period, fetal involvement and adaptive functioning in nature [9]. This reflects the multifactorial nature of this condition and the need to apply strategies that are capable of identifying multiple markers simultaneously in parallel with the assessment of clinical and biophysical risk factors.

There are many clinical [10–13] and biochemical risk factors [14–16] associated with sPTB and it is likely that these biochemical markers are present in the maternal blood long before the onset of a preterm labour. However, although certain candidate-driven approaches to studying these changes show promise, this has not resulted in effective predictive biomarkers for the general pregnant population. Due to these complex and dynamic characteristics of sPTB syndrome, it remains a difficult task to identify women and babies at risk.

Currently, the selection of women likely to deliver prematurely from clinical risk factors alone lacks the sensitivity required to effectively identify the majority of patients at risk of idiopathic sPTB [14]. Furthermore, parameters derived from previous obstetric history cannot be applied to nulliparous women. The association of biophysical predictors such as cervical length and/or vaginal biomarkers (fibronectin and phosphorylated insulin-like growth factor binding protein-1) enhances accuracy for prediction and enables more effective interventions for selected women. There are therapeutic interventions available for the prevention of sPTB, such as the use of progesterone [10, 17, 18] and cervical pessary [19]. Despite advances in selection of eligible women for such therapeutic interventions, the efficacy of cervical length or fetal fibronectin levels in asymptomatic women are still limited and seem to be more capable of discriminating women at lower risk than those at higher risk [20–22]. Owen et al. showed that almost 50 % of women with cervical length between 15 and 25 mm did not deliver before 35 weeks, as well as approximately 70 % with cervical length between 25–30 mm [23].

The development of a predictive test for spontaneous preterm birth would help to accurately identify a high-risk population. To be effective, therapies need to be commenced at a gestational age in which they are likely to be of benefit. A sensitive early pregnancy-screening test would facilitate the timely administration of prophylactic treatments to those women at highest risk. The development of physics, biology and medicine translational research can provide a comprehensive approach for biological processes with complex pathways and regulations. Metabolomics offers an unbiased hypothesis generating approach to identify and validate potential candidate metabolomic biomarkers [24, 25].

We propose a multi-strategy approach to biomarker discovery and validation through the establishment of a large early pregnancy biobank of appropriate samples, in conjunction with the application of analytical methods capable of quantifying multiple blood-borne species simultaneously, and using some clinical and epidemiological markers to identify women at highest risk of spontaneous preterm birth.

The development of predictive tests that translate into clinical care can be divided into two distinct phases; (i) hypothesis generation after acquisition of data, a non-biased process where no or limited biological knowledge is required and (ii) validation of generated hypotheses [26]. The Preterm SAMBA study goal spans both phases and aims to identify a clinically useful early pregnancy-screening test to ascertain which pregnancies are at risk of developing sPTB. Discovery-based methods will be applied to blood and hair samples taken from carefully matched phenotypes in both cohorts (preterm and term deliveries) to develop a predictive algorithm to identify those women at increased risk of sPTB and test the effectiveness of such an algorithm in a prospective cohort.

Methods/design

Preterm SAMBA, an international collaborative multicentre study for the development of predictive tests that translate into clinical care, can be divided into two distinct phases: The first component (Discovery phase) is a case–control study that aims to identify clinical and metabolomics biomarkers related to spontaneous preterm birth. For this initial phase, untargeted metabolomics techniques will be employed to identify and quantify potential predictor's metabolites that can be associated to potential clinical predictors. The second component (Validation phase) is a cohort study developed to validate the algorithm of prediction using the clinical and metabolomics biomarkers discovered in

the first component of the study. Thus, to evaluate the performance of the prediction model developed at the first phase, targeted metabolomics techniques will be employed to analyse participants' blood and hair samples to quantify those specific metabolites identified as potential predictors of preterm birth.

Discovery phase

The initial phase of the project consists of a case–control study utilizing data and samples collected for the SCOPE study (Screening for Pregnancy Endpoints study). The SCOPE consortium was an international effort to determine the causes and potential predictors for pregnancy complications and its methodology had already been previously published [27–29]. Briefly, the cohort comprised 5690 healthy pregnant women recruited between November 2004 and August 2008 in New Zealand, Australia, Ireland and United Kingdom. Inclusion and exclusion criteria for the SCOPE study are described in Tables 1 and 2, respectively. Exclusion criteria include major fetal anomaly, chronic hypertension, diabetes, renal disease, systemic lupus erythematous, and antiphospholipid syndrome. These will therefore be the same criteria for the current study.

Extensive sociodemographic and physical data will be collected including age, ethnicity, socio-economic status, dietary and lifestyle questionnaire, parity, BMI (body mass index) and cigarette smoking.

Plasma and serum samples will be collected at 20 weeks of gestation using stringent standard operating procedures designed for metabolomics studies, barcoded and stored at −80 °C within 2–4 h; the timing between collection and freezing will be known for all specimens.

Several Standard Operating Procedures (SOP) for sample preparation by removal of proteins via ultrafiltration were developed and validated. The analysis of deproteinized plasma samples will be performed employing gas chromatography and liquid chromatography mass spectrometry (GC-MS and LC-MS). GC-MS and LC-MS techniques will be performed as described previously [30, 31]. Quality control samples (acquired by pooling plasma from all subjects) will be interspersed in every 5th run to assess reproducibility and validity. It is envisaged that the socioeconomic/physical/biomarker discovery phase of the Preterm SAMBA study will identify several candidate markers and predictive multivariate models. Discriminatory metabolites will be translated to a targeted triple quad MS (QQQ-MS) platform, to be used in the validation phase.

Table 1 Inclusion criteria of Preterm SAMBA validation phase – Brazilian cohort

• Singleton pregnancy
• Nulliparous (no previous delivery ≥20 weeks)
• Up to 21 weeks of gestational age

Table 2 Exclusion criteria of Preterm SAMBA validation phase – Brazilian cohort

• Unsure LMP and unwilling to have dating US	• Major Uterine Anomaly
• ≥3 Miscarriages	• Cervical Suture
• Major Foetal Anomaly/Abnormal Karyotype	• Knife cone biopsy
• Essential Hypertension Treated Pre-pregnancy	• Ruptured membranes now
• Mod-Severe Hypertension at booking (≥160/100 mmHg)	• Long term Steroids
• Pre-pregnancy Diabetes	• Low-dose Aspirin
• Renal Disease	• Calcium (>1 g/24 h)
• Systemic Lupus Erythematosus	• Eicosapentaenoic acid (fish oil)
• Anti-phospholipid Syndrome	• Vit. C ≥1000 mg & Vit. E ≥400 UI
• Sickle Cell Disease	• Heparin/LMW Heparin
• HIV or Hep B or Hep C positive	

The proportion of pregnancies complicated by any preterm birth is approximately 10 %. Preterm SAMBA Discovery Phase will randomly select 100 women (n = 100) whose pregnancies reached term as compared to fifty randomly sampled pregnancies (n = 50) complicated by spontaneous preterm birth prior to 37 weeks gestation, in each of the Cork Ireland and Auckland New Zealand SCOPE cohorts (Fig. 1). The 20th week samples and data will be analysed to identify sPTB potential predictors. Considering there were no previous studies on this topic for preterm birth, these numbers were empirically estimated using a similar study performed for pre-eclampsia [31]. Using a type I error of 0.01, type II error of 0.10, a ratio between controls and cases of 1:1, an AUC of 0.9 and an OR of 10, the estimated sample of preterm birth is 49. We then anticipated around 50 preterm birth for each of the two centers.

Validation phase – the Brazilian multicentre cohort study

The Preterm SAMBA validation phase consists of a Brazilian multicentre cohort study with 1150 low-risk pregnant nulliparous women. Five of the 27 members of the Brazilian Network for Studies on Reproductive and Perinatal Health (BNSRPH), were chosen to participate in the Brazilian cohort (Table 3). Previous excellence performance in epidemiological and translational studies and diversity of cultural, ethnical and sociodemographic population characteristics were criteria for centre selection. Therefore, there are participating centres in three of the five regions of Brazil, which are the three most populated regions of the country: Northeast, Southeast and South.

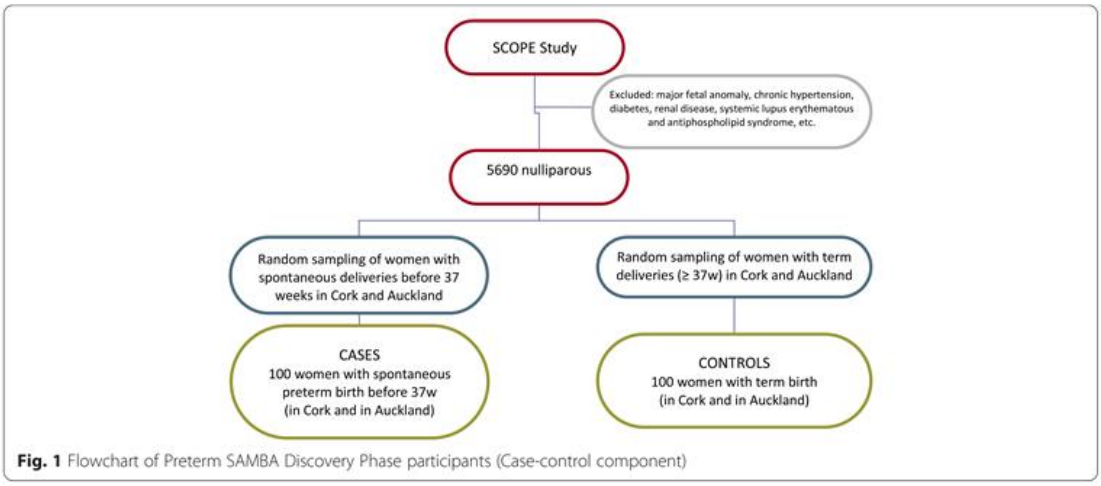


Fig. 1 Flowchart of Preterm SAMBA Discovery Phase participants (Case-control component)

Assuming a type I error rate, α, of 5 % and an estimated area under ROC curve of at least 0.68, then in order to test hypotheses to a suitable level of power (80 % power, $\beta = 0.2$), the sample size sufficient should approximate to 80 cases of spontaneous preterm birth (<34 weeks gestation), calculated using MEDCALC®. Based on a minimum expected preterm birth rate of 7 %, the total cohort size should therefore be of approximately 1150 subjects, around 230 women at each participating centres.

Recruitment and data collection

The recruitment strategies include approaching existing pregnant women in participating facilities during prenatal care visits and with website/internet, flyers and local community advertisings. After the identification of potential participants, the research assistant will invite women and obtain an informed consent form of those who meet the inclusion criteria and agree to participate. Maternal age and ethnicity will be recorded from all approached women to facilitate a comparison of those who are recruited and those who decline.

Table 3 Participating centres in the Preterm-SAMBA study validation phase – Brazilian cohort

Maternity of CAISM, University of Campinas, in Campinas, São Paulo.
Maternity of the School of Medicine from UNESP, in Botucatu, São Paulo.
Maternity of the Clinic Hospital, Federal University of Rio Grande do Sul, in Porto Alegre, Rio Grande do Sul.
Maternity of the Clinic Hospital, Federal University of Pernambuco, in Recife, Pernambuco.
MEAC – School Maternity of the Federal University of Ceará, in Fortaleza, Ceará.

All collected data will preferably be entered directly into the database, but printed forms will also be available in case of inability to access the internet-based database. In such cases, the data will be then entered later and completed printed forms will be stored, according to the required ethical principles.

Sociodemographic, physical data and pregnancy outcomes

First Visit (19–21 weeks): similarly to the SCOPE study, detailed information of sociodemographic characteristics (age, socioeconomic status, education, ethnicity, occupation and type of maternity care), maternal medical and obstetric history, infertility history, drugs and medications use, family medical and obstetric history and current pregnancy (occurrence and details of infection, vaginal bleeding, dipstick proteinuria, intercourse and hospital admission) will be collected.

Anthropometric measurements of maternal body mass index, height, weight, head circumference, arm circumference and triceps, biceps subscapular and suprailiac skinfolds will be performed according to standardized techniques. Height and weight of lightly clothed women will be measured to the nearest 0.1 mm and 0.1 kg respectively. Head and arm circumferences will be measured with an inelastic tape and skinfold thicknesses will be measured on the same side of the body to the nearest 0.2 mm using Harpenden (and/or Lange) skinfold calliper. The calliper is placed 1 cm distal to the firmly grasped skinfold, using the thumb and the index finger, at 90° to the skin. A single measurement is taken after 2 s.

Dietary intake will be assessed using a 24-h dietary recall administered by a trained professional who will query participants about food and beverage consumption

in the previous 24 h. A trained nutritionist will then estimate calories, macro and micronutrient intake using computer-based standard tables allowing for appropriate ethnic, social and regional variations.

Furthermore, three consecutive manual blood pressure measurements will be recorded, using an appropriate cuff size for different arm circumferences and using Korotkoff phase V for diastolic blood pressure.

Second and third Visit (27–29 weeks and 37–39 weeks; both optional): three consecutive manual blood pressure measurements, anthropometric parameters (weight, height, head and arm circumference and triceps, biceps, subscapular and suprailiac skinfolds) and occurrence and characteristics of infection, vaginal bleeding, dipstick proteinuria, intercourse and hospital admissions will be recorded.

Postpartum data: data will be collected from the participant's medical record, the prenatal chart and/or from a personal interview with the participant during hospital admission to minimize missing information. The main outcome is spontaneous preterm birth, defined as a birth before 34 weeks of gestational age due to preterm labour or premature rupture of membranes. Secondary outcomes will also be evaluated: spontaneous preterm birth alternatively defined as a birth before 37 weeks of gestational age due to preterm labour or premature rupture of membranes, provider-initiated preterm birth, defined as preterm birth due to medical indication on account of maternal or fetal conditions; pre-eclampsia, defined as having systolic blood pressure ≥ 140 or systolic blood pressure ≥ 90 mmHg after 20 weeks gestation on at least two occasions apart of 20 min, and/or proteinuria (24-h urinary protein ≥ 300 mg or urine dipstick ≥ ++) and/or severe maternal complications [32]; gestational diabetes mellitus according to ADA guidelines [33]; fetal growth restriction (FGR) defined as having birthweight below 10th percentile based on GROW customised birthweight centiles [34]. Clinical data will also be collected regarding the occurrence of preterm labour, cervical cerclage, deep vein thrombosis, infection, vaginal bleeding, dipstick proteinuria, intercourse, hospital admission, deep vein thrombosis during pregnancy and puerperium, and maternal mortality and the use of progesterone and/or pessary, tocolytic, antibiotic for preterm labour or pPROM, corticosteroids for fetal maturation, magnesium sulphate for neuroprotection during pregnancy. The occurrence of severe maternal morbidity and near miss will also be reported according to WHO guidelines [35]. Neonatal outcomes related to neonatal morbidity and mortality will be recorded until newborn discharge or death.

Sample collection, processing and storage

Non-fasting blood samples will be collected at 20 (between 19 and 21) weeks of gestational age (Fig. 2). All research assistants will be trained according to specific and detailed Standard Operation Protocols (SOPs) developed for sample collection, processing and storage. One of the study coordinators was trained by the SCOPE team, to guarantee adequate understanding of all necessary procedures. A maximum of 20 mL of blood will be collected to provide serum and plasma specimens. The blood specimens will be stored in 250 μL 2-D barcoded cryovials after one centrifugation cycle for plasma specimens (2000×g for 10 min at 4 °C) and two centrifugation cycles for serum (2000×g for 10 min and 2400×g for 10 min at 4 °C). The blood samples will be processed and frozen at −80 °C within 2–4 h. The time interval between collection and freezing will be recorded for all specimens.

Although the Preterm SAMBA strategy and workflow is focussed around the analysis of blood samples, we recently reported a proof-of-concept study, which highlighted the potential use of the hair metabolome in the prediction of pregnancy complications [36]. Hair samples will also be collected at 20 weeks' gestation. Samples (20–30 hair strands for each participant) will be collected from the occipital area, 0.5 cm away from the scalp, using blunt scissors. Then, hair will be packed in aluminium foil and stored at room temperature [36]. A unique linear barcode will be pasted on each hair package. All specimens and quality control information will be registered in the database.

Database

A specific database for the Preterm SAMBA Brazilian Cohort was developed together with MedSciNet, a Swedish based company specialized in the design and

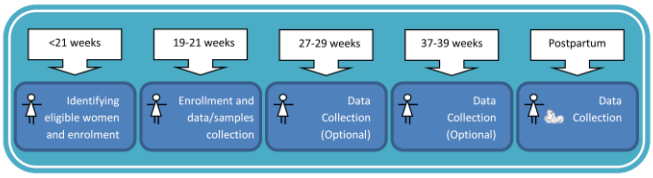


Fig. 2 Visits of Preterm-SAMBA Validation Phase

development of online database systems linked with biobanks management systems, similarly to the database previously used for the SCOPE. The preterm SAMBA database will be centralized, secure, internet-based and FDA (United States Food and Drug Administration) and HIPAA (Health Insurance and Accountability Act of 1996, United States Security and Privacy Rules) compliant, which allows continuous data entry and monitoring of study progress. Completeness of clinical data and specimen collection will be constantly monitored, with incomplete fields 'flagged' for attention. The database allows several monitoring procedures with hierarchical access licenses and tracking system for all specimen aliquots stored. To comply with biobank regulatory issues, patients will only be identified by a unique study number. Pseudoanonymised metadata and interim data will be stored using our laboratory information management system. The identifying information about participants will be kept in a separate and secure local database.

Data and sample quality

Several procedures to enhance and assure data and sample quality will be adopted. All entered data will be prospectively and retrospectively monitored. During data entry, internal consistency of variables is performed and error messages are automatically flagged. After completing the collection of data from a participant, all information needs to be reviewed by a local monitor. Then, the final form has to be signed by the local principal investigator (PI) in order to be incorporated in the final database. The coordinating centre (Campinas, Brazil) will also perform a centralized monitoring of data and samples. An initial meeting with all researchers from Brazilian participating centres has been held to discuss the final protocol, procedures to be implemented, their particular characteristics and necessary approaches to be used to guarantee the implementation of the study. Another general meeting at the end of study is planned in order to discuss results, strategies for manuscripts' writing and submission and other related topics.

The coordinating centre will randomly select approximately 10 % of printed completed forms to carry out a check and validation of data from the forms and database entry during the first and second half of the study. This double-check procedure enhances data quality and decreases typing errors.

The record of information regarding sample collection, processing (precentrifugation and centrifugation) and storage processes will follow the Standard Preanalytical Coding for biospecimens (SPRECs) protocol, developed and recommended by the International Society for Biological and Environmental Repositories Biospecimen (ISBER) Science Working Group [37]. This protocol enables standardization of preanalytical information, using standard codes to refer to the techniques and conditions to which the samples were submitted.

Metabolomics analysis

The precise methodology to be used in the validation phase will depend on the ongoing discovery studies. As detailed above we anticipate that it will be based on a targeted triple quad MS (QQQ-MS) platform, as previously described [38]. We will subsequently describe details relating to metabolomics analysis techniques and metabolomics statistical analyses. We anticipate that data analysis will be integrated into the relational database such that decision rules may combine both clinical and spectrometric data.

The performance of the final algorithm developed in the discovery phase will be evaluated by its capacity to predict spontaneous preterm birth occurrence in women from the Brazilian cohort. The validation will be performed using the average squared difference between predicted and observed outcome (R^2), adjusted R^2 (same as R^2, but penalizes for the number of predictors), sensitivity, specificity, positive and negative predictive values, likelihood ratio and the area under the ROC curve.

Ancillary studies

The Preterm SAMBA Brazilian cohort study will collect additional data regarding other relevant maternal and fetal obstetric complications. Detailed clinical data related to the occurrence and severity of pre-eclampsia, fetal growth restriction and gestational diabetes mellitus will be recorded. Fetal growth restriction will be diagnosed if birthweight is below 10th customised percentile. The occurrence of severe maternal morbidity, maternal near miss and maternal mortality during pregnancy or up to discharge after delivery will also be recorded, according to WHO definitions [35].

Ethical aspects

The SCOPE study, whose data and samples will be analysed for the Preterm SAMBA discovery phase, was approved by local ethics committees in New Zealand and Ireland and registered in the Australian and New Zealand Clinical Trial Registry (ACTRN12607000551493) [28]. All women who participated in the SCOPE study provided written informed consent and agreed to have their data and samples used in other studies. The Preterm SAMBA study has been reviewed and approved by the National Committee for Ethics in Research of Brazil (CONEP) and by the Institutional Review Board (IRB) of the coordinating centre (Letter of approval 1.048.565 issued on 28th April 2015) and of all other Brazilian participating centres. All women who will be enrolled in the

Preterm SAMBA Brazilian cohort (Validation phase) will sign an informed consent form, also allowing for future additional studies with their biological samples without any additional consent.

The ethical principles stated in the Brazilian National Heath Council (Resolution CNS 466/12) will be respected in every stage of this study. The anonymity of the source of information will be guaranteed and the care for the women will be provided independent of her agreement to participate in the study. All ethical principles related to biobank storage and transport will be followed according to national and international rules related to research with human beings. The study also complies with the Declaration of Helsinki amended in Hong Kong in 1989. The methodological and ethical aspects of Preterm SAMBA study protocol were developed following STROBE guidelines [39].

Discussion

The *"Omics"* Science comprises genomics, transcriptomics, proteomics and metabolomics technologies, which each provide valuable translational surveys in biological processes. A metabolomics approach enables the evaluation of metabolic pathways and the correlation of biochemical changes related to pathophysiology of disease, providing a downstream result of gene expression and higher sensitivity to phenotype of disease [40–45]. Underlying conditions and factors related to the occurrence, severity or prognosis of diseases with complex determinants may be assessed, bringing to light the final product of organism metabolism: the metabolome [42].

The development of a two-phase metabolomics research program that includes two large cohorts of nulliparous women is not an easy task. The network collaboration is essential to develop, implement and analyse such complex data and, more importantly, to achieve reliable results. Precise protocols for sample collection, processing, storage and biobank management will be essential to assure high quality data and results.

Metabolomics profiling requires different techniques to address the detection and quantification of different classes of metabolites once there is no current method capable to identify all of them. Preterm SAMBA study will employ different untargeted techniques that require very carefully and standardized protocols for sample preparation [24]. Studying the metabolome in blood samples requires invasive collection and immediate processing. As an alternative, hair samples are non-invasive, do not need processing methods and can be stored at room temperature. Hair can, theoretically, reflect endogenous compounds and environmental exposures from many days/weeks ago. The determination of the hair metabolome is a possible approach to identify biomarkers for spontaneous preterm birth. It has already been explored in gestational diabetes and fetal growth restriction, revealing potential endogenous mechanisms involved in those pathologic conditions [36, 45].

The identification of spontaneous preterm birth predictors using multi-ethnic data/samples and the evaluation of performance in a culturally and ethnically different population is desirable and meaningful for external validation. The use of quality control records and SPREC protocol is another important recommendation for metabolomics studies due to the necessity to evaluate confounders for analytical measures such as the time between sample collection, storage and processing conditions and the occurrence of haemolysis, lipaemia and metabolic degradation on account of inadequate temperature or solar exposure [37].

In the context of translational research, metabolomics may enhance understanding of the underlying pathways, which lead to obstetric complications. Preterm SAMBA aims to identify and validate a predictive model for spontaneous preterm birth, but will also develop a biobank and database that will enable research on pre-eclampsia (PE), fetal growth restriction (FGR) and gestational diabetes mellitus (GDM). The possibility to combine biochemical, genetics and clinical information that can be large-scale and replicable empowers the development of knowledge for clinical practice in preterm birth prevention. This would be especially worthwhile and helpful for countries with a high proportion and high absolute number of preterm births as is the case of Brazil where around 12 % of all births occur prematurely [46].

A recent clustered designed study showed that 30 % of all spontaneous preterm births do not have any maternal, fetal or placental conditions identified that could be related to its occurrence [47]. The application of metabolomics techniques could be a promising approach for spontaneous preterm birth prediction, all the more in those cases of silent phenotype in which there are no known predictors. Metabolomics have been already described in other obstetric conditions as pre-eclampsia, gestational diabetes mellitus and fetal growth restriction [31, 40, 41, 45, 48, 49], showing excellent performance in terms of a discriminatory algorithm. Therefore, we believe metabolomics is a powerful and strategic key not only for preterm birth prediction, but hopefully also for its prevention. The detection of metabolic pathways related to PTB syndrome may enable the development of more accurate therapies for primary or secondary prevention of pregnant women identified as at high-risk.

At the end of the study, if we are successful in the identification of such an effective algorithm, certainly several other topics should be carefully considered.

Can this knowledge be really translated into a commercially available kit for screening purposes? Would the costs derived from this process be acceptable for low and middle-income countries? How will this be made available for populations in public sector? For discussing a future implementation of such a screening strategy, the following necessary points to be covered are to know if a concrete package of interventions to reduce preterm birth among those women identified as high-risk is available, and if it is cost-effective to be supported by the public health system. Finally, in this study we are planning to transfer the technology developed for the algorithm from New Zealand to Brazil, including lab technologies for assessing the biomarkers identified by metabolomics for preterm birth. Hopefully, if this is proved to be feasible, we believe that an important step for reducing the burden of preterm birth will have been achieved.

Abbreviations

ADA, American Diabetes Association; BMI, body mass index; CONEP, Brazilian National Committee for Ethics in Research; FAME, fatty acid methyl esterification; FDA, Food and Drug Administration; FGR, fetal growth restriction; GC, gas chromatography; GDM, gestational diabetes mellitus; LC, liquid chromatography; MS, mass spectrometry; PE, pre-eclampsia; PI, principal investigator; pPROM, preterm premature rupture of membranes; PTB, preterm birth; SAMBA, Screening and Metabolomics in Brazil and Auckland; SCOPE, Screening for Pregnancy Endpoints study; SOP, standard operation procedures; sPTB, spontaneous preterm birth; WHO, World Health Organization

Acknowledgements

The Preterm SAMBA study group also included: Mary A. Parpinelli, Jussara Mayrink, School of Medical Sciences, University of Campinas, Brazil; Kleber G. Franchini, LNBio, Campinas, Brazil; Iracema M. Calderon, Bianca F. Cassettari, School of Medicine of Botucatu, UNESP, Brazil; Janete Vetorazzi, Lucia Pfitscher, School of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil; Edilberto P. Rocha Filho, Débora F Leite, School of Medicine, Federal University of Pernambuco, Recife, Brazil; Francisco E. Feitosa, Carolina L. Costa e Silva, School of Medicine, Federal University of Ceará, Fortaleza, Brazil.
The SCOPE study group also included: Lucilla Poston, King's College London and King's Health Partners, London, UK; Jenny E. Myers, Maternal & Fetal Health Research Centre, University of Manchester, UK; Nigel A.B. Simpson and James J Walker, University of Leeds, UK; Gus A. Dekker and Claire T. Roberts, University of Adelaide, South Australia.

Funding

This was one of the two big studies selected for sponsoring from the research call "Grand Challenges Brazil: Reducing the burden of preterm birth" number 05/2013 jointly issued by the Brazilian National Research Council (CNPq) and the Bill and Melinda Gates Foundation (Award 401636/2013-5). The funders played no role at all in the study design, writing the manuscript nor in the decision to submit the manuscript for publication. LC Kenny is supported by a Science Foundation Ireland Program Grant for INFANT (12/RC/2272). The SCOPE database is provided and maintained by MedSciNet AB (http://medscinet.com). The New Zealand SCOPE study was funded by the New Enterprise Research Fund, Foundation for Research Science and Technology; Health Research Council (04/198); Evelyn Bond Fund, Auckland District Health Board Charitable Trust. The Irish SCOPE study was funded by the Health Research Board of Ireland (CSA/2007/2; http://www.hrb.ie).

Authors' contributions

All authors contributed to the overall study design and specific methodologies. JGC, PNB, MLC and RCP conceived the study design. RTS, JGC, RCP and RP planned the implementation of the study. RTS and JGC drafted the manuscript. KS, LCK and SVB participated in the design of the metabolomics methods for essays. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Obstetrics and Gynecology, University of Campinas (UNICAMP) School of Medical Sciences, R. Alexander Fleming, 101, Campinas, SP, 13083-881, Brazil. [2]Gravida: National Centre for Growth & Development, Liggins Institute, University of Auckland, Auckland, New Zealand. [3]Irish Centre for Fetal and Neonatal Translational Research (INFANT), Department of Obstetrics and Gynaecology, University College Cork, Cork, Ireland. [4]South Auckland Clinical School, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand. [5]School of Biological Sciences, University of Auckland, Auckland, New Zealand. [6]LNBio–Brazilian Biosciences National Laboratory and School of Medical Sciences, University of Campinas (UNICAMP), Campinas, SP, Brazil.

Received: 5 March 2015 Accepted: 4 August 2016
Published online: 08 August 2016

References

1. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet. 2012;379:2151–61.
2. Blencowe H, Lee ACC, Cousens S, Bahalim A, Narwal R, Zhong N, et al. Preterm birth-associated neurodevelopmental impairment estimates at regional and global levels for 2010. Pediatr Res. 2013;74 Suppl 1:17–34.
3. Blencowe H, Vos T, Lee ACC, Philips R, Lozano R, Alvarado MR, et al. Estimates of neonatal morbidities and disabilities at regional and global levels for 2010: introduction, methods overview, and relevant findings from the Global Burden of Disease study. Pediatr Res. 2013;74 Suppl 1:4–16.
4. Beck S, Wojdyla D, Say L, Betran AP, Merialdi M, Requejo JH, et al. The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. Bull World Health Organ. 2010;88:31–8.
5. Copper RL, Goldenberg RL, Creasy RK, DuBard MB, Davis RO, Entman SS, et al. A multicenter study of preterm birth weight and gestational age-specific neonatal mortality. Am J Obstet Gynecol. 1993;168(1 Pt 1):78–84.
6. Costeloe K, Hennessy E, Gibson AT, Marlow N, Wilkinson AR. The EPICure study: outcomes to discharge from hospital for infants born at the threshold of viability. Pediatrics. 2000;106:659–71.
7. Petrou S. The economic consequences of preterm birth during the first 10 years of life. BJOG. 2005;112(Suppl):10–5.
8. Wood NS, Costeloe K, Gibson AT, Hennessy EM, Marlow N, Wilkinson AR. The EPICure study: associations and antecedents of neurological and developmental disability at 30 months of age following extremely preterm birth. Arch Dis Child Fetal Neonatal Ed. 2005;90:F134–40.
9. Di Renzo GC. The great obstetrical syndromes. J Matern Neonatal Med. 2009;22:633–5.
10. Da Fonseca EB, Bittar RE, Carvalho MH, Zugaib M. Prophylactic administration of progesterone by vaginal suppository to reduce the incidence of spontaneous preterm birth in women at increased risk: A randomized placebo-controlled double-blind study. Am J Obstet Gynecol. 2003;188:419–24.
11. Newnham JP, Dickinson JE, Hart RJ, Pennell CE, Arrese CA, Keelan JA. Strategies to Prevent Preterm Birth. Front Immunol. 2014;5:584.
12. Requejo J, Merialdi M, Althabe F, Keller M, Katz J, Menon R. Born Too Soon: Care during pregnancy and childbirth to reduce preterm deliveries and improve health outcomes of the preterm baby. Reprod Health. 2013;10 Suppl 1:S4.
13. Romero R, Nicolaides K, Conde-Agudelo A, Tabor A, O'Brien JM, Cetingoz E, et al. Vaginal progesterone in women with an asymptomatic sonographic short cervix in the midtrimester decreases preterm delivery and neonatal morbidity: a systematic review and metaanalysis of individual patient data. Am J Obstet Gynecol. 2012;206(124):e1–e19.
14. Goldenberg RL, Iams JD, Mercer BM, Meis PJ, Moawad A, Das A, et al. The Preterm Prediction Study: toward a multiple-marker test for spontaneous preterm birth. Am J Obstet Gynecol. 2001;185:643–51.
15. Goldenberg RL, Goepfert AR, Ramsey PS. Biochemical markers for the prediction of preterm birth. Am J Obstet Gynecol. 2005;192(5 Suppl):S36–46.
16. Torbé A, Czajka R. Proinflammatory cytokines and other indications of inflammation in cervico-vaginal secretions and preterm delivery. Int J Gynaecol Obstet. 2004;87:125–30.

17. Meis PJ, Klebanoff M, Thom E, Dombrowski MP, Sibai B, Moawad AH, et al. Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate. N Engl J Med. 2003;348:2379–85.
18. Romero R. Progesterone to prevent preterm birth in twin gestations: what is the next step forward? BJOG. 2013;120:1–4.
19. Goya M, Pratcorona L, Merced C, Rodó C, Valle L, Romero A, et al. Cervical pessary in pregnant women with a short cervix (PECEP): an open-label randomised controlled trial. Lancet. 2012;379:1800–6.
20. Abbott DS, Hezelgrave NL, Seed PT, Norman JE, David AL, Bennett PR, et al. Quantitative fetal fibronectin to predict preterm birth in asymptomatic women at high risk. Obstet Gynecol. 2015;125:1168–76.
21. Crane JM, Hutchens D. Transvaginal sonographic measurement of cervical length to predict preterm birth in asymptomatic women at increased risk: a systematic review. Ultrasound Obstet Gynecol. 2008;31:579–87.
22. Honest H, Forbes CA, Durée KH, Norman G, Duffy SB, Tsourapas A, et al. Screening to prevent spontaneous preterm birth: systematic reviews of accuracy and effectiveness literature with economic modelling. Health Technol Assess. 2009;13:1–627.
23. Owen J, Szychowski JM, Hankins G, Iams JD, Sheffield JS, Perez-Delboy A, et al. Does midtrimester cervical length ≥25 mm predict preterm birth in high-risk women? Am J Obstet Gynecol. 2010;203(393):e1–5.
24. Horgan RP, Clancy OH, Myers JE, Baker PN. An overview of proteomic and metabolomic technologies and their application to pregnancy research. BJOG. 2009;116:173–81.
25. Romero R, Espinoza J, Gotsch F, Kusanovic J, Friel L, Erez O, et al. The use of high-dimensional biology (genomics, transcriptomics, proteomics, and metabolomics) to understand the preterm parturition syndrome. BJOG. 2006;113:118–35.
26. Kell DB, Oliver SG. Here is the evidence, now what is the hypothesis? The complementary roles of inductive and hypothesis-driven science in the post-genomic era. Bioessays. 2004;26:99–105.
27. North RA, McCowan LM, Dekker GA, Poston L, Chan EHY, Stewart AW, et al. Clinical risk prediction for pre-eclampsia in nulliparous women: development of model in international prospective cohort. BMJ. 2011;342:d1875.
28. Australian New Zealand Clinical Trials Registry: ACTRN12607000551493. https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=82254. Accessed 6 Aug 2016.
29. McCowan LM, Roberts CT, Dekker GA, Taylor RS, Chan EHY, Kenny LC, et al. Risk factors for small-for-gestational-age infants by customised birthweight centiles: data from an international prospective cohort study. BJOG. 2010; 117:1599–607.
30. Wishart DS, Lewis MJ, Morrissey JA, Flegel MD, Jeroncic K, Xiong Y, et al. The human cerebrospinal fluid metabolome. J Chromatogr B Anal Technol Biomed Life Sci. 2008;871:164–73.
31. Kenny LC, Broadhurst DI, Dunn W, Brown M, North RA, McCowan L, et al. Robust early pregnancy prediction of later preeclampsia using metabolomic biomarkers. Hypertension. 2010;56:741–9.
32. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: Statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Hypertens Pregnancy. 2001;20:ix–xiv.
33. American Diabetes Association: 2. Classification and Diagnosis of Diabetes. Diabetes Care. 2015;38:S8–S16.
34. GROW Birthweight Centiles. Bulk Centile Calculator. https://www.gestation.net/index.htm. Accessed 6 Aug 2016.
35. Say L, Souza JP, Pattinson RC. Maternal near miss—towards a standard tool for monitoring quality of maternal health care. Best Pract Res Clin Obstet Gynaecol. 2009;23:287–96.
36. Sulek K, Han TL, Villas-Boas SG, Wishart DS, Soh SE, Kwek K, et al. Hair metabolomics: identification of fetal compromise provides proof of concept for biomarker discovery. Theranostics. 2014;4(9):953–9.
37. Betsou F, Lehmann S, Ashton G, Barnes M, Benson EE, Coppola D, et al. Standard preanalytical coding for biospecimens: defining the sample PREanalytical code. Cancer Epidemiol Biomarkers Prev. 2010;19:1004–11.
38. Bond L, Thomas G, Nolan C, Christensen P, Baker PN, Kenny LC, et al. Preeclampsia risk stratification early in pregnancy: First results of a new LC-MS based multiplex metabolite assay. 2nd Annual European Congress on Clinical Mass Spectrometry. 2015.
39. Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. PLoS Med. 2007;4:e296.
40. Dessi A, Marincola FC, Fanos V. Metabolomics and the great obstetrical syndromes – GDM, PET, and IUGR. Best Pract Res Clin Obstet Gynaecol. 2014;29:156–64.
41. Fanos V, Atzori L, Makarenko K, Melis GB, Ferrazzi E. Metabolomics application in maternal-fetal medicine. Biomed Res Int. 2013;2013:720514.
42. Putri SP, Nakayama Y, Matsuda F, Uchikata T, Kobayashi S, Matsubara A, et al. Current metabolomics: practical applications. J Biosci Bioeng. 2013;115:579–89.
43. Dharuri H, Demirkan A, van Klinken JB, Mook-Kanamori DO, van Duijn CM, 't Hoen PA, et al. Genetics of the human metabolome, what is next? Biochim Biophys Acta. 2014;1842:1923–31.
44. Romero R, Mazaki-Tovi S, Vaisbuch E, Kusanovic JP, Chaiworapongsa T, Gomez R, et al. Metabolomics in premature labor: a novel approach to identify patients at risk for preterm delivery. J Matern Fetal Neonatal Med. 2010;23:1344–59.
45. He X, de Seymour JV, Sulek K, Qi H, Zhang H, Han T-L, et al. Maternal hair metabolome analysis identifies a potential marker of lipid peroxidation in gestational diabetes mellitus. Acta Diabetol. 2016;53(1):119–22.
46. Passini Jr R, Cecatti JG, Lajos GJ, Tedesco RP, Nomura ML, Dias TZ, for the Brazilian Multicentre Study on Preterm Birth study group, et al. Brazilian Multicentre Study on Preterm Birth (EMIP): prevalence and factors associated with spontaneous preterm birth. PLoS One. 2014;9(10):e109069.
47. Barros FC, Papageorghiou AT, Victora CG, Noble JA, Pang R, Iams J, et al. The Distribution of Clinical Phenotypes of Preterm Birth Syndrome: Implications for Prevention. JAMA Pediatr. 2015;169:220–9.
48. Kenny LC, Black MA, Poston L, Taylor R, Myers JE, Baker PN, et al. Early pregnancy prediction of preeclampsia in nulliparous women, combining clinical risk and biomarkers: the Screening for Pregnancy Endpoints (SCOPE) international cohort study. Hypertension. 2014;64:644–52.
49. Huynh J, Xiong G, Bentley-Lewis R. A systematic review of metabolite profiling in gestational diabetes mellitus. Diabetologia. 2014;57:2453–64.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at
www.biomedcentral.com/submit

BioMed Central

4.6. Artigo *Planning, implementing and running a multicentre preterm birth study with biobank resources in Brazil: the Preterm SAMBA study*

Hindawi
BioMed Research International
Volume 2019, Article ID 5476350, 8 pages
https://doi.org/10.1155/2019/5476350

Research Article

Planning, Implementing, and Running a Multicentre Preterm Birth Study with Biobank Resources in Brazil: The Preterm SAMBA Study

Renato T. Souza,[1] Jose G. Cecatti,[1] Maria L. Costa,[1] Jussara Mayrink,[1] Rodolfo C. Pacagnella,[1] Renato Passini Jr.,[1] Kleber G. Franchini,[2] Francisco E. Feitosa,[3] Iracema M. Calderon,[4] Edilberto A. Rocha Filho,[5] Débora F. Leite,[5] Janete Vettorazzi,[6] Louise C. Kenny,[7] Philip N. Baker,[8,9] and The Preterm SAMBA Study Group[1]

[1] *Department of Obstetrics and Gynecology, University of Campinas (UNICAMP), School of Medical Sciences, Campinas, SP, Brazil*
[2] *LNBio-Brazilian National Laboratory of Biosciences and University of Campinas (UNICAMP) School of Medical Sciences, University of Campinas (UNICAMP), Campinas, SP, Brazil*
[3] *MEAC–Maternity School of The Federal University of Ceará, Fortaleza, CE, Brazil*
[4] *Department of Obstetrics and Gynecology, Botucatu Medical School, Sao Paulo State University–Unesp, Botucatu, Brazil*
[5] *Department of Maternal and Child Health, Maternity of The Clinics Hospital, Federal University of Pernambuco, Recife, PE, Brazil*
[6] *Department of Obstetrics and Gynecology, Maternity of The Clinics Hospital, Federal University of RS, Porto Alegre, RS, Brazil*
[7] *Irish Centre for Fetal and Neonatal Translational Research (INFANT), Department of Obstetrics and Gynaecology, University College Cork, Cork, Ireland*
[8] *College of Medicine, Biological Sciences and Psychology, University of Leicester, Leicester, UK*
[9] *Gravida: National Centre for Growth & Development, Auckland, New Zealand*

Correspondence should be addressed to Jose G. Cecatti; cecatti@unicamp.br

Received 4 November 2018; Revised 16 December 2018; Accepted 27 December 2018; Published 16 January 2019

Academic Editor: Marco Scioscia

Copyright © 2019 Renato T. Souza et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. Our aim was to describe the steps in planning, implementing, and running a multicentre cohort study of maternal and perinatal health using a high-quality biobank comprised of maternal serum, plasma, and hair samples collected from five sites in Brazil. The Preterm SAMBA study, conducted by the Brazilian Network for Studies on Reproductive and Perinatal Health, was an innovative approach used to identify women at higher risk for preterm birth. It is also of great importance in the study of other maternal and perinatal complications in the context of Brazil, which is a middle-income country. *Methods.* We described phases of planning, implementing, and running the Preterm SAMBA study, a multicentre Brazilian cohort study of low-risk nulliparous pregnant women, to validate a set of metabolite biomarkers for preterm birth identified in an external cohort. Procedures and strategies used to plan, implement, and maintain this multicentre preterm birth study are described in detail. Barriers and experience cited in the current narrative are not usually discussed in the scientific literature or published study protocols. *Results.* Several barriers and strategies were identified in different phases of the Preterm SAMBA study at different levels of the study framework (steering committee; coordinating and local centres). Strategies implemented and resources used in the study are a legacy of the Brazilian Network, aimed at training collaborators in such complex settings. *Conclusion.* The Brazilian Network for Studies on Reproductive and Perinatal Health has gained some experience in conducting a multicentre cohort study using a resourceful biobank which may be helpful to other research groups and maternal/perinatal health networks that plan on employing a similar approach to a similar background.

1. Background

The Brazilian Network for Studies on Reproductive and Perinatal Health (BNSRPH) is a national research initiative that has enabled a number of multicentre studies within the country. It has had a major impact on maternal and perinatal health in the last decade [1–4]. The BNSRPH was officially launched in 2008, when twenty-seven institutions from five regions of Brazil agreed to participate in the first multicentre study coordinated by this network. These institutions were provided with funding to perform surveillance of severe maternal morbidity [5–7]. For the conception of this Brazilian initiative [5], it was particularly important to have experience as partners and data collectors in multicentre studies headed by international initiatives such as the University of Cincinnati, USA [8], and most importantly the University of Toronto, Canada [9].

Experience and effectiveness of epidemiological studies have evolved to the possibility of scaling up and planning studies that meet translational research requirements. It is an approach to propose interventions that may have a potential impact on maternal and perinatal health. Current infrastructure has enabled adequate clinical data collection, along with biological sample storage. The Preterm SAMBA project represents this initiative.

The Preterm SAMBA cohort study (Preterm Screening and Metabolomics in Brazil and Auckland) enrolled around 1,150 low-risk nulliparous pregnant women from 5 different Brazilian centres. Participants received follow-up care at three study visits during pregnancy (20, 27, and 37th weeks gestation). Blood and hair samples were collected at 20 weeks of gestational age (+/- 1wk). Comprehensive clinical data including blood pressure levels, BMI (body mass index), medical history, clinical complications such as infectious diseases, ultrasound data, and maternal and perinatal outcomes were standardised, collected, and recorded online in an electronic database. Biological samples were collected, processed, and immediately stored in the laboratory, under specific conditions and following a very precise operational biobank protocol [4]. The main objective was to validate biological predictors of preterm birth based on the metabolomics approach.

Planning, implementing, and running a multicentre preterm birth study with biobank resources in Brazil represents a challenge that should be acknowledged. Experience that is not always reported in research protocols can serve as an example, especially in under-resourced settings where difficulties in pursuing these studies are known to be enormous. We aim to tackle all issues involved in this initiative.

2. Methods

2.1. Planning. Research projects can only thrive with proper funding. In 2013, the Preterm SAMBA project proposal was submitted to a joint research call from the Bill and Melinda Gates Foundation (BMGF), the Brazilian Ministry of Health and the Brazilian National Research Council (CNPq), an agency under the Ministry of Science and Technology. This grant was part of a program called "Reducing the burden of preterm birth,, an agenda of shared priorities to address the occurrence and consequences of preterm birth through innovative approaches in locations where the burden of disease is heaviest. At that time, it seemed very appropriate to form a partnership with international collaboration. The chosen international group had pioneered the application of metabolomics technologies in maternal and perinatal health [10–13] and could help build a very high-quality biobank.

Interestingly, the full proposal of this study was only developed during the selection process of this research call. Initially, a 5-page letter of intention was presented and selected in the first round. Then, a full proposal was presented for the second round, when a technical examining committee was in charge of assessing proposals and making recommendations. Finally, in the third round the same committee was responsible for checking changes in the proposal, according to previous suggestions and selecting the final grantees. This process allowed for some improvements in the research proposal while the selection process was running.

The Preterm SAMBA project aims to develop a predictive algorithm based on biomarker screening tests (involving both phases of discovery and validation) that may translate into clinical care. Ultimately, a panel of biomarkers can be identified as an early pregnancy screening test to ascertain which pregnant women are at higher risk of developing preterm birth (PTB), thus facilitating timely and appropriate interventions.

2.2. Ethics, Consent and Permission. The establishment of systematic sample collection and biobank storage requires compliance with rigorous ethical regulations. Ethical aspects for biorepository/biobank (Resolution № 441/11), sample transport (Resolution № 1306/14), shipping samples abroad to international partners (Resolution № 292/99), and subsequent sample use in future studies/analyses (Resolutions № 347/05 and 441/11) were all regulations considered and respected.

The first step was to submit the research project to the Institutional Review Board (IRB) of the coordinating centre for assessment and approval, followed by its registration in the Brazilian National Research Registry platform (*Plataforma Brasil*), where the project was appreciated by each local IRB of the remaining participating centres. Ethical approval was then confirmed by the National Committee for Ethics in Research (CONEP) through Letter of approval number 912.714 from the IRB of the University of Campinas issued on 12/13/2014. Although the coordinating centre had only most recently approved its Institutional Biobank by the Brazilian National Committee for Ethics in Research, the project started after a biorepository and not a biobank was launched and established, according to Brazilian regulation. However, it benefited from Biobank infrastructure, as well as its technical and human resources, assuring quality control for all necessary procedures. No other participating institution where samples were collected and temporarily stored had obtained formal biobank approval. In this case, the local PI and not the institution was the curator responsible for

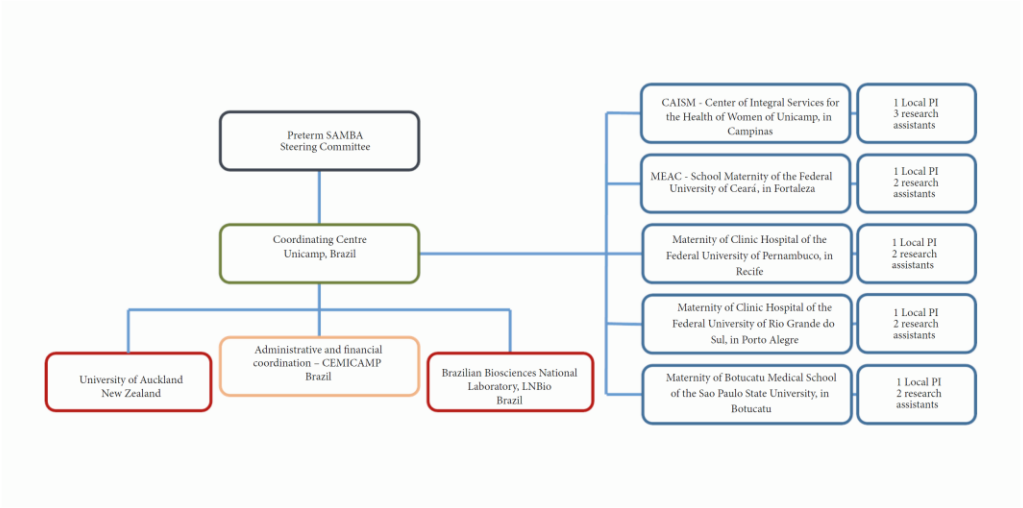


Figure 1: Framework of Preterm SAMBA study.

the samples and periodical renovation of ethical approval as required. A clear and elucidative consent form was applied to all participating women, clarifying that, in addition to trivial points, part of the samples would be shipped abroad and future investigations could be conducted using their samples. It was accepted that these participants could agree partially or fully. Furthermore, another important aspect of ethical consideration was the commitment of international partners to follow Brazilian rules.

The coordinating centre (University of Campinas, UNICAMP) and the steering committee had a major role in the framework of the study (Figure 1). The steering committee, represented by the senior investigators of both research groups, played a significant and strategic role throughout the project. The committee was responsible for reviewing, approving and coordinating study data and sample analyses and supervising study progress. As outlined by the flowchart, international partnership was initially established with the University of Auckland, which was hosting the international PI of the study, Prof. P Baker. Shortly afterwards, our second international partner was the University of Cork, Ireland, followed by the University of Leicester, UK. The international PI had moved to the last location during the study.

The five participating centres were selected according to previous performance and commitment to the Brazilian network of multicentre studies, minimal existing infrastructure for biological sample collection and storage, and strategic geographical location, to address populations of different cultural and ethnic background. Each participating centre worked with a local PI and one or more assistant researchers to cover study requirements for subject enrolment, clinical follow-up and laboratory procedures. Team selection is particularly relevant to the development and sustainability of the study. The commitment of the staff exceeded that foreseen by the financial support available and was mainly driven by research interests.

3. Results

The general study manager, based in the coordinating centre in Brazil, visited the University of Auckland and University of Cork before implementing the study. Experience and know-how were shared following standard protocols used in the SCOPE and IMPROvED studies [14, 15] by the Preterm SAMBA international partners. It was crucial to standardise equipment and supplies that would be used in the Brazilian cohort and develop the Preterm SAMBA Standard Operating Procedures (SOPs). One week spent at each university provided the general manager with detailed training in all steps of data and sample collection, processing and storage. Overall, this training allowed transfer of expertise from international partners to the Brazilian team, providing resources for the development of Case-Report Forms (CRF), database and protocols for the Preterm Samba Study. Concern about the temperature control system, institutional adaptation to biobank monitoring (development of maintenance service protocols) and emergency protocols for freezer failure were also considered at this point in time.

Seven different SOPs were designed to standardise several procedures for all related topics, in addition to registering and providing detailed information on such procedures. The following SOPs were created in press and pdf files, with images, photographs, and print screens and provided to all assistants and researchers in all participating centres: (1) Operation Manual: introduction to the database system and details of its functions; (2) Interviewer: information on study design, clinical visits, eligibility criteria, invitation and inclusion procedures, data collection, and definition of all variables; detailed support guidelines on how to administer a 24-h dietary recall (with charts on portion size) and skinfold measurements (with detailed information on technical procedures); (3) sample collection: detailed information about sample collection, biological safety procedures,

quality control and ethical aspects; (4) sample processing and storage: detailed information on different steps and quality control regarding the biorepository; (5) division of biobank samples: after sample processing, samples from each woman participating in the Preterm SAMBA study generated serum and plasma aliquots.

Samples were temporarily stored in the local centres, and then shipped to the coordinating centre, which was responsible for dividing the biobank in two—Biobank Brazil and International Biobank. Aliquots from the international biobank were shipped to the international project collaborator for use in previously designed research analyses and also for future discussion by the project steering committee [4]. Careful safety procedures were employed to avoid misclassifying aliquot tracking positions, according to the respective participant and also assure quality control. Such procedures had to be planned and carried out carefully, without any haste. Two serum vacutainer tubes, for instance, were collected from each participant. After processing, serum samples from the second tube were stored in positions immediately after the first tube. Since the biobank was divided in two, alternate aliquots were selected for the Brazilian biobank instead of selecting the first half of the aliquots, for example. Although tricky, this procedure ensured that fibrin or haemolysis of one of the serum vacutainers tube would not compromise the entire Brazilian or International biobank; (6) sample transportation: details on sample packing, parcel configuration, proportion of boxes/aliquots, and dry ice and data logger disposal were described. A certified company in compliance with international rules and recommendations for biological transport in ultra-low temperatures was responsible for national and international sample transport.

3.1. Implementing. The Preterm SAMBA biorepository was based on the Institutional Biobank of the Maternity Hospital of the University of Campinas. We believe that institutional support with technical equipment and human resources are crucial to establish a high-quality biobank and allow implementation of the clinical study. A strong commitment of the institution facilitates the following: (1) general and specific arrangements for project implementation and progression: some institutional arrangements are usually required for project implementation such as private rooms dedicated to clinical visits and interviews for the study, changes in patient care workflow, adaptation of routine antenatal care agenda with activities foreseen in the project (sample collection, interviews, etc.), and temporary clinical licence for research assistants (respecting adequate background), allowing their access to medical records at different sites of the institution; (2) identification of possible new assistants throughout the study, to allow for needed reposition: the Brazilian Network experience showed us that despite an occasional replacement of research assistants, this may have a huge impact on study implementation and progress due to possible delays in identifying new suitable collaborators and the need for new training sessions and accreditation. Part-time contract employees from the institution and postgraduate students linked to the project are the most common assistants that collaborate with the project. In our opinion, the main barriers to identify new assistants and the reasons for discontinuation may be related to the perspective that research collaboration may require more strict commitment in terms of workload, quality control, and responsibilities compared to other more profitable activities. Therefore, adequate funding allocated for research assistants and availability of institutional staff to conduct research activities is a priority of great importance; (3) optimal use of resources: the acquisition of new equipment or consumables that will be part of the heritage of the institution should take into consideration existing infrastructure. After the end of the study, the institution will be responsible for the maintenance and supply of consumables.

Some of the equipment and consumables for the biobank were imported. There were few suppliers that provided new solutions in biobank technology in Brazil. Cryovials made of polypropylene, RNase/DNase-free, and pyrogen-free, containing a unique 2-D Data-Matrix Bar Code Insert at the bottom of the vial were used to store serum and plasma aliquots. This technology permits scanning of a whole cryobox with up to 96 cryovials in less than 10 seconds, saving time, and minimizing human errors during the process. In addition, the compact cryobox size results in at least 50% more space in the freezer when compared to ordinary biobank boxes available in Brazil. Depending on the configuration of freezer racks (using low profile racks), space can improve up to 150%. The timeframe to complete all customs clearance procedures for imported equipment and consumables can take months, sometimes years, depending on the item. It is considered a bureaucratic barrier for the Brazilian scientific community. In 2004, an alternative to the conventional import procedure was launched by a joint of Brazilian organisations (the Brazilian official postal service, the Brazilian Ministries of Science, Technology and Innovation and Finance) termed "Science Easy Import system." Although there are some restrictions on certain items, including value of the commercial invoice, size and weight of the parcel, etc., the system provides a much quicker, less bureaucratic, and more suitable operation. We have translated our experience with the Science Import into a tutorial document to assist and encourage other researchers to use the system [16].

For adequate referral of study subjects, the public health system was strongly involved, raising awareness about the study. It also developed mechanisms aimed at identifying eligible cases, especially within primary healthcare, which was the targeted study population. This strategy was effective, since participating maternities are referral centres for high-risk pregnancies. The Brazilian public health system is decentralised and municipalities provide women with comprehensive, free and universal access to healthcare [17]. Primary healthcare facilities are crucial to the provision of medical care. These units are the basis for the entire healthcare system and a referral for screening. Therefore, antenatal care for low-risk pregnant women should be provided at this level of care. During study implementation, the participating centres arranged local agreements and obtained respective approval of the municipalities to identify eligible women seeking prenatal care at primary healthcare units.

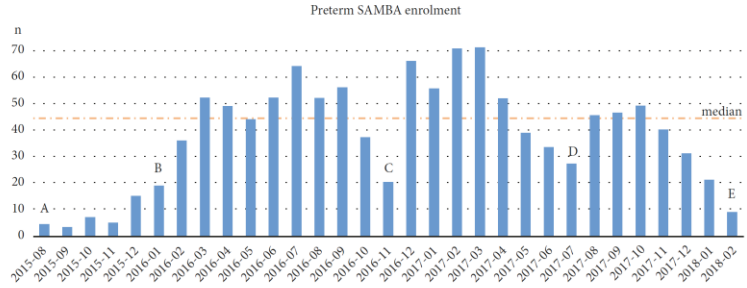


FIGURE 2: Enrolment rate of the Preterm SAMBA study. Marks A, B, C, D, and E were remarkable set points of the study. The dashed line corresponds to the median – 45.0.

All multicentre studies carried out by the Brazilian Network were launched during the initial meeting for implementation and training with all research assistants, local PIs, research collaborators and funders. Although some training sessions could be organised by webinar meetings, we believe that the interaction and participation of the whole group can transform a seminar into a much more collaborative meeting, with a greater commitment of everyone involved. For the current study, a two-day meeting was held at the University of Campinas to discuss the final version of an electronic CRF within the database and potential local adaptation of any protocol procedure. Furthermore, the study protocol, study equipment, platform for data collection, and a general view of SOPs were also introduced. On-site visits prior to the study were also performed in all participating centres, since sample collection, processing, and storage training sessions required hands-on practice.

A test version of the database, very similar to one that is routinely implemented, was used for training research assistants. In addition, the coordinating centre arranged webinars to discuss more detailed information on the definition of variables and monitored data entered by assistants in the test database during the training period. Only then would the assistants and local PI receive the live database login and password. Passwords were issued following hierarchy, depending on the role of each staff in the study. For instance, a local assistant only had access to data of the correspondent centre, and not to full data.

It is noteworthy that no matter how well-planned a study is, there are possible surprises and challenges while the study is running. In our case, an important fact that could potentially have an impact on the cohort was the Zika virus outbreak that occurred in the country from 2015 to 2016 [18, 19]. As soon as the issue arose, the steering committee decided to include variables that could address the occurrence of the infection, such as history of fever, exanthema, conjunctivitis, and fetal malformations [18, 19]. A few of these variables were already part of routine collection. Nevertheless, the centres were further contacted to pay careful attention to these variables.

3.2. Running. We regard the onset of participant recruitment as the key phase of a prospective study. Breaking through the barrier of initial inertia, reaching the expected enrolment rate, and ensuring that trained procedures are adequately employed are the first challenges to overcome. Figure 2 demonstrates the enrolment rate of Preterm SAMBA study. Set points, defined as A, B, C, D, and E in the figure, are most important to understand how enrolment progress occurred. We decided to first run the study in only one centre and chose the coordinating centre (A–Figure 2). We were able to monitor the database, recruitment strategies and standard procedures, learn the proper approach to eligible women who might participate in the cohort study with biobank sample collection and the identification and management of potential barriers. This experience enabled more effective on-site training sessions of the remaining participating centres in the following months (months between A and B – Figure 2). As soon as all participating centres started running the project, fortunately it did not take long to reach the expected enrolment rate plateau (B–Figure 2). There were three moments of noticeable decrease in enrolment rates (C, D, and E–Figure 2). Reasons for the decreased inclusion rate of participants in these periods were as follows: C–temporary absence of one of the two research assistants in one centre and a hospital staff strike in another centre; D–change of research assistants in two participating centres and a technical issue with the freezer in one of these centres (new equipment had to be bought); E–a decrease in enrolment rate was evident in the last few months of the study, most likely due to the long period of data collection (over two years) and distance from sites where visits or group meetings took place. We believe that constant monitoring and surveillance, not only remote feedback, are important to maintain the inclusion rate of cases in such cohorts. Furthermore, ongoing shifts in initially defined strategies to identify and recruit eligible women should also be considered.

Television, radio, and newspaper media and a website containing general information about the study, profile of eligible women, and contact of all participating centres were the main strategies employed to draw attention to the project and reach a local and regional audience.

A general project manager was designated to perform continuous monitoring of quality control parameters (data and sample collection; sample processing and storage), monitoring of freezer temperature records, inclusion, and loss

to follow-up rates and inconsistent dataset. Two or three monitoring visits were made to each centre during the study to provide feedback on study procedures, difficulties, and barriers to run the study and repeat some training sessions, according to the performance of the centre and the evaluation of the monitor. A standard monitoring report was written after each visit and shared with all local researchers, assistants and also the coordinating centre, to register established commitments and decision making on how to overcome difficulties encountered. Main parameters reported were the number of included participants per month, loss to follow-up, missing data, data inconsistencies, SPREC (Standard PREanalytical Code) pre-analytical quality parameters for biological samples [20], number of aliquots generated per tube, occurrence of fibrin, time between collection and storage, strategies for identifying eligible women, and others. In addition to on-site monitoring visits, several web-conference meetings were performed to discuss the details of study progress and data inconsistencies, enabling continuous monitoring and a learning atmosphere which facilitated the identification and correction of human errors and unproductive work processes.

4. Discussion

4.1. Monitoring. An open, two-way communication between research assistants of all participating centres and the general study monitor was of the utmost importance for the establishment of an effective organisation and coordination of the study. We did not expect the lack of mistakes during procedures or human errors discordant from standard protocols. Nevertheless, it is not only important to prevent these errors, but also to recognise, report, and adequately record the issues. It is helpful to accept errors in a nonpunitive atmosphere to enhance the confidence of collaborators. In terms of communication, cell-phone group chats were the quickest and most objective method employed to assure clear and quick-response communication. Following ethical principles, no data of participants were ever shared through message chats.

The database system was developed in the MedSciNet® platform, a resourceful and very helpful online system for study management. It securely links clinical data to biobank data, providing hierarchical access, a biosample tracking system, and multiple functions for data monitoring. At the end of the study, the system provides a case-control function through which the user can design clinical experiments, selecting women whose sample positions will be provided.

According to study protocol, all women who were invited to participate in the study and refused to undergo complete or partial sample collection were properly registered. Contrary to what we had anticipated, eligible women rarely declined or reported feeling uncomfortable with blood and/or hair collection.

Regardless of research team support or antenatal care provided by the participating institution, a few women preferred to maintain their antenatal care visits in primary healthcare facilities or continue with their own private doctors. Reasons were either emotional because of a good relationship previously established with healthcare providers or more practical due to a closer distance from the primary care unit. This caused a little but noticeable impact on rates of inclusion and loss to follow-up, owing to delays in attending scheduled visits for sample collection. In addition, the staff found it a much more difficult and time-consuming task to complete information on women delivering outside the participating facility, and required specific local strategies. We noticed that centres which provided ANC visits had better performance in rates of inclusion and loss to follow-up.

Public health facilities where eligible women were identified and invited to participate had different reactions to the study. Some units were helpful and highly committed to the task, facilitating study processes and establishing clear boundaries between research and healthcare activities. According to strategies designed to ascertain adequate follow-up of included cases, the centres were encouraged to provide ANC support. Although complete healthcare was not the responsibility of the study, as previously mentioned, the proportion of women who agreed to participate and adhere to the study was traditionally higher in centres that offer a personalised ANC assistance.

Regardless of the ANC provided, research assistants were trained to adequately report several maternal and perinatal outcomes. Standard outcomes—PE (pre-eclampsia), GDM (gestational diabetes mellitus), PTB (preterm birth), and FGR (fetal growth restriction)—were consistently checked against previously standardised diagnostic criteria. Retrospective data review to confirm or rule out an outcome can be a challenge, since it may underestimate or overestimate the incidence of the outcome. Taking into account GDM, PTB, FGR, and PE, we believe that PE is the most challenging outcome to review and double-check. Registration of high blood pressure levels and urine protein values during study visits might not suffice to assure its occurrence [21]. Participating institutions have to follow an adequate standard obstetric protocol to ensure that late pregnancy and delivery/postpartum data will be appropriate to confirm PE cases. Nonstandardised recording may introduce bias and generate uncertainty with compromised data. In summary, the concept "do it right the first time" is legitimate and strongly recommended, as well as the application of international standard definitions during the study protocol. For these data, it is also important to know the clinical protocol of each institution and reinforce the importance of its use.

The provision of national and international biosample transport was planned in two steps. First, for national sample arrangement, each participating centre sent biosamples to the coordinating centre when 60% of the sample size was attained. Then, a second transport was arranged, containing the remaining samples. After shipment from participating centres arrived, the position of each cryobox containing biosamples was updated in the database system, according to the freezer of the coordinating centre. Transportation was provided by an experienced company in compliance with IATA (International Air Transport Association) and international ethical regulations. Samples were maintained in dry ice and monitored by a temperature data logger in each parcel. The preparation for international shipping

entailed further splitting of the samples into International Biobank and Biobank Brazil, as previously detailed. The first international transport contained 80% of the International Biobank samples. Consequently, the second contained 20% of samples.

The coordinating centre held a final meeting with all local investigators, research assistants and collaborators to discuss the plan for analysis of primary and secondary outcomes. The participation of all collaborators from all participating centres was truly important not only to put into practice the opportunity to collaborate among all researchers and assistants involved in the study but also extend the exploration of secondary analysis by using a comprehensive dataset. Investigators that built their careers in different areas of maternal and perinatal health research were able to share their experience and suggest innovative approaches to examine some specific complications that occur during pregnancy. Moreover, participating centres of the Brazilian Network contributed to the discussion about the implementation of the next big study, termed the Maternal Actigraphy Exploratory Study–I (MAES-I). The future study is an extension of the Brazilian multicentre cohort study. It will investigate physical activity and sleep patterns throughout pregnancy, in association with maternal complications using actigraphy data (study protocol to be published). Human and technological resources allocated for the Brazilian multicentre cohort using biobank resources may facilitate the implementation of new studies and application of innovative approaches including wearable technologies, such as the actigraphy device to monitor women during pregnancy.

5. Conclusions

Planning, implementing and running a multicentre study with standardised clinical data and biosample collection is challenging, although possible in low-income and middle-income settings. Proper funding, with a detailed developed proposal and well-established network, is pivotal to the development of such an initiative. Enhancement of capacity and training in settings most commonly affected by the burden of disease in maternal and perinatal health will strengthen findings and provide a venue for future research.

The Brazilian Network, created almost a decade ago, with prior conduction of successful studies was fundamental to support the current initiative. Furthermore, funders willing to collaborate throughout the study create an opportunity for more than study results and reports. There is room for more effective learning to disseminate findings, raise awareness about research impact, and add new perspectives and collaborations.

Abbreviations

ANC: Antenatal care
BMGF: Bill and Melinda Gates Foundation
BMI: Body mass index
BNSRPH: Brazilian Network for Studies on Reproductive and Perinatal Health
CEP: Local Institutional Review Board
CNPq: Brazilian National Research Council
CONEP: National Committee for Ethics in Research
CRF: Case-report forms
FGR: Fetal growth restriction
GDM: Gestational diabetes mellitus
IATA: International Air Transport Association
IMPROvED: IMproved Pregnancy Outcomes by Early Detection
IRB: Institutional Review Board
PE: Preeclampsia
PI: Principal investigator
PTB: Preterm birth
Preterm SAMBA: Preterm Screening and Metabolomics in Brazil and Auckland
SCOPE: Screening for Pregnancy Endpoints study
SOP: Standard Operating Procedures
SPREC: Standard PREanalytical Code
UNICAMP: University of Campinas
Wk: Week.

Data Availability

The current manuscript describes several topics related to methodological aspect and the implementation of a multicentre cohort study and does not involve data to be available.

Ethical Approval

The Institutional Review Board (IRB) of the coordinating centre assessed and approved the research project, which was registered in the Brazilian National Research Registry platform (Plataforma Brasil), through which the project was appreciated by each local IRB of the remaining participating centres. This ethical approval was then confirmed by the National Committee for Ethics in Research (CONEP) through letter of approval number 912.714 from the IRB from the University of Campinas issued on 12/13/2014.

Disclosure

Membership of the Preterm SAMBA study group is provided in the Acknowledgments.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Jose G. Cecatti and Renato T. Souza conceived the manuscript. Renato T. Souza, Jose G. Cecatti, and Renato Passini Jr. discussed the content. Renato T. Souza, Maria L. Costa, Jussara Mayrink, and Jose G. Cecatti drafted the first version of the manuscript, which was revised by Rodolfo C. Pacagnella and Renato Passini Jr. Then, all authors read, reviewed, and approved the final manuscript.

Acknowledgments

The Preterm SAMBA study group also included Mary A. Parpinelli, Karayna G Fernandes, José P Guida, Danielly Santana, Ricardo M Barbosa, and Rafael B F Galvao, School of Medical Sciences, University of Campinas, Brazil; Bianca F. Cassettari, School of Medicine of Botucatu, UNESP, Brazil; Lucia Pfitscher, School of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil; Daisy Lucena de Feitosa, School of Medicine, Federal University of Ceará, Fortaleza, Brazil. The authors also acknowledge the following research assistants, collaborators, and staff members of the institution who made a remarkable contribution to run the Preterm SAMBA study: Elias de Melo Ferreira Júnior, and Danilo Anacleto, School of Medicine, Federal University of Pernambuco, Recife, Brazil; Vilma Zotareli and Marcia Alice Silva, from Cemicamp, who provided administrative and financial support for the study. The authors also acknowledge Global Health Strategies and FioCruz (Oswaldo Cruz Foundation, Rio de Janeiro), the institutions responsible for financial and clinical advisory on behalf of BMGF, which made a remarkable contribution to the identification of barriers for study sustainability, disruptiveness, and potentiality to scale.

References

[1] J. G. Cecatti, M. L. Costa, S. M. Haddad et al., "Network for Surveillance of Severe Maternal Morbidity: A powerful national collaboration generating data on maternal health outcomes and care," *British Journal of Obstetrics and Gynaecology*, vol. 123, no. 6, pp. 946–953, 2016.

[2] R. Passini, J. G. Cecatti, G. J. Lajos et al., "Brazilian Multicentre Study on Preterm Birth (EMIP): Prevalence and Factors Associated with Spontaneous Preterm Birth," *PLoS ONE*, vol. 9, no. 10, Article ID e109069, 2014.

[3] L. Katz, M. Amorim, J. P. Souza, S. M. Haddad, and J. G. Cecatti, "COHELLP: collaborative randomized controlled trial on corticosteroids in HELLP syndrome," *Reproductive Health*, vol. 10, no. 1, 2013.

[4] J. G. Cecatti, R. T. Souza, K. Sulek et al., "Use of metabolomics for the identification and validation of clinical biomarkers for preterm birth: Preterm SAMBA," *BMC Pregnancy and Childbirth*, vol. 16, no. 1, 2016.

[5] J. G. Cecatti, C. Silveira, R. T. Souza, K. G. Fernandes, and F. G. Surita, "Experience with the brazilian network for studies in reproductive and perinatal health: the power of collaboration in postgraduate programs," *Revista do Colégio Brasileiro de Cirurgiões*, vol. 42, pp. 89–93, 2015.

[6] J. G. Cecatti, J. P. Souza, M. A. Parpinelli et al., "Brazilian network for the surveillance of maternal potentially life threatening morbidity and maternal near-miss and a multidimensional evaluation of their long term consequences," *Reproductive Health*, vol. 6, no. 1, article 15, 2009.

[7] S. M. Haddad, J. G. Cecatti, M. A. Parpinelli et al., "From planning to practice: building the national network for the surveillance of severe maternal morbidity," *BMC Public Health*, vol. 11, no. 283, 2011.

[8] J. A. Spinnato, S. Freire, J. L. Pinto e Silva, M. V. Cunha Rudge, S. Martins-Costa, M. A. Koch et al., "Antioxidant therapy to prevent preeclampsia," *Obstetrics & Gynecology*, vol. 110, pp. 1311–1318, 2007.

[9] J. F. Barrett, M. E. Hannah, E. K. Hutton et al., "A Randomized Trial of Planned Cesarean or Vaginal Delivery for Twin Pregnancy," *The New England Journal of Medicine*, vol. 369, no. 14, pp. 1295–1305, 2013.

[10] L. C. Kenny, W. B. Dunn, D. I. Ellis, J. Myers, P. N. Baker, and D. B. Kell, "Novel biomarkers for pre-eclampsia detected using metabolomics and machine learning," *Metabolomics*, vol. 1, no. 3, pp. 227–234, 2005.

[11] X. He, J. V. de Seymour, K. Sulek et al., "Maternal hair metabolome analysis identifies a potential marker of lipid peroxidation in gestational diabetes mellitus," *Acta Diabetologica*, vol. 53, no. 1, pp. 119–122, 2016.

[12] K. Sulek, T.-L. Han, S. G. R. Villas-Boas et al., "Hair metabolomics: identification of fetal compromise provides proof of concept for biomarker discovery," *Theranostics*, vol. 4, no. 9, pp. 953–959, 2014.

[13] L. C. Kenny, D. I. Broadhurst, W. Dunn et al., "Robust early pregnancy prediction of later preeclampsia using metabolomic biomarkers," *Hypertension*, vol. 56, no. 4, pp. 741–749, 2010.

[14] L. M. E. McCowan, C. T. Roberts, G. A. Dekker et al., "Risk factors for small-for-gestational-age infants by customised birthweight centiles: data from an international prospective cohort study," *British Journal of Obstetrics and Gynaecology*, vol. 117, no. 13, pp. 1599–1607, 2010.

[15] K. Navaratnam, Z. Alfirevic, P. N. Baker et al., "A multicentre phase IIa clinical study of predictive testing for preeclampsia: improved pregnancy outcomes via early detection (IMPROvED)," *BMC Pregnancy and Childbirth*, vol. 13, no. 1, 2013.

[16] "Easy Science Import Tutorial Version 1," 2016 [cited 28 Feb 2018], https://www.fcm.unicamp.br/fcm/pos-graduacao-em-tocoginecologia/tutoriais.

[17] C. G. Victora, E. M. Aquino, M. Do Carmo Leal, C. A. Monteiro, F. C. Barros, and C. L. Szwarcwald, "Maternal and child health in Brazil: Progress and challenges," *The Lancet*, vol. 377, pp. 1863–1876, 2011.

[18] N. Wikan and D. R. Smith, "Zika virus: history of a newly emerging arbovirus," *The Lancet Infectious Diseases*, vol. 16, no. 7, pp. e119–e126, 2016.

[19] P. Brasil, J. P. Pereira Jr., C. Raja Gabaglia et al., "Virus infection in pregnant women in rio de janeiro," *The New England Journal of Medicine*, pp. 2321–2334, 2016.

[20] F. Betsou, S. Lehmann, G. Ashton et al., "Standard Preanalytical Coding for Biospecimens: Defining the Sample PREanalytical Code," *Cancer Epidemiology Biomarkers & Prevention*, vol. 19, no. 4, pp. 1004–1011, 2010.

[21] M. A. Brown, M. D. Lindheimer, M. de Swiet, A. van Assche, and J. M. Moutquin, "The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP)," *Hypertens Pregnancy*, vol. 20, no. 1, pp. ix–xiv, 2001.

4.7. Artigo *Trace biomarkers associated with spontaneous preterm birth from the maternal serum metabolome of asymptomatic nulliparous women – parallel case-control studies from the SCOPE cohort*

Gmail Renato Souza <doutorrenatosouza@gmail.com>

Manuscript submission SREP-19-02329

scientificreports@nature.com <scientificreports@nature.com> 28 January 2019 at 13:32
Reply-To: scientificreports@nature.com
To: renatotsouzasp@gmail.com

Dear Dr T Souza,

Thank you for submitting your manuscript "Trace biomarkers associated with spontaneous preterm birth from the maternal serum metabolome of asymptomatic nulliparous women – parallel case-control studies from the SCOPE cohort" to Scientific Reports. We are pleased to inform you that it has passed through the initial Quality Check process and has been submitted successfully. Your manuscript will be assigned to an Editorial Board Member shortly. Following initial assessment by the Editorial Board Member, manuscripts that are within the scope of the journal, appear to make an original contribution to the literature, and appear to be technically sound and scientifically valid, will be sent to external reviewers.

You may check the status of your submission by selecting the "Check manuscript status" link at the following URL:

http://mts-srep.nature.com/cgi-bin/main.plex?el=A1CG1BtHQ5A4FqTk4F2A9ftdZWC8FzWClCZHkWbjHQwMcwZ

Your paper is at the stage “Manuscript Submitted”. The next stage, “Manuscript Assigned to Editor” will begin when we assign an appropriate Editorial Board Member to your submission, and the status will be updated further when peer-reviewers are assigned. You will be notified by email when a decision has been reached.

Best regards,

Manuscript Administration
Scientific Reports
4 Crinan Street
London N1 9XW
E-mail: scientificreports@nature.com

Springer Nature offers an open access support service to make it easier for our authors to discover and apply for APC funding. For further information please visit http://www.springernature.com/gp/open-research/funding

This email has been sent through the Springer Nature Tracking System NY-610A-NPG&MTS

Confidentiality Statement:

This e-mail is confidential and subject to copyright. Any unauthorised use or disclosure of its contents is prohibited. If you have received this email in error please notify our Manuscript Tracking System Helpdesk team at http://platformsupport.nature.com .

Details of the confidentiality and pre-publicity policy may be found here http://www.nature.com/authors/policies/confidentiality.html

Privacy Policy | Update Profile

RESEARCH ARTICLE

Trace biomarkers associated with spontaneous preterm birth from the maternal serum metabolome of asymptomatic nulliparous women – parallel case-control studies from the SCOPE cohort

Authors

Renato T. Souza[1], Elizabeth J. McKenzie[2], M. Beatrix Jones[2], Jamie V. de Seymour[2], Melinda M. Thomas[2], Erica Zarate[2], Ting Li Han[2], Lesley McCowan[2], Karolina Sulek[2], Silas Villas-Boas[2], Louise C. Kenny[3], José G. Cecatti[1], Philip N. Baker[2,4]

Affiliations

1- Department of Obstetrics and Gynecology, University of Campinas, Campinas, Brazil.
2- The University of Auckland, Auckland, New Zealand.
3- The Department of Women's and Children's Health, Institute of Translational Medicine, Faculty of Health and Life Sciences, University of Liverpool, UK
4- College of Life Sciences, University of Leicester, Leicester, United Kingdom.

Short Title

Serum metabolomic biomarkers of spontaneous PTB

Corresponding Author

Renato T Souza, MD, MSc
Department of Obstetrics and Gynecology
University of Campinas
R. Alexander Fleming, 101
Campinas, SP, 13083-881
Brazil
E-mail: renatotsouzasp@gmail.com

Abstract

Prediction of spontaneous preterm birth (sPTB) in asymptomatic women remains a great challenge; accurate and reproducible screening tools are still not available in clinical practice. We aimed to investigate whether the maternal serum metabolome together with clinical factors could be used to identify asymptomatic women at risk of sPTB. We conducted two case-control studies using gas chromatography-mass spectrometry to analyse maternal serum samples collected at 15- and 20-weeks' gestation from 164 nulliparous women from Cork, and 157 from Auckland. Smoking and vaginal bleeding before 15 weeks were the only significant clinical predictors of sPTB for Auckland and Cork subsets, respectively. Decane, undecane, and dodecane were significantly associated with sPTB (FDR <0.05) in the Cork subset. The odds ratio associated with a 1 sd increase in log (undecane) in a multiple logistic regression also including vaginal bleeding was 1.9. In summary, elevated serum levels of the alkanes decane, undecane, and dodecane were associated with sPTB in asymptomatic nulliparous women from Cork, but not in the Auckland cohort. The association is not strong enough to be a useful clinical predictor, but suggests that further investigation of the association between oxidative stress processes and sPTB risk is warranted.

Keywords: preterm birth, spontaneous preterm birth, metabolomics, prediction, biomarker, metabolites, mechanism, maternal serum, decane, dodecane, undecane, alkanes.

Funding: This study was jointly supported by the Brazilian National Research Council (CNPq) (Award 401636/2013-5), and the Bill and Melinda Gates Foundation (Award OPP1107597- Grand Challenges Brazil: Reducing the burden of preterm birth).

Introduction

Spontaneous preterm birth (sPTB) due to spontaneous onset of labour or premature rupture of membranes (PROM) is a major cause of neonatal mortality and morbidity[1-3]. Although sPTB is prevalent in both high and low/middle-income countries, the major burden of sPTB is concentrated in Asian and African countries where about 85% of preterm births occur [1,4,5]. Short- and long-term consequences of preterm birth include bronchopulmonary dysplasia, neurodevelopment and cognitive impairment, retinopathy, as well as substantial impact on the functional, mental and social health of the infant and its family [6-8]. Despite an increase in the research conducted into sPTB and advancements in the implementation of management and prevention strategies, significant reductions of sPTB have not been achieved[9,10].

Natural progesterone (oral or vaginal), 17α-hydroxyprogesterone (intra-muscular), pessary and/or cervical cerclage have been variously recommended to prevent sPTB in some guidelines [11-14], but their benefits are disputed. According to systematic reviews and meta-analyses, the use of such interventions in selected women can reduce the risk for sPTB, but they are less likely to reduce the risk for perinatal morbidity [15-19]. The identification of a more accurate screening tool to predict which women are most at risk of sPTB could improve the selection of women that would benefit from interventions, increasing the likelihood of success and reducing the costs to the healthcare system. However, current screening tools used to identify women at risk of developing sPTB fail to accurately predict preterm birth in asymptomatic women.

Cervical length is possibly the most employed indicator for sPTB risk in clinical practice. While there is evidence for an increased risk of sPTB in women with a shortened cervix [11,12], approximately 2/3 of women with short cervical length will have a term birth [20,21]. Chemical biomarkers for sPTB have also been sought, with fetal fibronectin (fFN), insulin-

like growth factor-binding protein 1 (IGFPB-1), and interleukin-6 (IL-6) [22-27] among the most studied. A systematic review of sPTB biomarkers found more than 200 studies published between 1965 and 2008, reporting more than 100 potential biomarkers [27]. However, no single biomarker has proven to be a reliable predictor of sPTB. The authors of the systematic review concluded that there are many heterogeneities between the studies in terms of their experimental study design, timepoint of sample collection, and sample processing methods. One of the largest prospective cohort studies to date evaluated the performance of serial transvaginal cervical length measurements and quantitative vaginal fFN levels for predicting sPTB in a sample of approximately 10,000 nulliparous women with singleton pregnancies [28]. Despite determination of cervical length and fibronectin levels at three different gestations, the model still showed low predictive accuracy for sPTB.

sPTB is a condition with a multifactorial aetiology, a long pre-clinical phase, and adaptive mechanisms during pregnancy [29]. For instance, the cervical remodelling process, which involves softening and ripening of the cervix, invariably occurs in the spontaneous onset of labour and is considered its endpoint [30]. However, it does not occur in the same period of pregnancy for all women, which limits its potential as a marker for the prediction of sPTB [31]. The underlying complexity of the drivers of sPTB demands a robust analytical technique capable of taking into consideration the multiple pathways potentially affected in the development of sPTB.

Metabolomics, the study of low molecular weight compounds in a biological system, has previously been used to successfully investigate pregnancy complications such as fetal growth restriction, preeclampsia, and gestational diabetes mellitus [32-35]. Metabolomic measurements provide a biochemical snapshot of the physiological state of the organism [36]; their close relationship with the biological phenotype means they have the potential to

reveal underlying mechanisms of disease. Due to the biochemical complexity of the metabolome, hyphenated analytical techniques are used. This involves subjecting sample extracts to a high-resolution separation technique such as gas chromatography (GC), high-pressure liquid chromatography (HPLC), or capillary electrophoresis (CE). Identification is then carried out using mass spectrometry (MS), or nuclear magnetic resonance (NMR).

The Preterm SAMBA study (Preterm Screening and Metabolomics in Brazil and Auckland) has been established to identify and validate metabolomic biomarkers for sPTB [37], in collaboration with the SCOPE (Screening for Pregnancy Endpoints) study. SCOPE was an international multicentre prospective cohort study that collected standardized data and samples from more than 5,690 low-risk pregnant women [38-40]. The main objective of SCOPE was to develop a robust database and biobank to identify predictors and develop screening tests for preeclampsia, sPTB and small-for-gestational-age newborns. The current study describes the results of Preterm SAMBA phase 1, which used the SCOPE biobank to investigate the metabolomic profile, at 15- and 20-weeks' gestation, amongst SCOPE participants who went on to have sPTB, when compared to matched controls. Parallel analyses were performed using participants from the Irish and New Zealand´s centres of the SCOPE study.

Methods

Study design and participants

Two case-control studies were conducted, using a sub-set of women from the Auckland, New Zealand and Cork, Ireland centres of the Screening for Pregnancy Endpoints (SCOPE) study [41].

The SCOPE study recruited 5,690 nulliparous low-risk women with a singleton pregnancy from New Zealand, Australia, Ireland and the United Kingdom between November 2004

and August 2008. Ethical approval was obtained from the local ethics committees in both Ireland and New Zealand (Cork: study number ECM5 (10) 05/02/08, Clinical Research Ethics Committee of the Cork Teaching Hospitals; New Zealand: study number AKX/02/00/364, Northern X Reginal Ethics Committee) and all participants gave their written informed consent. Details on enrolment and inclusion criteria for the SCOPE study have been published elsewhere [38-40]. Collection of data and biological samples complied with standardized procedures in all participating centres and was conducted in accordance with the principles of the Declaration of Helsinki. Cases of sPTB were defined as those women who delivered before 37 weeks' gestation, and controls as those who delivered at or after 37 weeks' gestation.

a) Cork, Ireland

The subset of SCOPE participants included in this study from the Cork, Ireland centre consisted of 55 cases of sPTB and 102 controls, matched to cases according to maternal age (± 3 years) and maternal body mass index (BMI; ± 5 kg/m^2)*.

b) Auckland, New Zealand

The subset of SCOPE participants included in this study from the Auckland, New Zealand centre consisted of 55 cases of sPTB and 109 controls, comprising 56 controls matched to cases according to maternal age (± 3 years), and 53 controls matched to cases according to both maternal age (± 3 years) and BMI (± 5 kg/m^2)*.

(* Our intention was to match each case to two controls, however, in Cork eight control subjects were excluded due to misclassification or lack of data; in Auckland one maternal age and BMI control was reclassified to a maternal age control and another was excluded due to technical difficulties).

Data collection

Demographic information including maternal age and BMI was collected at the time of recruitment (15 ±1 weeks' gestation), along with information on family medical history. History of vaginal bleeding and any infections during the pregnancy were also recorded at the initial visit. The infant characteristics were recorded at birth and included birth weight, customised birth weight centile, and whether the infant was small, normal, or large for gestational age.

Outcome

Spontaneous preterm birth was defined as any birth that occurred before 37 weeks due to spontaneous onset of labour or premature rupture of membranes. Gestational age (GA) was estimated by last menstrual period (LMP) and confirmed by an ultrasound dating before 16 weeks of gestation. A discordance of seven or more days between LMP and ultrasound dating or an unsure LMP led to the estimation of GA exclusively by early ultrasound parameters. Term birth for the control group was determined as delivery after 37 weeks of gestation. Early sPTB before 34 weeks was considered for subgroup analysis.

Sample collection and storage

Maternal blood samples were collected in two 6 mL vacutainers by venepuncture at 15 and 20 weeks (± 1 week). Following clot formation, the vacutainers were centrifuged at 3,000 rpm for 10 min at 4^{o}C, followed by a second centrifugation at 4,000 rpm for 10 min at 4^{o}C. After centrifugation, the resulting serum (supernatant) was pipetted into a sterile tube and aliquots of 250 µL were dispensed into cryotubes. All samples were stored at -80^{o}C. The study followed standard best practice procedures for repositories in all steps of sample

collection and storage, registering all SPREC (Standard PREanalytical Coding) data accordingly in an online database [42].

For this study, two 250 µL maternal serum samples were obtained from the biobank for each participant, one from each time point; 15- and 20-weeks' gestation (± 1 week).

Sample preparation and extraction

The serum samples underwent extraction and derivatization procedures based on the 2010 protocol of Smart et al. [43]. Briefly, samples were thawed on ice at 4ºC and transferred from cryotubes to 1.5 mL microcentrifuge tubes. An internal standard (IS; 20 µL of 10 mM L-Alanine-2,3,3,4-d4) was added to all samples and the sample-IS mix was vortexed for 1 min. Samples were dried for 4h at 0.8 HPa in a centrifugal vacuum concentrator with a -104°C refrigerated vapour trap (Thermo Fisher Scientific Savant SC250EXP SpeedVac Concentrator with Savant SP5121P Refrigerated Vapour trap). Metabolites were extracted using 50% and 80% cold methanol-water solution (-20ºC, v/v). Specifically, 500 µL of 50% cold methanol-water solution was added to all samples, followed by vortexing for 1 min and centrifugation at 3,000 rpm for 5 min at -4°C. After centrifugation, the supernatant was transferred to a fresh chilled microcentrifuge tube (kept on dry ice). Then, 500 µL of 80% methanol-water solution was added to the pellet and this was centrifuged at 3,000 rpm for 5 min at -4°C. The supernatants obtained from both extraction steps were combined and dried in the centrifugal vacuum concentrator for 4 hours at 0.8 HPa, with a -104ºC refrigerated vapour trap. Dried extracted samples were stored at -80°C prior to derivatisation.

Negative controls were produced by subjecting an empty microcentrifuge tube to the same processing as the samples. Pooled quality control samples (QC) were produced by pooling

a small amount from every sample, mixing, and then making aliquots of the same volume as the samples.

Derivatization was carried out using methyl chloroformate (MCF). Samples were derivatised in batches of 18-24, on the same day that they were analysed on the GC-MS. Samples were re-suspended with 400 µL of 1 M sodium hydroxide and were transferred to silanised glass tubes, followed by addition of 334 µL of methanol and 68 µL of pyridine. The sample was placed on a vortexer for the remainder of the derivatization process at ~1,500 rpm. The rate limiting step began from the addition of 40 µL of MCF. A second addition of 40 µL of MCF was made, 30 sec later. After another 30 sec, 400 µL of chloroform was added to extract the alkylated derivatives from the reaction mixture. After 10 sec, 400 µL of sodium bicarbonate (50 mM) was added. Centrifugation was used to separate the aqueous layer from the chloroform layer. After centrifugation, the aqueous layer was removed and the remaining chloroform extract was dehydrated by the addition of sodium sulphate (~0.3g). The remaining liquid was then transferred to an amber glass GC-MS vial with a glass 33 µL insert.

Gas chromatography – mass spectrometry (GC-MS) analysis

The GC-MS instrument parameters were based on Smart et al. [43], with modifications. One microliter of sample was injected for analysis. The injector was set to 290 °C in splitless mode. The column flow was maintained at 1.0 mL min-1 in constant flow mode. The column was a fused silica ZB-1701 30 m long, 0.25 mm inside diameters, with a 0.15 µm stationary phase constituting of 86% dimethylpolysiloxane and 14% cyanopropylphenyl (Phenomenex). Instrument grade helium (> 99.99 %, BOC) was used as the carrier gas for the analysis. The detector was run in positive-ion, electron-impact ionisation mode, at 70eV electron energy. Identification of compounds was carried out using mass spectra acquired

in scan mode from 38 to 550 atomic mass units. The Cork samples were analysed on an Agilent 7890A gas chromatograph coupled to an 5975C inert mass spectrometer. The Auckland samples were analysed on a Thermo Scientific Trace GC Ultra gas chromatograph coupled to an ISQ mass spectrometer.

Data extraction and compound identification

Data processing was semi-automated. The raw files obtained from the GC-MS were converted into common data form (cdf) format for analysis and were deconvoluted and identified using the Automated Mass Spectral Deconvolution and Identification System (AMDIS - http://www.amdis.net/) from an in-house mass spectral library for MCF-derivatised metabolites (~210 compounds) developed by Silas Villas-Boas (SVB). The library contained mass spectra predominantly from certified reference standards. In addition to the in-house library, the National Institute of Standards and Technology (NIST) mass spectral library (NIST14, 163,198 compounds) was also used to identify peaks in the raw chromatograms. Since AMDIS is not able to batch deconvolute with the entire NIST library, a NIST subset library was constructed employing a method developed by Elizabeth McKenzie using pooled quality controls.

The NIST subset was produced using the top five results for each feature from the Agilent Chemstation PBM (Probability Based Matching) deconvolution program. MassOmics (version 2.3) was used to create the subset library. MassOmics is an R script based on the XCMS R package, with a Windows graphic user interface (GUI) developed by Ting-Li Han. MassOmics used XCMS and the AMDIS report to integrate the peak areas for each of the identified metabolites. The summary report obtained from running the MassOmics script was then checked, and peaks with low ID hits, or with large retention time shift, and laboratory contaminants were removed.

Data was checked against negative controls to identify and remove background contaminants. Peaks that were not extracted correctly with XCMS were integrated separately using the Ion Extractor feature in MassOmics. Co-eluting peaks underwent manual integration. The relative abundance obtained for each metabolite was normalised to the internal standard (Alanine-d4). After internal standard normalisation, the remaining technical variation was corrected for by analytical batch median centering using the samples. An analytical batch was defined as ~25 injections, comprising ~18 samples, 4 QC´s, one Alkane series, one negative control, and one standard mixture.

Statistical analysis

Statistical analysis of the normalised mass spectral data was performed using R 3.4.3 [44] (https://www.R-project.org). Data was analysed separately for each site. Clinical predictors in Table 2 were analysed for univariate associations with preterm birth, and a multivariate logistic regression model predicting preterm birth with these predictors was selected using stepwise logistic regression with AIC, starting from an intercept only model, followed by backward elimination of variables with multivariate p> 0.05. Mann-Whitney tests were used to analyse the difference in metabolite levels between cases and controls; ratios between the 15 and 20-week levels of each metabolite were also assessed in this way. To adjust for multiple comparison testing, false discovery rates (FDR) were calculated for each comparison using the Benjamini-Hochberg procedure [45]. Metabolites with an estimated FDR < 0.05 are reported. We then assessed whether a logistic regression model including these metabolites, individually or in combination, improved the area under the receiver operator characteristic curve compared to the model based on clinical predictors alone [46,47]. To assess whether a more complex model might improve prediction, the sparse partial least

squares discriminant analysis (PLS-DA) method from the mixOmics package was employed [48].

The clinical variables, log transformed 15 and 20-week metabolite intensities, and 15 to 20-weeks ratios were used as candidate predictors. Up to three components with five predictors each were used. The number of components was selected based on the Malhalanobis distance balanced error rate using 10-fold cross validation, averaged over 10 repeats. The significance of this model was assessed by a permutation test. Specifically, the balanced error rate estimated by cross validation was compared between the actual data, and data where the response category was permuted to destroy any true association with the predictors. The error rate was computed for 1000 permutations; the model was considered significantly better than random if the balanced error rate for the real data was better than 95% of the permuted replicates ($p < 0.05$).

The above procedures were repeated for comparing preterm birth <34 weeks to the entire control group.

Results

Participants

Participant demographic and outcome characteristics are shown in Table 1. These show the success of the matching strategy (similar BMI and age in case and control groups) and the expected association between birthweight and gestational age category. Potential clinical predictors are shown in Table 2. For Auckland data, smoking was the only significant clinical predictor in univariate models, and the multivariate model selection also chose a model with smoking only (odds ratio = 4.9; p-value = 0.03). When considering preterm birth <34 weeks, smoking was no longer a significant predictor comparing preterm cases

<34 weeks to the entire control group. For the Cork data, having vaginal bleeding before 15 weeks' was the only significant univariate predictor of sPTB (<37weeks); multivariate model selection again chose a model with this variable only (odds ratio = 2.8; p-value = 0.006). The selected multivariate model for preterm birth <34 weeks included vaginal bleeding, smoking, and history of miscarriage (Table 4).

Metabolites identified

In the Cork samples, 176 compounds were detected. Of these, 77 were identified using an in-house library of reference standards and the remaining compounds were identified using mass spectrum alone (NIST 2014 mass spectral library). Of the in-house library matches, 61 were putatively identified (80-100% match to a reference standard) and 15 were tentatively identified (60-79% mass spectral match). Of the NIST14 library identifications, 25 were putatively identified (80-100% mass spectral match), 58 were tentatively identified (60-79% mass spectral match), and three were unknown (< 60% mass spectral match).

In the Auckland samples, 142 compounds were detected. Of these, 50 were identified using an in-house library of reference standards and the remaining compounds were identified using mass spectrum alone (NIST 2014). Of the in-house library matches, 41 were identified, eight were tentatively identified, and one was unknown. Of the NIST14 library identifications, 51 were putatively identified, 34 were tentatively identified, and seven were unknown.

Metabolites and performance of predictive models

Table 3 shows the metabolites associated with preterm birth < 37 weeks' and preterm birth < 34 weeks', at each location and at each gestational time point (15 weeks or 20 weeks); ratios between the 15 and 20 week values were also assessed. Three metabolites detected at 20 weeks of gestation in the Cork subset were found to be significantly associated with

sPTB (FDR < 0.05) when compared to term births: undecane, dodecane and decane. All three were found to have higher abundance in sPTB cases. Adding the natural log intensity of undecane to the clinical predictors in a logistic regression model increased the area under the curve from 0.60 to 0.73 (Fig. 1), significantly improving the model (p=0.0007). This model estimates an odds ratio of 1.9 for a 1 standard deviation increase in log (undecane). The other metabolites were correlated with undecane (Pearson´s correlation for log intensities r=0.87 decane, r=0.89 dodecane). Consequently, results were similar for the other metabolites added individually to the model but adding multiple metabolites did not improve the model further. Fig. 1 shows the receiver operating characteristic curves for the clinical model only, and the clinical model with the addition of undecane. Sparse PLS-DA also produced a 1-component model for the Cork preterm birth data with an error rate that was significantly better than error rates produced for random permutations (p=0.01). This model again included undecane, dodecane, decane, and vaginal bleeding. A fifth predictor, stearic acid measured at 15 weeks' gestation, was present; however, its loading was small, and incorporating it into the logistic regression did not significantly improve the area under the curve (p=0.37).

No metabolites or 15-20 week ratios met the false discovery rate threshold for the Cork <34 weeks data, for either preterm <37 weeks or <34 weeks in the Auckland data, nor were the sparse PLS-DA models significant (p = 0.07, preterm birth <37 weeks; p= 0.48 preterm birth <34 weeks).

Discussion

We have analysed potential predictors of sPTB using serum samples and clinical data from Cork and Auckland participants of the SCOPE study, an international cohort of low-risk nulliparous women. An untargeted metabolomics approach was applied to serum samples collected at 15 and 20 weeks of gestation. More than one hundred metabolites were

identified in each subset (Cork and Auckland), but only three metabolites from the 20-week serum of Cork participants were found to be significantly associated with sPTB. As expected for the metabolomics method employed, the most common classes of metabolites were fatty acids, followed by amino acids. Vaginal bleeding before 15 weeks' and smoking during pregnancy were the only clinical factors associated with sPTB in Cork and Auckland subsets, respectively. In the Cork cohort, adding undecane to a multivariate logistic regression model for predicting sPTB improved its performance over a model with vaginal bleeding alone. We note that, as depicted in Fig. 1, there are clear average differences in undecane levels between case and control groups, but also substantial overlap, limiting the utility of the metabolite measurements in clinical practice.

There is a biologically plausible explanation for observing elevated alkanes (decane, undecane, dodecane) in the serum of mothers who had sPTB. Oxidative stress may lead to degradation of cell membranes by lipid peroxidation, followed by conversion of polyunsaturated fatty acids to volatile alkanes. The association of several oxidative stress-associated processes and sPTB have been previously reported [49-51], and oxidative stress has been associated with elevated alkane levels in gastroenteric disease, lung disease, and other chronic diseases of metabolism [52-54]. Glutathione, an important intracellular antioxidant, has been found to be decreased in maternal and umbilical cord blood of very low preterm neonates and their mothers [55]. Preterm birth seems to be associated with depletion of glutathione, reinforcing a possible increased oxidative status and lower antioxidant capacity. However, failure to demonstrate elevated alkanes among sPTB cases in the Auckland cohort limits our confidence, and there was no evidence these alkanes were associated with early sPTB (<34 weeks) at either study site. In addition, hypotheses describing the role of reactive oxygen species generation, metabolic and inflammatory imbalance and many other downstream mechanisms (telomerase reduction, cell apoptosis

and senescence, etc.) caused by oxidative stress activation [49] do not clarify whether those mechanisms are trigger factors or consequences of underlying conditions/alterations resulting in preterm PROM and/or spontaneous onset of preterm labour.

The differences across study sites in the associations between sPTB and clinical factors such as smoking and vaginal bleeding in this case-control analysis also suggest that there are differences in the Cork and Auckland populations. The alkanes elevated in the Cork preterm birth group are present in outdoor and indoor air contaminants, which could potentially differ across study sites, providing an alternative explanation for the observation. Association of environmental exposures with maternal and perinatal health have been reported for many years but are not yet well established [56,57]. Further investigation of individual pollutant exposure would be necessary to confirm whether elevated alkanes are associated with environmental exposure. It is also possible that technical rather than biological variability accounts for some of the differences observed across sites. Samples from the different sites, while analysed using the same protocols, were run on different platforms by different technical personnel. Thirty-four fewer metabolite species were detected in the Auckland samples, suggesting reduced sensitivity.

We also examined prediction of very preterm birth (<34 weeks). While no clinical predictors were significant in the Auckland cohort, the selected multivariate model for Cork included vaginal bleeding, smoking, and previous miscarriage. While caution is needed because of the small sample size, 14 out of the 16 cases of early sPTB from Cork had at least one of these risk factors.

To the best of our knowledge, there are few studies in the literature applying metabolomics techniques to understand sPTB in asymptomatic pregnant women [33,58-60]. Previous studies have used between 20 and 70 samples of a variety of biofluids (amniotic and cervicovaginal fluid, as well as serum), equally divided between cases and controls. There is also a

diversity of pregnancy stages and analytical techniques, so it is not surprising that there is little overlap in the specific compounds identified. However, Virgiliou et al. also suggested that the changes in amino acids and lipids they observed could be related to oxidative stress [60].

Our study has strengths and limitations. Cases and controls were selected from a large cohort comprised of low-risk nulliparous women enrolled in early pregnancy and containing a high standard biobank. Several procedures were employed to assure data, sample, and analysis compliance and reliability according to Standard Operating Protocols. Shortcomings of our study include lack of data regarding cervical length, a previously reported risk factor, and assaying of the metabolome of the two cohorts at different times on different equipment. In addition, we have not investigated predictive metabolites for the different preterm birth subtypes (spontaneous onset of preterm labour or preterm premature rupture of membranes), focusing instead on an early sPTB (<34 weeks) subgroup, due to the increased morbidity in this specific group. Using data and samples from women of different populations (Cork, Ireland; Auckland, New Zealand), enabled us to compare reproducibility of our technique and also to discuss possible local drivers for sPTB (Alkane pollutants in Cork, Ireland, for instance). Cork and Auckland samples were analysed by different laboratory experts and instruments and as such, the reproducibility and sensitivity differed across the sites.

While our finding of an association between elevated alkanes and sPTB at the Cork site is preliminary, it raises several interesting questions to pursue in the future. What differences are typical or expected across geographically distant study sites? What role might exposure to exogenous pollution sources, including alkanes, play in preterm birth? And finally, how might oxidative stress trigger, or be triggered by, processes leading to spontaneous preterm birth?

References

1 Blencowe, H. *et al.* Born Too Soon: The global epidemiology of 15 million preterm births. *Reproductive health* **10**, S2-S2, doi:10.1186/1742-4755-10-S1-S2 (2013).

2 Blencowe, H. *et al.* Preterm birth-associated neurodevelopmental impairment estimates at regional and global levels for 2010. *Pediatric research* **74 Suppl 1**, 17-34, doi:10.1038/pr.2013.204 (2013).

3 Lawn, J. E., Cousens, S. & Zupan, J. 4 million neonatal deaths: when? Where? Why? *Lancet* **365**, 891-900, doi:10.1016/S0140-6736(05)71048-5 (2005).

4 Beck, S. *et al.* The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. *Bulletin of the World Health Organization* **88**, 31-38, doi:10.2471/BLT.08.062554 (2010).

5 Blencowe, H. *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* **379**, 2162-2172, doi:10.1016/S0140-6736(12)60820-4 (2012).

6 Patel, R. M. Short- and Long-Term Outcomes for Extremely Preterm Infants. *Am J Perinatol* **33**, 318-328, doi:10.1055/s-0035-1571202 (2016).

7 Natarajan, G. & Shankaran, S. Short- and Long-Term Outcomes of Moderate and Late Preterm Infants. *Am J Perinatol* **33**, 305-317, doi:10.1055/s-0035-1571150 (2016).

8 Saigal, S. & Doyle, L. W. An overview of mortality and sequelae of preterm birth from infancy to adulthood. *Lancet* **371**, 261-269, doi:10.1016/S0140-6736(08)60136-1 (2008).

9 Chang, H. H. *et al.* Preventing preterm births: analysis of trends and potential reductions with interventions in 39 countries with very high human development index. *Lancet* **381**, 223-234, doi:10.1016/S0140-6736(12)61856-X (2013).

10 Zeitlin, J. *et al.* Preterm birth time trends in Europe: a study of 19 countries. *BJOG* **120**, 1356-1365, doi:10.1111/1471-0528.12281 (2013).

11 *National Institute for Health and Care Excellence: Clinical Guidelines. Preterm Labour and Birth*. (2015 National Collaborating Centre for Women's and Children's Health., 2015).

12 ACOG Practice Bulletin. Assessment of risk factors for preterm birth. Clinical management guidelines for obstetrician-gynecologists. Number 31, October 2001. . *Obstet Gynecol* **98**, 709-716 (2001).

13 Sentilhes, L. *et al.* Prevention of spontaneous preterm birth: Guidelines for clinical practice from the French College of Gynaecologists and Obstetricians (CNGOF). *Eur J Obstet Gynecol Reprod Biol* **210**, 217-224, doi:10.1016/j.ejogrb.2016.12.035 (2017).

14 Di Renzo, G. C. *et al.* Guidelines for the management of spontaneous preterm labor: identification of spontaneous preterm labor, diagnosis of preterm premature rupture of membranes, and preventive tools for preterm birth. *J Matern Fetal Neonatal Med* **24**, 659-667, doi:10.3109/14767058.2011.553694 (2011).

15 Saccone, G. *et al.* Cervical Pessary for Preventing Preterm Birth in Singleton Pregnancies With Short Cervical Length: A Systematic Review and Meta-analysis. *J Ultrasound Med* **36**, 1535-1543, doi:10.7863/ultra.16.08054 (2017).

16 Berghella, V. *et al.* Cerclage for sonographic short cervix in singleton gestations without prior spontaneous preterm birth: systematic review and meta-analysis of randomized controlled trials using individual patient-level data. *Ultrasound Obstet Gynecol* **50**, 569-577, doi:10.1002/uog.17457 (2017).

17 Jin, Z. *et al.* Cervical pessary for preventing preterm birth: a meta-analysis. *J Matern Fetal Neonatal Med*, 1-7, doi:10.1080/14767058.2017.1401998 (2017).

18 Romero, R. *et al.* Vaginal Progesterone for Preventing Preterm Birth and Adverse Perinatal Outcomes in Singleton Gestations with a Short Cervix: A Meta-Analysis of Individual Patient Data. *American Journal of Obstetrics & Gynecology*, doi:10.1016/j.ajog.2017.11.576 (in Press).

19 Jarde, A. *et al.* Effectiveness of progesterone, cerclage and pessary for preventing preterm birth in singleton pregnancies: a systematic review and network meta-analysis. *BJOG* **124**, 1176-1189, doi:10.1111/1471-0528.14624 (2017).

20 Berghella, V., Roman, A., Daskalakis, C., Ness, A. & Baxter, J. K. Gestational age at cervical length measurement and incidence of preterm birth. *Obstetrics and gynecology* **110**, 311-317, doi:10.1097/01.AOG.0000270112.05025.1d (2007).

21 Iams, J. D. *et al.* The length of the cervix and the risk of spontaneous premature delivery. National Institute of Child Health and Human Development Maternal Fetal Medicine Unit Network. *The New England journal of medicine* **334**, 567-572, doi:10.1056/NEJM199602293340904 (1996).

22 Conde-Agudelo, A., Papageorghiou, A. T., Kennedy, S. H. & Villar, J. Novel biomarkers for the prediction of the spontaneous preterm birth phenotype: a systematic review and meta-analysis. *BJOG* **118**, 1042-1054, doi:10.1111/j.1471-0528.2011.02923.x (2011).

23 Abbott, D. S. *et al.* Quantitative fetal fibronectin to predict preterm birth in asymptomatic women at high risk. *Obstetrics and gynecology* **125**, 1168-1176, doi:10.1097/AOG.0000000000000754 (2015).

24 Conde-Agudelo, A. & Romero, R. Cervical phosphorylated insulin-like growth factor binding protein-1 test for the prediction of preterm birth: a systematic review and metaanalysis. *American journal of obstetrics and gynecology* **214**, 57-73, doi:10.1016/j.ajog.2015.06.060 (2016).

25 van der Ven, J. *et al.* The capacity of mid-pregnancy cervical length to predict preterm birth in low-risk women: a national cohort study. *Acta Obstetricia et Gynecologica Scandinavica* **94**, 1223-1234, doi:10.1111/aogs.12721 (2015).

26 Celik, E. *et al.* Cervical length and obstetric history predict spontaneous preterm birth: development and validation of a model to provide individualized risk assessment. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology* **31**, 549-554, doi:10.1002/uog.5333 (2008).

27 Menon, R. *et al.* Biomarkers of Spontaneous Preterm Birth: An Overview of The Literature in the Last Four Decades. *Reproductive Sciences* **18**, 1046-1070, doi:10.1177/1933719111415548 (2011).

28 Esplin, M. S. *et al.* Predictive Accuracy of Serial Transvaginal Cervical Lengths and Quantitative Vaginal Fetal Fibronectin Levels for Spontaneous Preterm Birth Among Nulliparous Women. *JAMA* **317**, 1047-1056, doi:10.1001/jama.2017.1373 (2017).

29 Di Renzo, G. C. The great obstetrical syndromes. *The journal of maternal-fetal and neonatal medicine* **22**, 633-635, doi:10.1080/14767050902866804 (2009).

30 *Preterm Birth: Causes, Consequences, and Prevention*. (National Academy of Sciences., 2007).

31 Word, R. A., Li, X. H., Hnat, M. & Carrick, K. Dynamics of cervical remodeling during pregnancy and parturition: mechanisms and current concepts. *Semin Reprod Med* **25**, 69-79, doi:10.1055/s-2006-956777 (2007).

32 Romero, R. *et al.* The use of high-dimensional biology (genomics, transcriptomics, proteomics, and metabolomics) to understand the preterm parturition syndrome. *BJOG: An International Journal of Obstetrics & Gynaecology* **113**, 118-135, doi:10.1111/j.1471-0528.2006.01150.x (2006).

33 Horgan, R. P., Clancy, O. H., Myers, J. E. & Baker, P. N. An overview of proteomic and metabolomic technologies and their application to pregnancy research. *BJOG* **116**, 173-181, doi:10.1111/j.1471-0528.2008.01997.x (2009).

34 Sulek, K. *et al.* Hair metabolomics: identification of fetal compromise provides proof of concept for biomarker discovery. *Theranostics* **4**, 953-959, doi:10.7150/thno.9265 (2014).

35 He, X. *et al.* Maternal hair metabolome analysis identifies a potential marker of lipid peroxidation in gestational diabetes mellitus. *Acta diabetologica*, doi:10.1007/s00592-015-0737-9 (2015).
36 Hollywood, K., Brison, D. R. & Goodacre, R. Metabolomics: current technologies and future trends. *Proteomics* **6**, 4716-4723, doi:10.1002/pmic.200600106 (2006).
37 Cecatti, J. G. *et al.* Use of metabolomics for the identification and validation of clinical biomarkers for preterm birth: Preterm SAMBA. *BMC Pregnancy and Childbirth* **16**, 212-212, doi:10.1186/s12884-016-1006-9 (2016).
38 McCowan, L. M. E. *et al.* Risk factors for small-for-gestational-age infants by customised birthweight centiles: data from an international prospective cohort study. *BJOG : an international journal of obstetrics and gynaecology* **117**, 1599-1607, doi:10.1111/j.1471-0528.2010.02737.x (2010).
39 North, R. A. *et al.* Clinical risk prediction for pre-eclampsia in nulliparous women: development of model in international prospective cohort. *BMJ (Clinical research ed.)* **342**, d1875-d1875 (2011).
40 Australian New Zealand Clinical Trials Registry: ACTRN12607000551493. https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=82254. Accessed 27 Sep 2017., (2007).
41 Kenny, L. C. *et al.* Early pregnancy prediction of preeclampsia in nulliparous women, combining clinical risk and biomarkers: the Screening for Pregnancy Endpoints (SCOPE) international cohort study. *Hypertension* **64**, 644-652, doi:10.1161/HYPERTENSIONAHA.114.03578 (2014).
42 Betsou, F. *et al.* Standard preanalytical coding for biospecimens: defining the sample PREanalytical code. *Cancer epidemiology, biomarkers & prevention* **19**, 1004-1011, doi:10.1158/1055-9965.EPI-09-1268 (2010).
43 Smart, K. F., Aggio, R. B., Van Houtte, J. R. & Villas-Bôas, S. G. Analytical platform for metabolome analysis of microbial cells using methyl chloroformate derivatization followed by gas chromatography-mass spectrometry. *Nat Protoc* **5**, 1709-1729, doi:10.1038/nprot.2010.108 (2010).
44 (2006).
45 Benjamini, Y. & Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B (Methodological)* **57**, 289-300 (1995).
46 DeLong, E. R., DeLong, D. M. & Clarke-Pearson, D. L. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* **44**, 837-845 (1988).
47 Robin, X. *et al.* pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics* **12**, 77, doi:10.1186/1471-2105-12-77 (2011).
48 Le Cao, K. A., Boitard, S. & Besse, P. Sparse PLS discriminant analysis: biologically relevant feature selection and graphical displays for multiclass problems. *BMC Bioinformatics* **12**, 253, doi:10.1186/1471-2105-12-253 (2011).
49 Menon, R. Oxidative stress damage as a detrimental factor in preterm birth pathology. *Front Immunol* **5**, 567, doi:10.3389/fimmu.2014.00567 (2014).
50 Venkatesh, K. K. *et al.* Inflammatory and oxidative stress markers associated with decreased cervical length in pregnancy. *Am J Reprod Immunol* **76**, 376-382, doi:10.1111/aji.12545 (2016).
51 Moore, T. A., Ahmad, I. M. & Zimmerman, M. C. Oxidative Stress and Preterm Birth: An Integrative Review. *Biol Res Nurs* **20**, 497-512, doi:10.1177/1099800418791028 (2018).
52 Phillips, M. *et al.* Volatile organic compounds in breath as markers of lung cancer: a cross-sectional study. *Lancet* **353**, 1930-1933, doi:10.1016/s0140-6736(98)07552-7 (1999).

53 Phillips, M. *et al.* Detection of lung cancer with volatile markers in the breath. *Chest* **123**, 2115-2123 (2003).
54 Phillips, M. *et al.* Volatile biomarkers of pulmonary tuberculosis in the breath. *Tuberculosis (Edinb)* **87**, 44-52, doi:10.1016/j.tube.2006.03.004 (2007).
55 Küster, A. *et al.* Cord blood glutathione depletion in preterm infants: correlation with maternal cysteine depletion. *PLoS One* **6**, e27626, doi:10.1371/journal.pone.0027626 (2011).
56 Ferguson, K. K., O'Neill, M. S. & Meeker, J. D. Environmental contaminant exposures and preterm birth: a comprehensive review. *J Toxicol Environ Health B Crit Rev* **16**, 69-113, doi:10.1080/10937404.2013.775048 (2013).
57 Royal College of Obstetricians and Gynaecologists. Chemical Exposures During Pregnancy: Dealing with Potential, but Unproven, Risks to Child Health. (2016).
58 Parfieniuk, E., Zbucka-Kretowska, M., Ciborowski, M., Kretowski, A. & Barbas, C. Untargeted metabolomics: an overview of its usefulness and future potential in prenatal diagnosis. *Expert Rev Proteomics*, doi:10.1080/14789450.2018.1526678 (2018).
59 Ghartey, J., Bastek, J. A., Brown, A. G., Anglim, L. & Elovitz, M. A. Women with preterm birth have a distinct cervicovaginal metabolome. *Am J Obstet Gynecol* **212**, 776.e771-776.e712, doi:10.1016/j.ajog.2015.03.052 (2015).
60 Virgiliou, C. *et al.* Amniotic Fluid and Maternal Serum Metabolic Signatures in the Second Trimester Associated with Preterm Delivery. *J Proteome Res* **16**, 898-910, doi:10.1021/acs.jproteome.6b00845 (2017).

Acknowledgements

We would like to acknowledge Rennae Taylor for assistance accessing the Auckland SCOPE biobank, and Emma Snapes, Ali Khashan and the SCOPE team at University College Cork for assistance with shipping samples. Thanks also to the staff at the Centre for Genomics, Proteomics & Metabolomics (University of Auckland), and at the Liggins Institute.

Authors Contributions

Baker PN, Kenny LC, and McCowan L participated in the primary cohort study design and project implementation. Baker PN, Kenny LC, and Cecatti JG conceived and enabled the current study. Thomas MM carried out sample preparation and instrumental analysis and Sulek K directed and supervised the analysis for the Auckland samples. Villas-Boas S developed the analytical method for sample analysis. Zarate E carried out sample

preparation and instrumental analysis and McKenzie EJ directed and supervised the analysis for the Cork samples. Han TL provided significant help in data acquisition and instrumental analysis, and produced the software used for data extraction. Thomas MM processed the Auckland data and McKenzie EJ processed the Cork data; Jones MB and de Seymour JV carried out the statistical analysis for the study. de Seymour JV collated material written by Thomas MM, McKenzie EJ, Jones MB, and Souza RT for the first draft of the manuscript, and Souza RT coordinated the final draft of the manuscript. All the authors read, reviewed and approved the final version of the manuscript.

Competing Interest Statement

Baker PN and Kenny LC are minority shareholders of Metabolomics Diagnostics Ltd, a company dedicated to developing innovative screening tests using metabolomics technology.

Funding

The SCOPE database is provided and maintained by MedSciNet AB (http://medscinet.com). The New Zealand SCOPE study was funded by the New Enterprise Research Fund, Foundation for Research Science and Technology; Health Research Council (04/198); Evelyn Bond Fund, Auckland District Health Board Charitable Trust. The Australian SCOPE study was funded by the Premier's Science and Research Fund, South Australian Government (http://www.dfeest.sa.gov.au/science- research/premiers-research-and-industry-fund). The Irish SCOPE study was funded by the Health Research Board of Ireland (CSA/2007/2; http://www.hrb.ie). The UK SCOPE study was funded by National Health Service NEAT Grant (Neat Grant FSD025), Biotechnology and Biological Sciences Research council (www. bbsrc.ac.uk/funding; GT084) and University of Manchester Proof of Concept Funding (University of Manchester); Guy's and St. Thomas'

Charity (King's College London) and Tommy's charity (http://www. tommys.org/; King's College London and University of Manchester); and Cerebra UK (www.cerebra.org.uk; University of Leeds).

The current analyses were designed, sponsored and developed as part of the Preterm SAMBA study, a collaborative study group on Preterm Birth Research jointly issued by the Brazilian National Research Council (CNPq) and the Bill and Melinda Gates Foundation (Award 401636/2013-5 - Grand Challenges Brazil: Reducing the burden of preterm birth, number 05/2013).

Data availability statement

The analysis in this study used the information from two centres from the SCOPE Consortium that has the ownership of data. Any requirement regarding this data can be directed to Prof. Philip N Baker.

Figure Legends

Figure 1. Prediction model for sPTB <37 weeks: Comparison of ROC curves using clinical predictors only (vaginal bleeding) and clinical predictors with log undecane intensity for the Cork cohort; with comparison of log_2 intensity for undecane across case (Preterm Birth <37w) and control (Term birth) individuals.

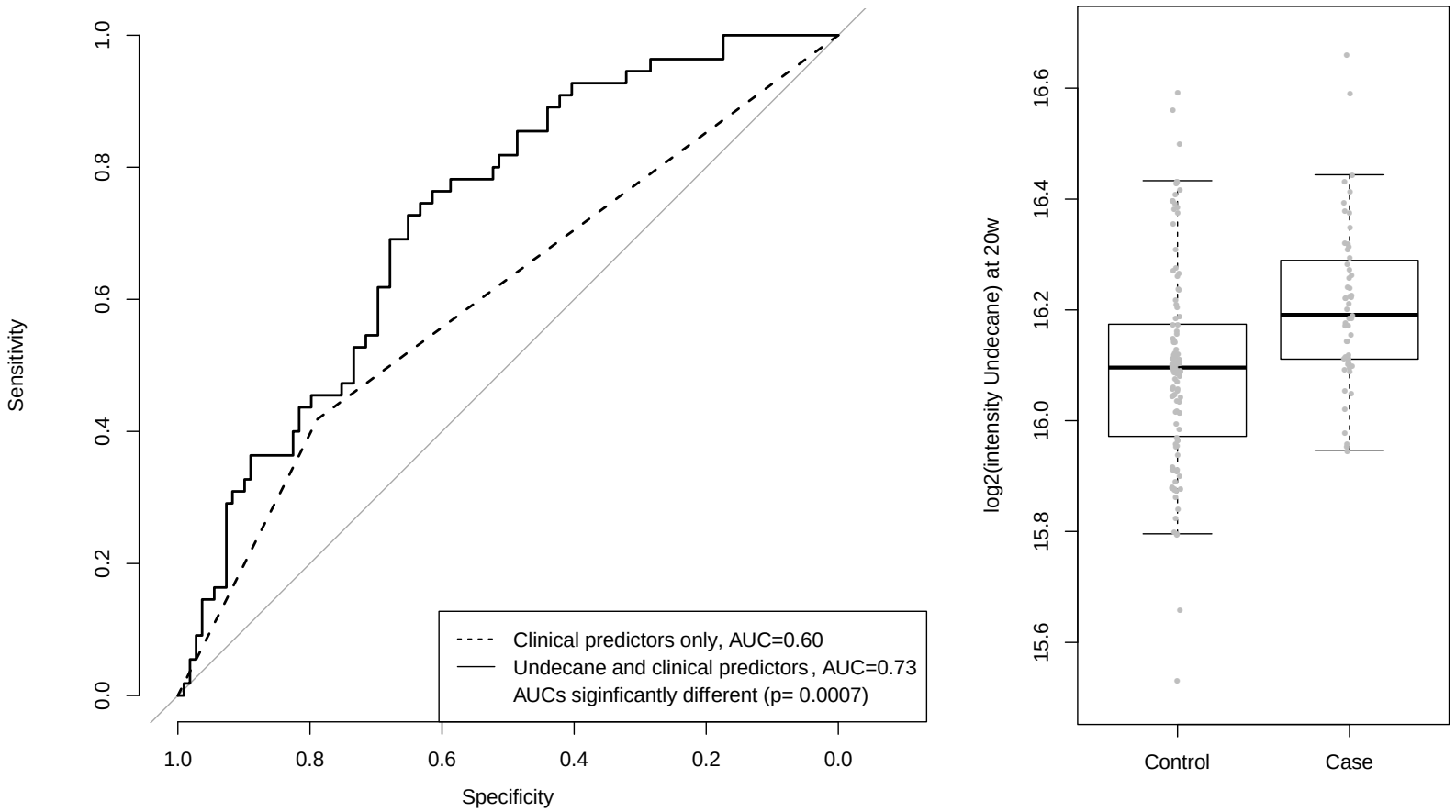


Fig 1. Prediction model for sPTB <37 weeks: Comparison of ROC curves using clinical predictors only (vaginal bleeding) and clinical predictors with log undecane intensity for the Cork cohort; with comparison of log_2 intensity for undecane across case (Preterm Birth <37w) and control (Term birth) individuals.

Table 1. Medians (Lower and Upper Quartiles) or Counts (%) for Matching and Outcome Variables

	Cork		Auckland	
	Term Birth N= 109	Spontaneous Preterm Birth N= 55	Term Birth N=102	Spontaneous Preterm Birth N=55
Maternal BMI	23.4 (21.8, 25.8)	24.0 (21.6, 26.3)	23.5 (21.7, 26.0)	23.5 (21.5,27.0)
Maternal Age (years)	30 (27, 32)	30 (27, 34)	31 (28, 33)	32 (29, 34)
Birthweight (g)	3600 (3350, 3830)	2520 (2045, 2795)	3560 (3320, 3884)	2540 (2173, 2995)
Gestational age at delivery (weeks)	40.6 (39.9, 41.3)	35.3 (33.7, 36.4)	40.4 (39.3, 41.1)	35.7 (34, 36.4)
Gestational age at delivery				
< 28 weeks	N/A	3 (6%)	N/A	3 (6%)
28-32 weeks		4 (7%)		2 (4%)
32-34 weeks		9 (16%)		8 (15%)
34-37 weeks		39 (71%)		42 (76%)
Preterm Premature Rupture of Membranes				
Yes	N/A	29 (53%)	N/A	25 (45%)
No		26 (47%)		30 (55%)

Table 2. Medians (Lower and Upper Quartiles) or Counts (%) for potential clinical predictors for sPTB <37 weeks and <34weeks

	Cork					Auckland				
	Term Birth N= 109	**Spontaneous Preterm Birth <37w N= 55**	p-value <37w vs term	**Spontaneous Preterm Birth <34w N=16**	p-value <34w vs term	**Term Birth N=102**	**Spontaneous Preterm Birth <37w N=55**	p-value <37w vs term	**Spontaneous Preterm Birth <34w N=13**	p-value <34w vs term
Maternal height (cm)	165 (161, 168)	165 (160, 168)	0.99	167 (160, 169)	0.48	165 (161, 169)	165 (162, 168)	0.80	164 (162, 168)	0.53
Infant sex										
Female	47 (43%)	24 (44%)	>0.99	7 (44%)	1 *	47 (46%)	24 (44%)	0.90	4 (31%)	0.38*
Male	62 (57%)	31 (56%)		9 (56%)		55 (54%)	31 (56%)		9 (69%)	
Smoking in pregnancy										
no smoking	82 (75%)	40 (72%)	0.78 §	10 (62%)		92 (90%)	44 (80%)	0.06* §	12 (92%)	
quit	17 (16%)	8 (15%)		3 (19%)	0.33*	7 (7%)	4 (7%)	**0.03* ǂ**	1 (8%)	1 *§
current smoker	10 (9%)	7 (13%)	0.66 ǂ	3 (19%)		3 (3%)	7 (13%)		0	
Fertility Treatment	7 (6%)	2 (4%)	0.72*	2 (13%)	0.32*	11 (11%)	7 (13%)	0.92	2 (15%)	0.64*
Previous Miscarriage	15 (14%)	10 (18%)	0.61	4 (25%)	0.26*	15 (15%)	6 (11%)	0.67	2 (15%)	1 *
Gravidity										
1	91 (83%)	43 (78%)		12 (75%)		73 (72%)	37 (67%)		8 (62%)	
2	13 (12%)	10 (18%)	0.55*	2 (13%)	0.39*	23 (23%)	11 (20%)	0.36*	3 (23%)	0.35*
3 or more	5 (5%)	2 (4%)		2 (13%)		6 (6%)	7 (13%)		2 (15%)	
Vaginal bleeding before 15 w visit	23 (21%)	23 (42%)	**0.01**	9 (56%)	**0.01***	22 (22%)	14 (25%)	0.72	3 (23%)	1 *
Any infection before 15 w visit[a]	26 (24%)	11 (20%)	0.72	5 (31%)	0.54*	45 (44%)	24 (44%)	>0.99	6 (46%)	1*

P-values are from tests of case-control differences using the Mann-Whitney test (Continuous variables), Chi-squared test (categorical variables with adequate counts) or Fisher's exact test (*). § p-value for the three categories. ǂ p-value for current smoker vs no smoking in pregnancy. [a]Any infection during pregnancy means upper respiratory or urinary tract infection, pyelonephritis, gastrointestinal infection, vaginal candidiasis or other infections.

Table 3. Metabolites significantly associated with sPTB <37 weeks and <34 weeks

SCOPE site	Gestational age at sample collection	Metabolite	CAS number	P-Value*	FDR	Direction of association and PTB category
Auckland	15 weeks	None	-	-	-	-
Auckland	20 weeks	None	-	-	-	-
Auckland	Ratio between 15 and 20 weeks' gestation	None	-	-	-	-
Cork	15 weeks	None	-	-	-	-
Cork	20 weeks	Undecane	1120-21-4	3.73E-05	0.007	Higher in sPTB <37weeks
		Dodecane	112-40-3	8.11E-05	0.007	Higher in sPTB <37weeks
		Decane	124-18-5	7.06E-04	0.040	Higher in sPTB <37weeks
Cork	Ratio between 15 and 20 weeks' gestation	None	-	-	-	-

* Mann Whitney U test

Table 4. Clinical predictors of preterm birth <34 weeks in the Cork data, compared to controls (n=124)

Variables	Odds Ratio	P-value
Vaginal Bleeding	9.17	0.002
Smoking—current	9.47	0.015
Any miscarriage	4.54	0.047

4.8. Artigo *Clinical and epidemiological factors associated with spontaneous preterm birth: a multicentre cohort of low risk nulliparous women*

Gmail

Renato Souza <doutorrenatosouza@gmail.com>

Fwd:

José Guilherme Cecatti <cecatti@unicamp.br>
To: Renato Souza <renatotsouzasp@gmail.com>

5 February 2019 at 22:48

---------- Forwarded message ---------
From: **Chronos** <support@chronos-oa.com>
Date: ter, 5 de fev de 2019 às 20:50
Subject:
To: <cecatti@unicamp.br>

Dear **José Guilherme Cecatti,**

Thank you for submitting the following article:

- Article title: **Clinical and epidemiological factors associated with spontaneous preterm birth: a multicentre cohort of low risk nulliparous women**
- Journal: **BJOG: An International Journal of Obstetrics and Gynaecology**
- Primary author: **José Guilherme Cecatti**
- Date submitted: **February 05, 2019**
- Chronos reference number: **3546AEB3-19BA-4C7E-8799-0C384273AC20**

Chronos will transfer the information you submitted to the journal based on the journal's submission process. The journal publisher will contact you with next steps. Upon article acceptance, Chronos will check compliance with the Gates Foundation's Open Access Policy, coordinate payment directly with the publisher, and ensure that your article is deposited in PubMed Central.

If you have any questions about your submitted article, please email support@chronos-oa.com.

Thank you,

The Chronos Support Team

BILL & MELINDA GATES foundation

SIMPLIFYING RESEARCH PUBLISHING
CHRONOS

CLINICAL ARTICLE

Clinical and epidemiological factors associated with spontaneous preterm birth: a multicentre cohort of low risk nulliparous women

Renato T Souza [1], Maria L Costa [1], Jussara Mayrink [1], Francisco E. Feitosa [2], Edilberto A Rocha Filho [3], Débora F Leite [1,3], Janete Vettorazzi [4], Iracema M Calderon [5], Maria H Sousa [6], Renato Passini Jr [1], Philip N Baker [7], Louise Kenny [8], Jose G. Cecatti [1], for the Preterm SAMBA study group

Affiliations

[1] Department of Obstetrics and Gynaecology, University of Campinas (UNICAMP) School of Medical Sciences, Campinas, SP, Brazil

[2] MEAC – School Maternity of the Federal University of Ceará, Fortaleza, CE, Brazil

[3] Department of Maternal and Child Health, Clinics Hospital, Federal University of Pernambuco, Recife, PE, Brazil

[4] Department of Obstetrics and Gynaecology, Maternity of the Clinics Hospital, Federal University of RS, Porto Alegre, RS, Brazil

[5] Department of Obstetrics and Gynaecology, Botucatu Medical School, Unesp, Botucatu, SP, Brazil

[6] Statistics Unit, Jundiai School of Medicine, Jundiaí, SP, Brazil

[7] College of Life Sciences, University of Leicester, Leicester, United Kingdom.

[8] Faculty of Health and Life Sciences, University of Liverpool, UK

*Membership of the Preterm SAMBA study group are provided in the Acknowledgments.

Short title: Risk factor for spontaneous preterm birth in nulliparous women

Corresponding author
José G Cecatti
Department of Obstetrics and Gynaecology
University of Campinas, Brazil
E-mail: cecatti@unicamp.br

Abstract

Objective: Determine incidence and risk factors associated with spontaneous preterm birth (sPTB).

Design: Prospective multicentre cohort.

Setting: Five Brazilian referral maternity hospitals.

Population: Nulliparous low-risk women.

Methods: Women enrolled at 19-21 weeks. Comprehensive maternal data collected during three study visits, addressed as potentially associated factors for sPTB. Bivariate and multivariate analysis estimated risk ratios.

Main outcomes measures: Birth before 37 weeks due to spontaneous preterm labour or premature rupture of membranes (sPTB). Control group were women with term birth (≥37weeks).

Results: Outcome data was available for 1,165 women, 6.7% of whom had sPTB, around 16% had consumed alcohol and about 5% had used other illicit drugs during the first half of pregnancy. Current drinking at 19-21 weeks (RR 3.96 95% CI [1.04-15.05]) and a short cervix from 18-24 weeks (RR 4.52 95% CI [1.08-19.01]) correlated with sPTB on bivariate analysis. Increased incidence of sPTB occurred in underweight women gaining weight below quartile 1 (14.8%), obese women gaining weight above quartile 3 (14.3%), women with a short cervix (<25 mm) at 18-24 weeks (31.2%) and those with a short cervix and vaginal bleeding in the first half of pregnancy (40%). Cervical length (RR_{adj} 0.93 95% CI [0.92-0.94]) and current drinking (RR_{adj} 6.61 95% CI [1.04-15.05]) correlated independently with sPTB.

Conclusion: The incidence of sPTB increased in some maternal phenotypes, representing potential groups of interest, the focus of preventive strategies. Similarly, targeted interventions for nulliparous women with a short cervix in the second trimester and alcohol use prevention during pregnancy require further exploration.

Funding: The Brazilian National Research Council (CNPq) (Award 401636/2013-5) and Bill and Melinda Gates Foundation (grant OPP1107597). Funders played no role in study design, data collection/analysis, decision to publish or manuscript preparation.

Tweetable abstract: Short cervix and alcohol use correlated with higher risk for spontaneous preterm birth in nulliparous women.

Keywords: preterm birth, risk factor, alcohol, cervical length, phenotype.

Intoduction

Spontaneous preterm birth (sPTB) is a common major maternal complication leading to perinatal morbidity and mortality as well as short- and long-term sequelae (1–3). Around two-thirds of deliveries that occur before 37 weeks are due to spontaneous preterm labour or preterm premature rupture of membranes (pPROM) and its pathophysiology remains unclear (3). The two main known risk factors for preterm birth (PTB) are a previous history of preterm birth and multiple pregnancies (1,3,4). These factors are highly associated with preterm birth, although they cannot be applied to all women, such as nulliparous with singleton pregnancies.

Several biophysical, biological and clinical markers have been studied to identify women at high risk in a timely manner. Assessment is aimed at providing a more specialized prenatal care in referral centres capable of optimizing preventive interventions such as progesterone, pessary or cerclage (5–8). Women with a short cervix in the second trimester, defined as a cervical length ≤ 25mm measured by transvaginal ultrasound between 18 to 24 weeks of gestation (9), are four to five times more likely to have a spontaneous preterm birth than women with a normal cervix (> 25mm) (10,11). Nevertheless, cervical length (CL) seems to vary according to ethnicity and parity (10,12), and possibly has a different impact on distinct populations.

Routine universal screening of cervical length remains controversial and it is not widely recommended (13,14). Nevertheless, determining its association with sPTB in different populations may be significant for a better investigation of appropriate preventive interventions in targeted subgroups of women at higher risk. Since sPTB is a multifactorial complex disease, other clinical risk factors for sPTB also remain controversial; initial or pre/pregnancy body mass index (BMI), weight gain during pregnancy and sociodemographic factors are lacking in consistency (15–17).

Therefore, our aim is to assess the incidence and clinical/ epidemiological risk factors associated with sPTB in nulliparous women in Brazil. The determination of risk factors is an important strategy to identify women at higher risk for this important complication which

represents a great burden for maternal and perinatal health. Earlier identification of women at increased risk is crucial for implementation of preventive strategies and planning adequate prenatal/childbirth care.

Methods

We conducted a nested case-control analysis derived from the Preterm-SAMBA study, a longitudinal multicentre cohort from July 2015 to July 2018 in five Brazilian obstetric centres located in three regions of Brazil. The research protocol and other methodological details were previously published (18,19). Briefly, the study was developed to identify clinical and biological predictors of sPTB, applying metabolomics techniques in maternal blood samples. The Brazilian cohort is the validation phase of the Preterm SAMBA study to assess the performance of a potential prediction model developed, using another international multicentre cohort – the SCOPE study (20). The study protocol was approved by the Institutional Review Board (IRB) of each centre and endorsed by the Brazilian National Committee for Ethics in Research (CONEP). This manuscript follows the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) Statement.

Participants and settings

Nulliparous low-risk women with a singleton pregnancy between 19 and 21 weeks of gestation were the inclusion criteria. Exclusion criteria included 3 or more previous abortions; cervical suture; fetal malformation; chronic hypertension requiring antihypertensive drugs and/or diabetes and/or renal disease; arterial blood pressure above 160x100 mmHg on enrolment; Systemic Lupus Erythematosus and/or antiphospholipid syndrome; sickle cell disease; HIV infection; Müllerian anomalies; history of cervical knife cone biopsy; chronic use of corticosteroids, aspirin, calcium, fish oil, vitamin C, vitamin E or heparin. Participating centres were five referral maternity hospitals from the Brazilian Network for Studies on Reproductive and Perinatal Health from the northeastern, southern and southeastern regions of Brazil. Nevertheless, eligible women underwent surveillance not only in maternity outpatient clinics but also in neighbouring primary healthcare units and private clinics.

Sample Size Estimation

The sample size for the Preterm SAMBA cohort was calculated taking into account the primary outcome - spontaneous preterm birth. Assuming a type I error of 5% and accuracy for the test by the area under the ROC curve of at least 0.68, and to test the hypotheses with adequate power (80% of power, β = 0.2), the required sample size should be near 80 sPTB cases. The expected minimum prevalence of this outcome in Brazil is 7%, resulting in a sample size calculation of 1,150 women for the cohort.

Procedures and data management

All steps of the clinical study have been previously described (18). Briefly, data were collected on three study visits. During the first visit, between 19 and 21 weeks of gestation, blood and hair samples were collected and stored appropriately for metabolomic assay and other possible future measurements. A comprehensive assessment was conducted to gather information on sociodemographic characteristics, reproductive family history, current or previous diseases, and habits, with a complete follow-up until delivery and immediate postpartum period. During the interview, data were entered on an internet-accessed central database with a complete audit trail (MedSciNet®). Anthropometric measurements of maternal body mass index (BMI), height, weight, head circumference, arm circumference and triceps, biceps, subscapular, suprailiac skinfolds plus a nutritional assessment based on a 24-hour diet recall were performed according to standardized methods described in the SOP. Between 27-29 weeks and between 37-39 weeks, women underwent the same evaluation, except for blood and sample collection performed only on the first visit. Late pregnancy and postpartum data were collected through medical records and prenatal chart review, or alternatively by personal interviews or phone calls in cases where childbirth occurred outside the five hospitals.

Outcome and variables

Spontaneous preterm birth was the main outcome, defined as any delivery before 37 weeks of gestation due to spontaneous onset of labour or preterm premature rupture of membranes (pPROM). Gestational age (GA) was estimated by the last menstrual period (LMP) and/or ultrasound (US) before 20 weeks of gestation. If discordance between LMP and US was below 7 days, GA was estimated by LMP. US was the preferred method for GA estimation

when discordance was above 7 days. The control group was comprised of women who had term birth, defined as any delivery ≥37 weeks of gestation.

Potential factors associated with sPTB included sociodemographic data, smoking, alcohol (self-reported and recorded if drinking or not: 3 months before pregnancy and during pregnancy until the first visit) and drug use (marijuana, cocaine, etc.; reported and recorded similarly to alcohol consumption), history of preterm birth of study participant's mother or sisters, sPTB or low birth weight reported by study participant, body mass index on the first visit, weight gain per week between the first and second visits, cervical length recorded by transvaginal ultrasound between 18 and 24 weeks of gestation, maternal conditions (self-reported anemia, depression or chronic hypertension), complications and infections during pregnancy, including asymptomatic bacteriuria and vaginal bleeding before the first visit (until 19-21 weeks). Maternal clinical phenotype was defined by the association with some maternal characteristics including maternal BMI, family income, weight gain, vaginal bleeding, urinary infection, short cervical length (<25mm, measured at 18-24 weeks of gestation), ethnicity and schooling level. The proportion of women in each quartile (below Q1, Q1-Q2, Q2-Q3 and above Q3) and percentile category (<p10, p10-p90, and >p90) of weight gain per week between the first and second visits were also addressed for both preterm and term groups.

Statistical analysis

We determined the general incidence of sPTB in the cohort and each gestational category according to the severity of prematurity: late sPTB (34-36weeks), moderate sPTB (32-33weeks), very sPTB (28-32weeks) and extreme sPTB (<28 weeks). Bivariate analysis was performed to assess potential factors associated with sPTB, calculating risk ratios (RR) and 95% confidence interval (CI). The incidence of sPTB and respective 95% CI were calculated for each clinical phenotype. We conducted a non-conditional logistic regression analysis to identify factors independently associated with sPTB using adjusted RR and 95% CI. Only variables with a p-value <0.25 entered the multivariate analysis model. Statistical analysis was performed using Stata v. 7.0 (StataCorp) and SPSS v. 20.0 (IBM). All analyses were adjusted

for the primary sampling unit (PSU) considering the heterogeneity of the five participating centres.

Results

In total, 1,373 nulliparous pregnant women were assessed for eligibility. Of these, 1,181 were included in the Preterm SAMBA study and had the first visit (Figure 1). Outcome data were available for 1,165 participants, 78 of whom had a spontaneous preterm birth (6.7%) and 1,040 had a term birth (89.3%). The overall preterm birth incidence, including provider-initiated preterm birth (pi-PTB) was 10.7% (Supplementary S1). The incidence of sPTB in the Northeastern and Southern/Southeastern centres was 6.0% and 7.3%, respectively, although this difference was not statistically different (p-value 0.387). The proportions of late, moderate, very and extreme sPTB were 70.5%, 12.8%, 10.3% and 6.4%, respectively (Supplementary S1). In around 98% of pregnancies, gestational age was estimated or confirmed before 20 weeks by ultrasound (Supplementary S2).

Table 1 shows that none of the assessed maternal socio-demographic characteristics was associated with sPTB. The majority of participating women were aged 20-34 years (69.2% sPTB women; 67.8% term birth women), non–white (59% sPTB; 60% term birth), had less than 12 years of schooling (66.7% sPTB; 67.9% term birth) and their annual family income was 3,000 to 12,000 US$ or more. Adolescents comprised 23.1% and 25.9% in sPTB and term births groups, respectively. More than 85% of participating women received prenatal care exclusively from the public health care system.

Table 2 shows that on the first visit, current drinking was almost four times more frequent in women who had sPTB than in those who had term births (RR 3.96, 95% CI [1.04-15.05]). On the first visit, 6.4% and 7.5% of women who had sPTB and term births, respectively, were current smokers or had ceased smoking during the first half of pregnancy. Around 16% of participants consumed alcohol in the first half of pregnancy and approximately 5% had used illegal drugs. Table 3 shows that 41.1% and 43.1% of women were overweight or obese in sPTB and term birth groups, respectively. Cervical length below 25 mm from 18 to 24 weeks was associated with a higher incidence of sPTB (RR 4.52, 95% CI [1.08-19.01]). However, the

mean cervical length of women who had sPTB (33.1mm ±9.96) or term birth (36.9mm ±6.35) was not statistically different (weighted mean difference of 3.76, 95% CI [(-2.17)-(9.69)]). Other maternal conditions evaluated were not associated with a higher risk for sPTB.

The incidence of sPTB in groups of women with clinical phenotypes including mixed maternal characteristics is shown in Table 4. Some of these women had a higher incidence of sPTB than the general study population. Examples were underweight women whose weight gain was below Q1 (14.8%), obese women with weight gain >Q3 (14.3%), women with a short cervical length (31.2%) and women with a short cervix that had vaginal bleeding in the first half of pregnancy (40%). None of the maternal phenotypes such as ethnicity, family income or schooling level showed a higher incidence of sPTB.

Cervical length (mm) measured between 18 and 24 weeks of gestation and alcohol use on the first visit were independently associated with sPTB on multivariate analysis (adjusted RR 0.93, 95%CI [0.92-0.94] and 6.61 [2.83-15.46], respectively) (Supplementary S3). Each increase of 1mm in cervical length decreases the risk of sPTB by 7%. Ethnicity and more than 3 days of vaginal bleeding in the first half of pregnancy were the only significantly different maternal characteristics among groups of women with varying cervical lengths (≤25 mm, 26-25 mm and >35 mm) (Supplementary S4). The proportion of white ethnicity and more than 3 days of vaginal bleeding in women with a cervix >35mm and <25mm were 47.7% and 36.8%, and 5.8% and 0%, respectively.

Discussion

Main Findings

The Preterm SAMBA multicentre cohort study found an overall PTB rate of 10.4%, where sPTB accounted for 62.4% of PTB cases (6.7% of all births). In addition, sPTB was highly associated with alcohol consumption and a short cervix at mid-pregnancy.

Strengths and limitations

We conducted a prospective multicentre cohort study of nulliparous women from five obstetric maternities in Brazil, representing a multi-regional and mixed population in an

upper-middle income country. Our study has some limitations: 1) the initial/pre-pregnancy weight was not recorded; 2) transvaginal ultrasound was recorded as ultrasound report or prenatal chart and not all participating women were offered the exam; 3) we did not use a standard instrument to address alcohol use during pregnancy. In addition to these limitations, our study represents local protocols and the reality of obstetric referral centres. Furthermore, a larger sample is presumed to confer more power to the study and other potential risk factors could be identified as significant.

Interpretations

The PTB rate was slightly lower in our study than in the general female Brazilian population. According to the EMIP study, a multicenter cross-sectional study that provided surveillance for over 33,700 births from 2011 to 2012 in 20 referral Brazilian maternity hospitals, the overall prevalence of PTB was 12.7% (4). sPTB was responsible for 65% of all PTB. The 2016 official data from SINASC, the national live birth information system, shows a PTB rate of 11.3% (21). Unfortunately, Brazilian official data does not distinguish PTB rates according to subtypes. A considerable proportion of women in our study, although classified as low-risk, were overweight or obese (291/1,165 - 41%) on the first visit (19-21 weeks), had previous chronic medical conditions including anemia, depression or chronic hypertension (145/1,165 - 12.4%), had an annual family income lower than 12,000 US$ (654/1,165 - 56.1%) and had less than 12 years of schooling (785/1,165 – 65.0%). Considering the characteristic of our population, the concept of “low-risk” pregnancy here may be controversial. The sampled population reflects the Brazilian population and is no different from another multicentre study in Brazil (4). In addition, we focused on nulliparous women to avoid confounders related to a previous history of preterm birth, because it is applicable to every woman (at least once).

A short cervix, defined as a cervical length below 25mm on second-trimester transvaginal ultrasound, is a significant risk factor for preterm birth. Since the ’90s, a growing body of evidence supports its association with PTB (10,22–24). Cervical lengths of nulliparous women seem to be statistically, but not clinically, different from cervical lengths of multiparous women. Iams et al. evaluating almost 3,000 North-American women, showed that median

cervical lengths were 34.0 mm and 36.1mm, respectively (10). More recently, van der Ven et al investigating over 11,000 Dutch women, reported that mean cervical lengths of nulliparous and multiparous were 43.1 mm and 45.1 mm, respectively (12). In our study, mean cervical lengths were 33.1 mm and 36.9 mm in women who had sPTB and term birth, respectively. Cervical length may not clinically differ according to parity, but this seems to vary widely among the population studied. We established 25mm as a cut-off point for women potentially at higher risk for sPTB. However, the distribution of cervical length in Brazilian women, including the 10^{th} percentile, remains undetermined. It is required for clarification of population characteristics and related adaptations to identify women at higher risk for sPTB.

Alcohol consumption during pregnancy is known to be associated with adverse maternal and perinatal outcomes, including preterm birth, small for gestational age infants and fetal alcohol spectrum disorder (FASD) (25–28). However, it is controversial whether there is a safe level of alcohol consumption during pregnancy and whether low alcohol intake could protect against PTB (26,28). Observational and experimental animal studies showed that ethanol seems to decrease oxytocin release in the neurohypophysis (29,30). A study on alcohol intake in nine large European cohorts showed that the proportion of women drinking alcohol during pregnancy decreased from 2000-04 (39%) to 2005-11 (14%) (31). Greater awareness of the harmful effects of alcohol and lower self-reporting are primary reasons for this decrease (31). Maternal alcohol use during pregnancy is a context-specific cultural challenge that needs to be confronted. In a meta-analysis by Larsen et al, over 90% of women were from Nordic countries (31). A systematic review with meta-analysis of studies from Latin American and Caribbean countries estimated that 15.2% of pregnant women in Brazil consume alcohol during pregnancy (32). Cuba and Mexico have prevalence rates of 4.8% and 1.2% respectively. Local and regional Brazilian studies showed that tobacco and illegal drug use, mental health disorders, low schooling, non-white race and lack of a partner are factors associated with alcohol consumption during pregnancy (33–37). Nevertheless, we did not find any Brazilian studies that comprehensively addressed maternal characteristics, lifestyle and other factors associated with alcohol use during pregnancy, specifically in nulliparous women.

Amelioration of preventive actions to avoid alcohol use in this specific maternal group is a key strategy.

Recently, a group of experts proposed a new conceptual framework for the study of preterm birth, using maternal clinical phenotypes (38). A clinical phenotype is characterized by a group of common clinical characteristics observed during pregnancy that is potentially associated with an outcome. Rather than separate women by outcome, the clinical phenotype intends to address the incidence and associated adverse outcomes in women who have common characteristics during pregnancy. It may improve recognition of groups at higher risk for adverse outcomes and enable implementation of targeted interventions for each specific group. We found four maternal phenotypes with at least double the incidence of sPTb compared to the overall population. These phenotypes were based on maternal BMI, weight gain from 20-27 weeks of gestation, cervical length, and vaginal bleeding in the first half of pregnancy. All these characteristics can be easily identified during pregnancy, especially in referral obstetric units where well-trained specialists perform transvaginal ultrasound. A standard threshold for weight gain during pregnancy, dependent on the initial or pre-pregnancy BMI, was not used in this study, since it was not available. More importantly, existing literature supports our findings. Obese women with higher weight gain and underweight women with lower weight gain are at increased risk for sPTB (39). Although each clinical phenotype was composed of a low number of women, these results are still useful for indicating groups of women that may benefit from further study and are also considered at higher risk for sPTB.

Conclusion

Our study reinforces that cervical length is a remarkable biophysical risk factor for sPTB. However, instead of routine screening for low-risk nulliparous women, we first suggest a better investigation of the benefits of preventive interventions for this population with a short cervix in the second trimester. In addition, subgroups that have a higher incidence of sPTB should also be further evaluated to find associated factors, perinatal related outcomes,

and preventive strategies. Alcohol use during pregnancy is known to be harmful. Strategies to identify and prevent alcohol consumption should be better explored in our context, especially in nulliparous women.

Acknowledgments

The Preterm SAMBA study group also included: Mary A. Parpinelli, Karayna G Fernandes, Rafael B Galvão, José Paulo Guida, Danielly S Santana, School of Medical Sciences, University of Campinas, Brazil; Kleber G. Franchini, LNBio, Campinas, Brazil; Bianca F. Cassettari, School of Medicine of Botucatu, UNESP, Brazil; Lucia Pfitscher, Luiza Brust, School of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil; Elias F Melo Junior, Danilo Anacleto, School of Medicine, Federal University of Pernambuco, Recife, Brazil; Daisy de Lucena, Benedita Sousa, School of Medicine, Federal University of Ceará, Fortaleza, Brazil.

Disclosure of Interests

Authors declare no competing interest for the current analysis.

Contribution to authorship

JGC, PNB, LCK and MLC conceived and planned the cohort. JGC, RTS, FEF, EARF, JV, IMC, JM and RPJr developed all related procedures, implemented and carried out the cohort. RTS, DFL, MHS and JGC designed and performed the current analysis. RTS, MLC and JGC wrote the manuscript. All author, including those from the Preterm SAMBA study group, read, reviewed and approved the final version of the manuscript.

Ethical Approval

The current study was approved by each local Institutional Review Board (IRB) and amended by the Brazilian National Committee for Ethics in Research (CONEP) - Letter of approval 1.048.565 issued on 28th April 2015. The study complies with national and international regulations for experiments in human beings, including resolution CNS 466/12 of the Brazilian National Heath Council and the 1989 Declaration of Helsinki.

Funding

This was one of the two big studies selected for sponsoring from the research call “Grand Challenges Brazil: Reducing the burden of preterm birth” number 05/2013 jointly issued by the Brazilian National Research Council (CNPq) (Award 401636/2013-5) and the Bill and Melinda Gates Foundation (grant OPP1107597). The funders played no role at all in the study design, writing the manuscript nor in the decision to submit the manuscript for publication.

References

1. Blencowe H, Cousens S, Chou D, Oestergaard M, Say L, Moller A-B, et al. Born Too Soon: The global epidemiology of 15 million preterm births. Reprod Health. 2013 Nov

15;10(Suppl 1):S2.

2. Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: when? Where? Why? Lancet. 2005 Jan;365(9462):891–900.
3. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371(9606):75–84.
4. Passini R, Cecatti JG, Lajos GJ, Tedesco RP, Nomura ML, Dias TZ, et al. Brazilian Multicentre Study on Preterm Birth (EMIP): Prevalence and Factors Associated with Spontaneous Preterm Birth. PLoS One. 2014 Jan;9(10):e109069.
5. Requejo J, Merialdi M, Althabe F, Keller M, Katz J, Menon R. Born Too Soon: Care during pregnancy and childbirth to reduce preterm deliveries and improve health outcomes of the preterm baby. Reprod Health. 2013 Jan;10 Suppl 1(Suppl 1):S4.
6. Dodd JM, Jones L, Flenady V, Cincotta R, Crowther CA. Prenatal administration of progesterone for preventing preterm birth in women considered to be at risk of preterm birth. Cochrane Database Syst Rev. 2013 Jul 31;(7):CD004947.
7. Goya M, Pratcorona L, Merced C, Rodó C, Valle L, Romero A, et al. Cervical pessary in pregnant women with a short cervix (PECEP): an open-label randomised controlled trial. Lancet. 2012 May 12;379(9828):1800–6.
8. American College of Obstetricians and Gynecologists. Practice Bulletin No. 142: Cerclage for the management of cervical insufficiency. Obstet Gynecol. 2014 Feb;123(2, PART 1):372–9.
9. To MS, Skentou C, Chan C, Zagaliki A, Nicolaides KH. Cervical assessment at the routine 23-week scan: standardizing techniques. Ultrasound Obstet Gynecol. 2001 Mar;17(3):217–9.
10. Iams JD, Goldenberg RL, Meis PJ, Mercer BM, Moawad A, Das A, et al. The length of the cervix and the risk of spontaneous premature delivery. National Institute of Child Health and Human Development Maternal Fetal Medicine Unit Network. N Engl J Med. 1996 Feb 29;334(9):567–72.
11. Berghella V, Roman A, Daskalakis C, Ness A, Baxter JK. Gestational age at cervical length measurement and incidence of preterm birth. Obstet Gynecol. 2007 Aug;110(2

Pt 1):311–7.

12. van der Ven J, van Os MA, Kazemier BM, Kleinrouweler E, Verhoeven CJ, de Miranda E, et al. The capacity of mid-pregnancy cervical length to predict preterm birth in low-risk women: a national cohort study. Acta Obstet Gynecol Scand. 2015 Nov;94(11):1223–34.
13. McIntosh J, Feltovich H, Berghella V, Manuck T, Manuck T. The role of routine cervical length screening in selected high- and low-risk women for preterm birth prevention. Am J Obstet Gynecol. 2016 Sep;215(3):B2–7.
14. Sentilhes L, Sénat M-V, Ancel P-Y, Azria E, Benoist G, Blanc J, et al. Prevention of spontaneous preterm birth: Guidelines for clinical practice from the French College of Gynaecologists and Obstetricians (CNGOF). Eur J Obstet Gynecol Reprod Biol. 2017 Mar;210:217–24.
15. Torloni MR, Betrán AP, Daher S, Widmer M, Dolan SM, Menon R, et al. Maternal BMI and preterm birth: a systematic review of the literature with meta-analysis. J Matern Fetal Neonatal Med. 2009 Nov;22(11):957–70.
16. Carnero AM, Mejía CR, García PJ. Rate of gestational weight gain, pre-pregnancy body mass index and preterm birth subtypes: a retrospective cohort study from Peru. BJOG. 2012 Jul;119(8):924–35.
17. Luke B. Nutrition for Multiples. Clin Obstet Gynecol. 2015;58(3):585–610.
18. Cecatti JG, Souza RT, Sulek K, Costa ML, Kenny LC, McCowan LM, et al. Use of metabolomics for the identification and validation of clinical biomarkers for preterm birth: Preterm SAMBA. BMC Pregnancy Childbirth. 2016 Aug;16(1):212.
19. Souza RT, Cecatti JG, Costa ML, Mayrink J, Pacagnella RC, Passini R, et al. Planning, Implementing, and Running a Multicentre Preterm Birth Study with Biobank Resources in Brazil: The Preterm SAMBA Study. Biomed Res Int. 2019 Jan 16;2019:1–8.
20. McCowan LME, Roberts CT, Dekker GA, Taylor RS, Chan EHY, Kenny LC, et al. Risk factors for small-for-gestational-age infants by customised birthweight centiles: data from an international prospective cohort study. BJOG. 2010 Dec;117(13):1599–607.

21. Ministério da Saúde. Brasil [Internet]. SINASC - Sistema de Informações sobre Nascidos Vivos. Available from: http://tabnet.datasus.gov.br/cgi/deftohtm.exe?sinasc/cnv/nvuf.def
22. Andersen HF, Nugent CE, Wanty SD, Hayashi RH. Prediction of risk for preterm delivery by ultrasonographic measurement of cervical length. Am J Obstet Gynecol. 1990 Sep;163(3):859–67.
23. Okitsu O, Mimura T, Nakayama T, Aono T. Early prediction of preterm delivery by transvaginal ultrasonography. Ultrasound Obstet Gynecol. 1992 Nov 1;2(6):402–9.
24. Sonek JD, Iams JD, Blumenfeld M, Johnson F, Landon M, Gabbe S. Measurement of cervical length in pregnancy: comparison between vaginal ultrasonography and digital examination. Obstet Gynecol. 1990 Aug;76(2):172–5.
25. Bhuvaneswar CG, Chang G, Epstein LA, Stern TA. Alcohol use during pregnancy: prevalence and impact. Prim Care Companion J Clin Psychiatry. 2007;9(6):455–60.
26. Patra J, Bakker R, Irving H, Jaddoe V, Malini S, Rehm J. Dose-response relationship between alcohol consumption before and during pregnancy and the risks of low birthweight, preterm birth and small for gestational age (SGA)-a systematic review and meta-analyses. BJOG An Int J Obstet Gynaecol. 2011 Nov;118(12):1411–21.
27. Pfinder M, Kunst AE, Feldmann R, van Eijsden M, Vrijkotte TGM. Preterm birth and small for gestational age in relation to alcohol consumption during pregnancy: stronger associations among vulnerable women? results from two large Western-European studies. BMC Pregnancy Childbirth. 2013 Dec 22;13(1):49.
28. Mamluk L, Edwards HB, Savović J, Leach V, Jones T, Moore THM, et al. Low alcohol consumption and pregnancy and childhood outcomes: time to change guidelines indicating apparently 'safe' levels of alcohol during pregnancy? A systematic review and meta-analyses. BMJ Open. 2017 Jul 3;7(7):e015410.
29. Dopico AM, Lemos JR, Treistman SN. Alcohol and the Release of Vasopressin and Oxytocin. In: Watson RR, editor. Alcohol and Hormones. Totowa, NJ: Humana Press; 1995. p. 209–26.
30. Fuchs A-R, Wagner G. Effect of Alcohol on Release of Oxytocin. Nature. 1963 Apr

6;198(4875):92–4.

31. Strandberg-Larsen K, Poulsen G, Bech BH, Chatzi L, Cordier S, Dale MTG, et al. Association of light-to-moderate alcohol drinking in pregnancy with preterm birth and birth weight: elucidating bias by pooling data from nine European cohorts. Eur J Epidemiol. 2017 Sep 12;32(9):751–64.
32. Lange S, Probst C, Heer N, Roerecke M, Rehm J, Monteiro MG, et al. Actual and predicted prevalence of alcohol consumption during pregnancy in Latin America and the Caribbean: systematic literature review and meta-analysis. Rev Panam Salud Publica. 2017 Jun 8;41:e89.
33. Guimarães VA, Fernandes KS, Lucchese R, Vera I, Martins BCT, Amorim TA de, et al. Prevalência e fatores associados ao uso de álcool durante a gestação em uma maternidade de Goiás, Brasil Central. Cien Saude Colet. 2018 Oct;23(10):3413–20.
34. Meucci RD, Saavedra JS, Silva ES da, Branco MA, Freitas JN de, Santos M dos, et al. Alcohol intake during pregnancy among parturients in southern Brazil. Rev Bras Saúde Matern Infant. 2017 Dec;17(4):653–61.
35. Baptista FH, Rocha KBB, Martinelli JL, Avó LR da S de, Ferreira RA, Germano CMR, et al. Prevalence and factors associated with alcohol consumption during pregnancy. Rev Bras Saúde Matern Infant. 2017 Jun;17(2):271–9.
36. Veloso LUP, Monteiro CF de S. Prevalence and factors associated with alcohol use among pregnant adolescents. Rev Lat Am Enfermagem. 2013 Feb;21(1):433–41.
37. Sbrana M, Grandi C, Brazan M, Junquera N, Nascimento MS, Barbieri MA, et al. Alcohol consumption during pregnancy and perinatal results: a cohort study. Sao Paulo Med J. 2016 Mar 18;134(2):146–52.
38. Villar J, Papageorghiou AT, Knight HE, Gravett MG, Iams J, Waller SA, et al. The preterm birth syndrome: a prototype phenotypic classification. Am J Obstet Gynecol. 2012 Feb;206(2):119–23.
39. Masho SW, Bishop DL, Munn M, Ogden C, Carroll M, Curtin L, et al. Pre-pregnancy BMI and weight gain: where is the tipping point for preterm birth? BMC Pregnancy Childbirth. 2013 Dec 24;13(1):120.

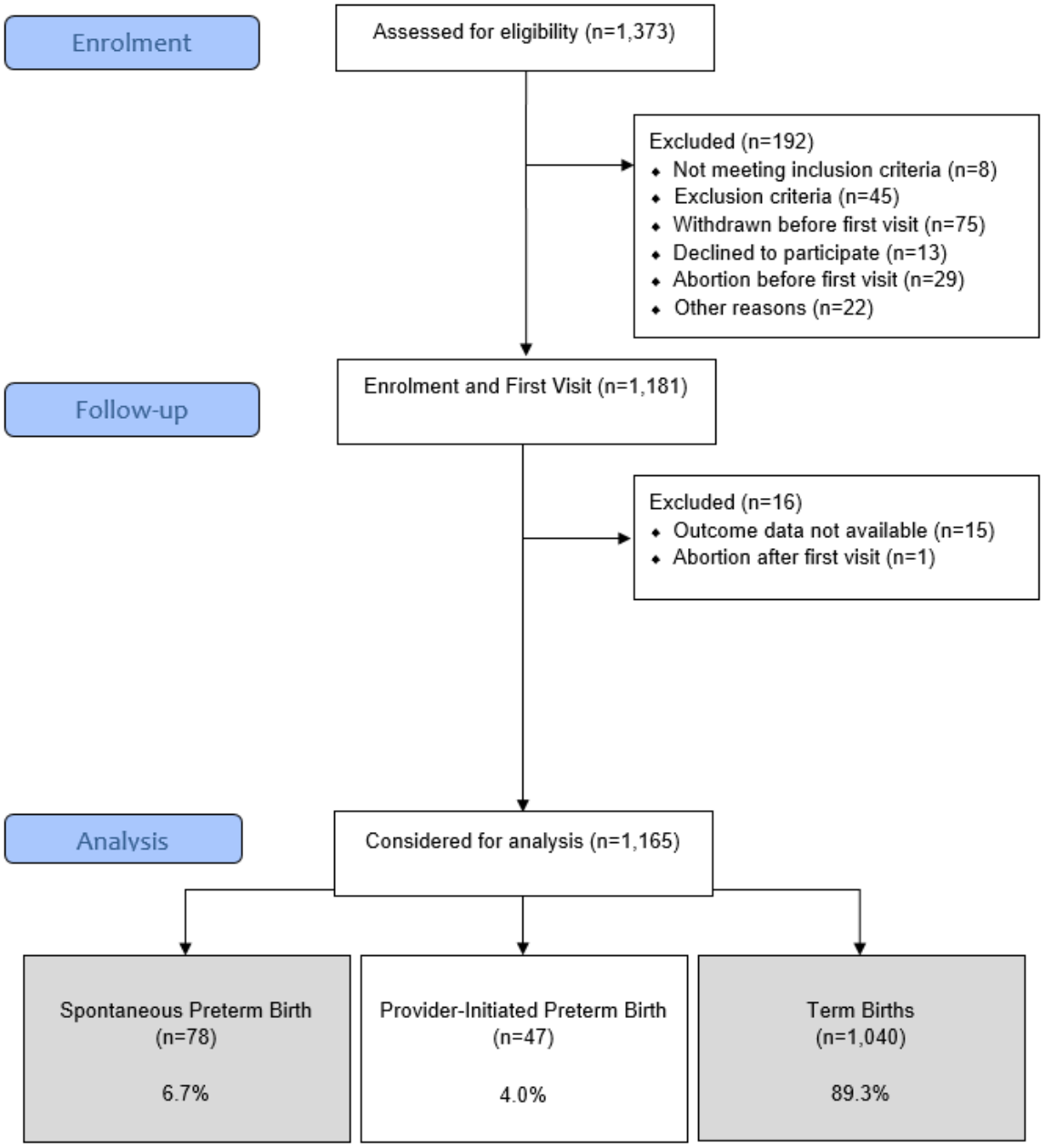


Figure 1. Preterm SAMBA Flowchart – Spontaneous preterm birth analysis

Table 1. Unadjusted risks for sPTB according to some socio-demographic characteristics

Characteristics	sPTB	Term Births	RR [95%CI]
Region			
Northeast	34 (43.6%)	506 (48.7%)	Ref.
South and Southeast	44 (56.4%)	534 (51.3%)	1.21 [0.69-2.12]
Maternal age (years)			
≤19	18 (23.1%)	269 (25.9%)	0.88 [0.55-1.42]
20-34	54 (69.2%)	705 (67.8%)	Ref.
≥35	6 (7.7%)	66 (6.3%)	1.17 [0.27-5.15]
Ethnicity			
White	32 (41.0%)	416 (40.0%)	Ref.
Non-white	46 (59%.0)	624 (60.0%)	0.96 [0.40-2.30]
Marital status			
With partner	52 (66.7%)	762 (73.3%)	Ref.
Without partner	26 (33.3%)	278 (26.7%)	1.34 [0.99-1,81]
Maternal Occupation			
Paid work	41 (52.6%)	512 (49.2%)	1.11 [0.74-1.66]
Housewife	13 (16.7%)	192 (18.5%)	0.95 [0.36-2.54]
Not working*	24 (30.7%)	360 (32.2%)	Ref.
Schooling (years)			
< 12	52 (66.7%)	706 (67.9%)	Ref.
≥ 12	26 (33.3%)	334 (32.1%)	1.05 [0.46-2.39]
Annual Family Income (US$)			
Up to 3,000	2 (2.6%)	48 (4.6%)	0.53 [0.21-1.32]
3,000 to 12,000	41 (52.5%)	563 (54.1%)	0.90 [0.55-1.47]
Above 12,000	35 (44.9%)	429 (41.3%)	Ref.
Source of prenatal care			
Entirely public	67 (85.9%)	899 (86.4%)	0.96 [0.54-1.70]
Private/insurance/mixed	11 (14.1%)	141 (13.6%)	Ref.
Total	**78**	**1,040**	

*students, unemployed and licensed.

Table 2. Unadjusted risks for sPTB according to some maternal medical history and habits

Characteristics	sPTB	Controls	RR (95%CI)
Smoking			
No smoking	73 (93.6%)	962 (92.5%)	Ref.
Ceased during pregnancy or smoker	5 (6.4%)	78 (7.5%)	0.85 [0.23-3.23]
Alcohol drinking [a]			
No alcohol	56 (83.6%)	757 (83.5%)	Ref.
Ceased before 1st visit	8 (11.9%)	142 (15.7%)	0.77 [0.34-1.75]
Current drinker at 1st visit	3 (4.5%)	8 (0.9%)	**3.96 [1.04-15.05]**
Other Drugs [b]			
Never	61 (95.3%)	839 (94.6%)	Ref.
Ceased before 1st visit	1 (1.6%)	39 (4.4%)	0.37 [0.03-5.18]
Current user at 1st visit	2 (3.1%)	9 (1.0%)	2.68 [0.86-8.33]
Previous maternal conditions			
Yes	15 (19.2%)	130 (12.5%)	1.60 [0.89-2.86]
No	63 (80.8%)	910 (87.5%)	Ref.
Previous abortion			
Yes	9 (11.5%)	117 (11.3%)	1.03 [0.33-3.19]
No	69 (88.5%)	923 (88.7%)	Ref.
Mother's History of PTB [c]			
Yes	8 (11.0%)	98 (9.9%)	1.11 [0.24-5.18]
No	65 (89.0%)	890 (90.1%)	Ref.
Mother's History of LBW [d]			
Yes5	5 (7.2%)	111 (11.7%)	0.61 [0.17-2.20]
No	64 (92.8%)	839 (88.3%)	Ref.
Sister's History of PTB [e]			
Yes	4 (12.5%)	22 (7.3%)	1.69 [0.52-5.48]
No	28 (87.5%)	279 (92.7%)	Ref.
Sister's History of LBW [e]			
Yes	4 (12.5%)	32 (10.6%)	1.18 [0.16-8.91]
No	28 (87.5%)	269 (89.4%)	Ref.
Total	**78**	**1,040**	

Missing information for: a) 144; b) 167; c) 57; d) 99; e) 17.
LBW: low birth weight; PTB: preterm birth. Values in bold mean they are statistically significant.

Table 3. Unadjusted risks for sPTB according to some maternal medical conditions during pregnancy

Characteristics	sPTB	Controls	RR [95%CI]
Body Mass Index* at first visit (19-21weeks) [a]			
Underweight (<21.5kg/m^2)	14 (17.9%)	181 (17.4%)	0.99 [0.28-3.55]
Normal weight (21.5-26.2 kg/m^2)	32 (41.0%)	410 (39.5%)	Ref.
Overweight (26.3-30.9 kg/m^2)	21 (26.9%)	270 (26.0%)	1.00 [0.54-1.85]
Obesity (>30.9 kg/m^2)	11 (14.2%)	178 (17.1%)	0.80 [0.52-1.23]
Quartile of weight gain rate per week (kg/week) [b]			
≤Q1 (≤0.33)	15 (27.3%)	211 (24.9%)	1.01 [0.47-2.20]
Q1-Q2 (0.34-0.49)	14 (25.5%)	200 (23.5%)	Ref.
Q2-Q3 (0.50-0.66)	15 (27.3%)	216 (25.5%)	0.99 [0.43-2.30]
≥Q3 (≥0.67)	11 (20.1%)	221 (26.1%)	0.72 [0.25-2.08]
Percentile of weight gain rate per week (kg/weeks) [b]			
<p10 (<0.18)	6 (10.9%)	85 (10.0%)	1.15 [0.60-2.23]
p10-p90 (0.18-0.82)	41 (74.5%)	675 (79.6%)	Ref.
>p90 (>0.82)	8 (14.5%)	88 (10.4%)	1.46 [0.97-2.17]
Cervical length from 18 to 24 weeks [c]			
Mean ±SD	33.1 ±9.96	36.9 ±6.35	3.76 [(-2.17)-(9.69)]#
≤ 25mm	5 (13.5%)	11 (2.5%)	**4.52 [1.08-19.01]**
> 25mm	32 (86.5%)	431 (97.5%)	Ref.
Urinary tract infection in the first half of pregnancy [d]			
Yes	8 (13.1%)	198 (25.6%)	0.46 [0.20-1.04]
No	53 (86.9%)	574 (74.4%)	Ref.
Asymptomatic bacteriuria in the first half of pregnancy [e]			
Yes	2 (3.5%)	69 (9.5%)	0.36 [0.04-3.25]
No	55 (96.5%)	654 (90.5%)	Ref.
Recurrence of any infection§ [f]			
Yes	6 (10.9%)	119 (14.0%)	0.76 [0.27-2.16]
No	49 (89.1%)	730 (86.0%)	Ref.
Vaginal bleeding in the first half of pregnancy			
Yes	24 (30.8%)	193 (18.6%)	1.85 [0.89-3.84]

No	54 (69.2%)	847 (81.4%)	Ref.
Number of days with vaginal bleeding in the first half of pregnancy			
1-3 days	14 (58.3%)	156 (80.8%)	Ref.
>3 days	10 (41.7%)	37 (19.2%)	2.58 [0.62-10.74]
Total	**78**	**1,040**	

*According to Atalah body mass index categories at 19weeks (Atalah E, Castillo C, Castro R, Aldea A. Rev Med Chil. 1997 Dec;125(12):1429-36) §Women who had any infection before first visit (19-21 weeks) and another any infection between first and second visits (between 19-21 weeks and 27-29 weeks); only calculated for women who attended both visits. Missing information for: a) 1; b) 215; c) 639; d) 285; e) 338; f) 32. #WMD, weighted mean difference [95% CI].

Values in bold mean they are statistically significant.

Table 4. Incidence of preterm birth according to some maternal clinical phenotypes

Maternal clinical phenotypes	Incidence of sPTB n/N (%)	[95% CI]
Underweight on enrolment (<21.5kg/m2)* and Weight gain rate per week <Q1	**4/27 (14.8%)**	[0.0 – 34.7]
Underweight on enrolment (<21.5kg/m2)* and Weight gain rate per week <Q2	4/52 (7.7%)	[0.0 – 18.7]
Obesity (>30.9)* and Weight gain rate per week >Q3	**3/21 (14.3%)**	[3.9 – 24.6]
Overweight or Obese* and Weight gain rate per week >Q3	6/76 (7.9%)	[4.1 – 11.7]
Obesity (>30.9)* and Weight gain rate per week >Q2	5/54 (9.3%)	[0.8 – 17.7]
Overweight or Obese* and Weight gain rate per week >Q2	11/165 (6.7%)	[3.6 – 9.7]
Vaginal bleeding and urinary infection in the first half of pregnancy	3/49 (6.1%)	[0.0 – 15.1]
Short Cervical Length from 18 to 24 weeks	**5/16 (31.2%)**	[0.0-77.2]
Short Cervical Length from 18 to 24 weeks and vaginal bleeding in the first half of pregnancy	**2/5 (40.0%)**	[0.0 – 91.6]
Low family income and schooling levels	0/42 (0%)	-
Low family income and schooling levels	14/261 (5.4%)	[2.6 – 8.1]
White, low family income and schooling levels (a)	0/6 (0%)	-
White, low family income and schooling levels (b)	3/48 (6.3%)	[0.0-12.5]
Non-white, low family income and schooling levels (a)	0/36 (0%)	-
Non-white, low family income and schooling levels (b)	11/213 (5.2%)	[2.0-8.3]
White, high family income and schooling levels (a)	11/169 (6.5%)	[3.4-9.7]
White, high family income and schooling levels (b)	13/198 (6.6%)	[3.9-9.2]
Non-white, high family income and schooling levels (a)	5/77 (6.5%)	[0.0-15.9]
Non-white, high family income and schooling levels (b)	9/129 (7.0%)	[0.0-14.8]
General population of the study	**78/1165 (6.7%)**	**[4.7 – 8.7]**

(a) Low income defined as income up to 3,000 US$. High income when above 12,000 US$. (b) Low income defined as income up to 6,000 US$. High income when above 6,000 US$. *(Atalah E, Castillo C, Castro R, Aldea A. Rev Med Chil. 1997 Dec;125(12):1429-36)

S1. Incidence of preterm birth in the Preterm SAMBA study

Incidence	**n/N (%)**
Preterm birth (overall)	125/1,165 (10.7%)
Spontaneous PTB	78/1,165 (6.7%)
Provider-initiated PTB	47/1,165 (4.0%)
sPTB by Region*	
Northeast (2 centres)	13/257 + 21/309 = 34/565 (6.0%)
South and Southeast (3 centres)	12/139 + 14/143 + 18/318 = 44/600 (7.3%)
sPTB categories/severity	78 (100%)
Late preterm birth (34-36w)	55 (70.5%)
Moderate PTB (32-33w)	10 (12.8%)
Very PTB (28-31w)	8 (10.3%)
Extreme PTB (<28w)	5 (6.4%)

*p-value: 0.387

PTB: preterm birth; sPTB: spontaneous preterm birth

S2. Methods for estimating gestational age in the Preterm SAMBA study

Method*	**sPTB**	**Term birth**
LMP only	1 (1.3%)	15 (1.4%)
LMP and US (LMP)	32 (41.0%)	449 (43.2%)
LMP and US (US)	21 (26.9%)	269 (25.9%)
US only	24 (30.8%)	307 (29.5%)

*p-value: 0.903

S3. Factors independently associated with sPTB: multivariate analyses by non-conditional logistic regression

Variables	**RR_{adj}**	**95% CI**	**p-value**
Cervical Length from 18 to 24 weeks (mm)	0.93	0.92-0.94	<0.001
Current drinker at 1st visit	6.61	2.83-15.46	0.004

Variables included in the model: age (years); marital status; alcohol drinking; previous maternal conditions; initial BMI; Cervical length (mm); vaginal bleeding in the first half of pregnancy; urinary tract infection in the first half of pregnancy; weight gain rate per week (kg) 20-27 weeks >p90.

S4. Maternal characteristics according to transvaginal cervical length measured between 18-24 weeks (n=497)

Characteristics	Cervical length ≤25mm	Cervical length 26-35mm	Cervical length >35mm	p-value
Maternal age (years)				0.125
≤19	3 (15.8%)	53 (26.4%)	40 (14.4%)	
20-34	15 (78.9%)	128 (63.7%)	208 (75.1%)	
≥35	1 (5.3%)	20 (10.0%)	29 (10.5%)	
Ethnicity				**0.034**
White	7 (36.8%)	78 (38.8%)	132 (47.7%)	
Non-white	12 (63.2%)	123 (61.2%)	145 (52.3%)	
Marital status				0.307
With partner	16 (84.2%)	145 (72.1%)	212 (76.5%)	
Without partner	3 (15.8%)	56 (27.9%)	65 (23.5%)	
Maternal Occupation				0.079
Paid work	13 (68.4%)	88 (43.8%)	166 (59.9%)	
Homemaker	4 (21.1%)	41 (20.4%)	47 (17.0%)	
Not working	2 (10.5%)	72 (35.8%)	64 (23.1%)	
Schooling (years)				0.768
< 12	12 (63.2%)	127 (63.2%)	173 (55.4%)	
≥ 12	7 (36.8%)	74 (36.8%)	104 (37.5%)	
Annual Family Income (US$)				0.295
Up to 3,000	0 (0%)	3 (1.5%)	5 (1.8%)	
3,000 to 12,000	7 (36.8%)	115 (57.2%)	122 (44.0%)	
Above 12,000	12 (63.2%)	83 (41.3%)	150 (54.2%)	
Source of prenatal care				0.500
Entirely public	16 (84.2%)	164 (81.6%)	220 (79.4%)	
Private/insurance/mixed	3 (15.8%)	37 (18.4%)	57 (20.6%)	
Smoking				0.316
No smoking	17 (89.5%)	188 (93.5%)	256 (92.4%)	
Ceased during pregnancy or smoker	2 (10.5%)	13 (6.5%)	21 (7.6%)	
Alcohol drinking [a]				0.571
No alcohol	15 (83.3%)	147 (81.7%)	189 (79.4%)	
Ceased during pregnancy or drinker	3 (16.7%)	33 (18.3%)	49 (20.6%)	
Other Drugs [b]				0.109
Never	16 (100%)	176 (95.7%)	245 (93.9%)	
Ceased during pregnancy or user	0 (0%)	8 (4.3%)	16 (6.1%)	

Previous maternal conditions	2 (10.5%)	40 (19.9%)	41 (14.8%)	0.382
Previous abortion	6 (31.6%)	31 (15.4%)	33 (11.9%)	0.142
Mother's History of PTB 29	1 (6.2%)	24 (12.9%)	38 (14.3%)	0.379
Mother's History of LBW [c]	2 (13.3%)	22 (12.6%)	36 (14.1%)	0.814
Sister's History of PTB	5 (100%)	63 (90%)	95 (88%)	0.502
Sister's History of LBW	5 (100%)	58 (82.9%)	91 (89.8%)	0.305
Body Mass Index on enrolment [d]				0.563
Underweight (<21.5kg/m2)	3 (15.8%)	35 (17.4%)	37 (13.4%)	
Normal weight (21.5-26.2)	6 (31.6%)	83 (41.3%)	118 (42.6%)	
Overweight (26.3-30.9)	6 (31.6%)	56 (27.9%)	69 (24.9%)	
Obesity (>30.9)	4 (21.0%)	27 (13.4%)	53 (19.1%)	
Quartiles of weight gain rate per week (kg) 20-27 weeks [d]				0.405
≤0.33 (≤Q1)	5 (33.3%)	28 (15.3%)	57 (23.3%)	
0.34-0.49 (Q1-Q2)	4 (26.7%)	56 (30.8%)	60 (24.5%)	
0.50-0.66 (Q2-Q3)	3 (20.0%)	58 (31.9%)	70 (28.6%)	
≥0.67 (≥Q3)	3 (20.0%)	40 (22.0%)	58 (23.7%)	
Percentiles of weight gain rate per week (kg) 20-27 weeks				0.522
<p10 (<0.18)	2 (13.3%)	12 (6.6%)	23 (9.4%)	
p10-p90 (0.18-0.82)	11 (73.4%)	151 (83.0%)	207 (84.5%)	
>p90 (>0.82)	2 (13.3%)	19 (10.4%)	15 (6.1%)	
Urinary infection in first the half of pregnancy [e]	2 (14.3%)	45 (30%)	50 (23.5%)	0.203
Asymptomatic Bacteriuria in the first half of pregnancy [f]	12 (85.7%)	105 (70%)	163 (76.5%)	0.521
Recurrence of any Infection§	0 (0%)	17 (12.3%)	20 (9.8%)	0.534
Vaginal bleeding in the first half of pregnancy	6 (31.6%)	36 (17.9%)	55 (19.9%)	0.372
Number of days with vaginal bleeding in the first half of pregnancy				**<0.001**
1-3	6 (100%)	31 (86.1%)	39 (70.9%)	
>3	0 (0%)	5 (13.9%)	16 (29.1%)	
Total	**19**	**201**	**277**	497

Missing information for: a) 61; b) 36; c) 51; d) 55; e) 120; f) 142.

4.9. Artigo *Perinatal outcomes from preterm and early term birth in a multicenter cohort of low risk nulliparous women*

CLINICAL ARTICLE

Perinatal outcomes from preterm and early term births in a multicenter cohort of low risk nulliparous women

Renato T Souza [1], Maria L Costa [1], Jussara Mayrink [1], Francisco E. Feitosa [2], Edilberto A Rocha Filho [3], Débora F Leite [1,3], Janete Vettorazzi [4], Iracema M Calderon [5], Maria H Sousa [6], Renato Passini Jr [1], Philip N Baker [7], Louise Kenny [8], Jose G. Cecatti [1], for the Preterm SAMBA study group*

Affiliations

[1] Department of Obstetrics and Gynaecology, University of Campinas (UNICAMP) School of Medical Sciences, Campinas, SP, Brazil

[2] MEAC – School Maternity of the Federal University of Ceará, in Fortaleza, CE, Brazil

[3] Department of Maternal and Child Health, Maternity of Clinic Hospital, Federal University of Pernambuco, in Recife, PE, Brazil

[4] Department of Obstetrics and Gynaecology, Maternity of the Clinic Hospital, Federal University of RS, Porto Alegre, RS, Brazil

[5] Department of Obstetrics and Gynaecology, Botucatu Medical School, Unesp, Botucatu, SP, Brazil

[6] Statistics Unit, Jundiai School of Medicine, Jundiaí, SP, Brazil

[7] College of Life Sciences, University of Leicester, Leicester, United Kingdom.

[8] Faculty of Health and Life Sciences, University of Liverpool, UK

*Membership of the Preterm SAMBA and SCOPE study groups are provided in the Acknowledgments.

Corresponding author
José G Cecatti
Department of Obstetrics and Gynaecology
University of Campinas, Brazil
E-mail: cecatti@unicamp.br

Abstract

Introduction: Preterm birth is the major contributor for neonatal and under-five years mortality rates and also accounts for a short- and long-term adverse consequences up to adulthood. Perinatal outcomes may vary according to lots of factors as preterm subtype, late prematurity, which account for the vast majority of cases, country and population characteristics. An under-recognition of the perinatal outcomes and its associated factors might have underpowered strategies to provide adequate care and prevent its occurrence.

Objective: To determine the incidence of maternal and perinatal outcomes according to preterm birth subtypes, and late preterm and early term births.

Methods: A multicentre prospective cohort in five maternities in Brazil between 2015 and 2018. Nulliparous low-risk women with singletons were included. Comprehensive data were collected during three antenatal visits (at 19-21weeks, 27-29 weeks and 37-39 weeks). Maternal and perinatal outcomes were also collected according to maternal and neonatal medical records. Women who had spontaneous (sPTB) and provider-initiated (pi-PTB) preterm birth were compared to those who had term birth. Also, late preterm birth (after 34 weeks), and early term (37-38 weeks) were compared to full term birth (39-40 weeks). Bivariate analysis estimated risk ratios for maternal and adverse outcomes. Finally, a multivariate analysis was conducted to address factors independently associated with any adverse perinatal outcome (APO).

Results: In total, 1,165 women had outcome data available, from which 6.7% had sPTB, 4.0% had pi-PTB and 89.3% had a term birth. sPTB and pi-PTb were associated with poorer perinatal outcomes, as well as late sPTB, late pi-PTB and early term neonates. pi-PTB (RR_{adj} 8.12, 95% CI [2.54-25.93], p-value 0.007), maternal weight gain between 20 and 27 weeks <p10 (RR_{adj} 2.04, 95% CI [1.23-3.38], p-value 0.018) and participants from the Northeast centres (RR_{adj} 2.35, 95% CI [1.11-4.95], p-value 0.034) were independently associated with APO.

Conclusion: sPTB and, especially, pi-PTB were associated with poorer perinatal outcomes in nulliparous low-risk women. Strategies to more accurately identify women at higher risk of PTB and to prevent perinatal adverse outcomes remain a great challenge in the Brazilian context, a middle-income country with perpetuated inequity in income, education and access to health care.

Key words: perinatal outcomes, spontaneous, provider-initiated, preterm birth, adverse perinatal outcome, late preterm birth

Introduction

Preterm birth (PTB) is associated with short- and long-term adverse outcomes for the neonate, including cerebral palsy. In addition, it is the leading cause of neonatal death and also a contributor to the under-five mortality rate (1–3). A secondary analysis of a World Health Organization study evaluating almost 300,000 deliveries in 29 countries showed that perinatal outcomes as stillbirth and early neonatal deaths vary according to the preterm birth subtypes (4). The rates are approximately 30% lower in spontaneous than in provider-initiated preterm birth.

Preterm birth subtype can be classified according to its motivation. Spontaneous preterm birth (sPTB) is defined as any preterm birth occurred due to spontaneous onset of labour or premature rupture of membranes (PROM) and provider-initiated preterm birth (pi-PTB) when preterm birth was indicated by health care providers due to maternal and/or fetal conditions (1). It can be also divided according to gestational age at delivery in extremely preterm (<28 weeks), very preterm (28-31 weeks), moderate preterm (32-33 weeks), and late preterm (34-36 weeks) (1,5). Not only preterm neonates are associated with adverse perinatal outcomes, but also early term neonates (37-38 weeks) (6–8). Both groups are close to 37 weeks and, because of that, related outcomes are usually underestimated, especially in provider-initiated deliveries. Neonatal and infant mortality rates are around 2 times higher in these groups compared to 39 weeks neonates (8).

Secondary analyses of the Birth in Brazil study, a hospital-based cross-sectional study that included women from 266 hospitals from February 2011 to October 2012, confirm that PTB is the leading cause of neonatal mortality in Brazil (9). The factors associated with higher rates of neonatal mortality included peregrination, not using a partograph, delivering before 32 weeks and delivering at a unit of the public unified health system (9). Less than 20% and 15% of the public and private maternities have an intensive care unit, respectively (10). In addition, 67.1% in the public and 16.5% in private maternities have ambulances for neonates.

Determining the incidence of perinatal and neonatal outcomes is important for allocating human and infrastructure resources to properly provide care for preterm neonates and

infants and planning adequate strategies to monitor PTB preventive interventions. We aim to address the incidence of maternal and perinatal outcomes in women with preterm and early term births.

Methods

We conducted a longitudinal multicentre cohort study in five referral obstetric centres in Brazil between July 2015 and July 2018, called Preterm SAMBA. The research protocol and others aspects of the study implementation and progress had already been detailed (11,12). In brief, the Preterm birth cohort was comprised of low-risk nulliparous women with singleton pregnancies. Exclusion criteria were repeated abortions (≥3), fetal major malformation, chronic hypertension (using antihypertensive drug or if moderate hypertension), diabetes type I or II, renal disease, HIV, sickle cell disease, uterine anomalies, history of cervical knife cone procedures, use of steroids (≥3 months), aspirin, calcium, fish oil, vitamin C or E or heparin. Participants were enrolled at 19-21 weeks of gestation. Then, antenatal visits were performed at 19-21 weeks, 27-29 weeks and 37-39 weeks. Data regarding sociodemographic characteristics, maternal and family medical history, habits, anthropometric measures, height, pregnancy characteristics, occurrence of complications and other maternal and fetal outcomes were collected and entered in an online database during the three study visits. Childbirth and postpartum data were retrospectively collected by reviewing maternal and neonatal medical records until discharge. Maternal weight gain rate per week (WGR) was calculated according to the difference of weight between the first two visits (19-21 weeks and 27-29 weeks).

The study was approved by the Ethical Review Board of each participant centre and was endorsed by the Brazilian National Committee for Ethics in Research (CONEP). The study complies with the 1989 Declaration of Helsinki and the Brazilian national regulations for studies in human beings stated by the National Health Council (Resolution CNS 466/12). All participating women signed an informed consent form before enrolment.

Outcomes and variables

Preterm birth was defined as any birth occurred before 37 weeks of gestation. A term birth group was comprised of all women who had birth ≥37 weeks of gestation. Then, three groups were established according to gestational age and preterm birth subtypes. Spontaneous preterm birth included women who had preterm birth due to spontaneous onset of labour or premature rupture of membranes. Provider-initiated preterm birth was defined as a preterm birth due to medical indication on account of maternal or fetal conditions/complications. In addition, neonatal outcomes from late sPTB, late pi-PTB (34-36 weeks) and early term (37-38 weeks) and post term (41-42 weeks) cases were compared to full term cases (39-40 weeks).

Maternal and neonatal outcomes included: onset of labour and mode of delivery; hyperglycaemia in pregnancy (HIP), defined by an initial fasting plasma glucose (FPM) ≥92 mg/dL or altered 75g oral glucose tolerance test performed between 24 and 28 weeks of gestation that means FPG ≥92 mg/dL or 1h-postglucose load ≥180 mg/dL or 2h-postglucose load ≥153 mg/dL; preeclampsia, defined as having systolic blood pressure ≥140 or systolic blood pressure ≥ 90 mmHg after 20 weeks gestation on at least two occasions apart of 20 min, and/or proteinuria (24-h urinary protein ≥ 300 mg or urine dipstick ≥ ++) and/or severe maternal complications; neonatal intensive care admission; phototherapy for jaundice; Apgar score <7 at 5 minutes; need of intubation after birth; length of maternal and NICU stay; fetal malformation diagnosed/confirmed after birth; neonatal sepsis (confirmed or suspected); adequacy of birthweight according to GROW customized birthweight centiles (13). A composite outcome “any adverse perinatal outcome” (APO) was operationally defined as one of the following adverse neonatal outcomes: NICU stay >7 days, intubation at birth, Apgar score<7 at 5 minutes, fetal or neonatal death, discharge home on oxygen, neonatal sepsis (early or late, suspected or confirmed), cyanosis, hypoglycaemia, birth asphyxia, respiratory distress or mechanical ventilation. Antenatal and peripartum management characteristics were also addressed, including vaginal progesterone (any dose), cerclage, pessary, steroids and tocolysis use. For mode of delivery, elective C-section was considered when it was indicated in women without labour, and also for women who failed induction (did not

initiated labour). Intrapartum C-section were considered when C-section was performed in women during labour, including women at any stage of labour following induction.

Statistical analysis

Maternal characteristics were compared between the sPTB, pi-PTB and term groups using chi-squared test (χ^2). Only p-values <0.05 were considered statistically significant. Bivariate analyses were carried out to calculate the risk ratios and 95% CI for maternal and perinatal outcomes, including pregnancy management characteristics in sPTB, pi-PTB compared to term and also to late sPTB, late pi-PTB, and early term compared to full term.

We used Stata v. 7.0 (StataCorp) and SPSS v. 20.0 (IBM SPSS Statistics, USA) to perform all statistical analysis. All the analyses (p-values and 95% CI of the RR) accounted for the primary sampling unit (PSU), i.e., they were design-based.

Results

Preterm SAMBA study included 1,181 participants, from which 1,165 were followed and had outcome data available (Figure 1). Preterm birth rate was 10.7% (n=125). From the 78 cases of sPTB and 47 of pi-PTB, 55 (70.5%) and 27 (57.4%) were late preterm births. From the 1,040 term births, 354 (34.0%) were early term (37-38 weeks), 575 (55.3%) full term (39-40) and 111 (10.6%) post-term (41-42 weeks). None of the studied maternal and sociodemographic characteristics were different between sPTB, pi-PTB and term births (Table 1). Before the admission when the birth occurred, women who had sPTB had significantly more cerclage (RR 3.62, 95% CI [1.07-12.22]), pessary use (RR 5.55, 95% CI [3.17-9.71]), history of preterm labour or pPROM (RR 8.27, 95% CI [3.70-18.51]), use of antenatal steroids (RR 9.45, 95% CI [7.19-12.42]) or tocolysis (RR 6.27, 95% CI [2.73-14.42]) compared to women who had term birth (Table 2). Women who had pi-PTB had pessary and antenatal steroids use approximately 6 and 26 times more frequent, respectively, than women with term births.

Table S1 shows that the frequency of use of antenatal steroids were 65.2% and 29.6% in women who had sPTB <34 weeks and 34-36 weeks, respectively. In pi-PTB, it was 73.7% and 56.0% for <34 weeks and 34-36 weeks, respectively. Tocolysis was performed in 43.5% and

14% of cases of women who had a sPTB <34 weeks and 34-36 weeks (p-value 0.001), respectively.

Table 3 shows maternal and neonatal outcomes according to sPTB, pi-PTB and term birth cases. All neonatal adverse outcomes were significantly more frequent in preterm birth groups than in term. In addition, pi-PTB showed higher risk for almost all adverse neonatal outcomes compared to sPTB, including APO (RR 6.17, 95% CI [3.72-10.22] for sPTB and RR 25.39, 95% CI [10.08-63.96] for pi-PTB). pi-PTB cases had had 7 and 5 times more preeclampsia [95% CI 2.39-12.21] and small for gestational age neonates [95% CI 3.15-16.99], respectively, than women with term birth.

Table 4 shows maternal and neonatal outcomes for late sPTB, late pi-PTB, and early and full-term birth categories. Longer maternal postpartum hospitalization, NICU admission, phototherapy for jaundice and APO were more frequent in late sPTB and pi-PTB groups than full term birth. Women who had preeclampsia were more frequent in late pi-PTB (RR 7.5 [2.48-22.67]) and early term birth cases (RR 1.7 [1.36-2.13]). There were no cases of fetal death in late sPTB, late pi-PTB, and term birth groups (data not shown). There were few cases of neonatal death (1 early term) and need for intubation after birth (1 late pi-PTB, 3 early term and 1 full term).

Elective C-section was much more frequent in pi-PTB (89.4%) and late pi-PTB (88.9%) than in overall term births (24.1%) or full-term birth (19.7%). Overall C-section (including elective and intrapartum) were performed in 91.5% of pi-PTB cases, 47.9% of term births and 26.9% of spontaneous preterm birth.

Table 5 shows that pi-PTB (RR_{adj} 8.12, 95% CI [2.54-25.93], p-value 0.007), maternal WGR (RR_{adj} 2.04, 95% CI [1.23-3.38], p-value 0.018) and women from the northeast participating centres (RR_{adj} 2.35, 95% CI [1.11-4.95], p-value 0.034) were independently associated with any adverse perinatal outcomes.

Discussion

In the Preterm SAMBA multicentre cohort, maternal and neonatal outcomes from 1,165 nulliparous low-risk women were evaluated. Spontaneous and, especially, provider-initiated

preterm neonates were associated with poorer short-term outcomes. Also, late preterm and early term were also associated with more adverse neonatal outcomes. pi-PTB, maternal weight gain rate between 20 and 27 weeks of gestation below the 10th percentile and women from the northeast centres were independently associated with any adverse perinatal outcome.

Despite being considered at term, early term neonates present poorer adverse outcomes when compared to full term and caution with “liberalization” in pregnancy resolution in this pregnancy interval should be taken (14,15). Similarly to late preterm, early term neonates are associated with higher prevalence of NICU admission, need for oxygen therapy, hypoglycaemia, neonatal mortality and other neonatal morbidities when compared to full term neonates (14). Neonatal mortality is around 2.3 times higher in 37 weeks compared to 39 weeks neonates (6). ACOG reinforced the importance of delaying, when possible, the elective resolution of pregnancy to after 39 weeks, rather than intervening at 37 or 38 weeks (7). In 2016, the Brazilian Federal Council of Medicine, an independent agency responsible for professional regulation of medical doctors, established a normative resolution establishing that elective C-section due to patient request should only be performed after 39 weeks of gestation (16). The concept of “too much, too soon” and “too little, too late” can be properly applied in this discussion (17). Adequate management of obstetric interventions (induction of labour, C-section, recognition of maternal/fetal complication, etc.) during late preterm and early term is a complex challenge in the Brazilian context, where there are disparities in access to intensive maternal and neonatal care units (10,18,19) and high rates of preventable severe maternal morbidities such as preeclampsia/eclampsia (20,21). Brazil shows to be a country where over-medicalization and misuse of obstetric interventions walk together with a lack of well-trained multidisciplinary team of health care providers and insufficient equipment and resources.

The use of tertiary preventive strategies such as antenatal corticosteroids (ACS) and tocolysis does not prevent preterm birth, but may improve associated neonatal outcomes (22–25). Around 65% of sPTB before 34 weeks and 30% of late sPTB used ACS. The EMIP study, a multicentre cross-sectional study in 20 referral maternities in Brazil, showed that ACS was

used in 54.0% of sPTB before 34 weeks and in 14.0% of late sPTB (26). In accordance with the evidence-based recommendation for using ACS between 34 and 36 weeks raised by ALPS study (27) in 2016 and by 2017 Cochrane Systematic review (22), an increase in the use of ACS can be observed between both Brazilian studies, EMIP (2011-2012) and Preterm SAMBA (2015-2018). In our study, half of women who had sPTB and around 70% who had pi-PTB had used ACS before the admission when birth occurred (Table S1). The effect of repeated doses and the benefits of ACS in low-resource settings remains controversial. The WHO reported that there is a need for further investigation of ACS effects in low-resource settings, where the estimate of gestational age may not be accurate enough (28). According to the Birth in Brazil study, information of an early ultrasound was available for only 44.5% of women (29).

Women who had a pi-PTB were independently associated with perinatal adverse outcomes. The EMIP study, a cross-sectional study that carried out surveillance of preterm births in 20 referral obstetric centres in Brazil, showed that hypertensive disorders motivated around 90% of pi-PTB due to maternal conditions (30). This study also showed that the neonatal mortality of extreme and late pi-PTB neonates before discharge were 200 and 6 times higher, respectively, in comparison to term neonates. A pi-PTB is a medical intervention to improve maternal and perinatal health condition, but it requires an evidenced-based decision-making process in order to avoid unnecessary prematurity and, consequently, more adverse neonatal outcomes. The HYPITAT II clinical trial showed that labour induction between 34 and 37 weeks of gestation in women with hypertensive disorders reduces adverse maternal complication (31). Respiratory distress, however, were more frequent in the intervention group (RR 3.3, 95% CI [1.4–8.2; p=0.005]). pi-PTB is associated with severe maternal morbidity and the decision-making process requires optimal resources to assure timely interventions, since any delay is also associated with more severe maternal outcomes (32,33). The evidence that maternal morbidity leads to such adverse perinatal outcomes related to preterm birth reinforces the need for monitoring the occurrence of maternal morbidity, maternal near miss and the effects of related interventions to reduce both maternal and perinatal adverse outcomes.

Maternal WGR between 20 and 27 weeks below the tenth percentile was independently associated with APO. The recommendations of gestational weight gain from the Institute of Medicine - 2009 remains controversial as it did not take into account different populations and the effect of weight gain to the different preterm birth subtypes (34). We did not use a standard definition for adequacy of weight gain as maternal early/pre-pregnancy body mass index was not available. Therefore, we addressed perinatal outcomes according to the different weight gain percentile and quintiles. We acknowledge the fact that 43% of women in Preterm SAMBA study were overweight or obese (data not shown) and only 39% were normal weight at 20 weeks according to the Atalah and cols´ reference ranges (35). Poor maternal weight gain during pregnancy has been associated with adverse perinatal outcomes such as small for gestational age and preterm birth. A study evaluating more than 500,000 normal weight women and 230,000 overweight women showed that deviations of weight gain are associated with small for gestational age (36). This association depends on how the exposure variable will be applied (total weight, rate of weight gain or adequacy according to IOM recommendation). Further studies including Brazilian population is required to better explore the risks for maternal and perinatal outcomes.

Although our findings are not innovative, we acknowledge the fact that it is a prospective low-risk nulliparous women cohort where, in theory, are expected to have low incidence of maternal and perinatal complications. Nevertheless, we have the opportunity to report important indicators as the real incidence of perinatal outcomes for this population, that can be used to plan and monitor strategies to ameliorate maternal and perinatal health care. For instance, almost 50% of women who delivered at term had a C-section. We did not evaluate the indication for elective or intrapartum C-section, but such high rates in this population (nulliparous women) requires a careful attention. There were only 12.8% of induction in pi-PTB cases. C-section can be a life-saving procedure for both mother and fetus, and a balance between risk and benefits might be context specific (37). In Brazil, C-section rate is certainly unbalanced.

A Cochrane systematic review showed that there are a plenty of non-clinical interventions to reduce unnecessary C-sections, including education programmes for women, training

programmes for professionals, implementation of midwifery-labourist care and clinical practice guidelines to better support its indication (38). The use of an institutional standardized classification of C-section to monitor its incidence is also a highly recommended approach (39,40). According to a systematic review, there are at least 27 classifications based on different factors including women´s characteristics, degree of urgency and indications (41). The Robson´s ten-group classification, based on obstetric characteristics as parity, previous C-section, preterm birth, onset of labour, fetal presentation and the number of fetuses, seems to be the most adequate (39,41); it can be easily implemented and used for longitudinal monitoring. A limitation is that the tenth group, comprised of all preterm birth cases, does not differentiate cases according to other obstetric characteristics (40). Nulliparous women is a priority group when avoiding unnecessary C-sections due to its consequences to the women´s reproductive and general health (42).

The antenatal use of steroids, tocolysis, pessary and cerclage were much more frequent in women who had sPTB than who had term births. This groups of women also presented a higher frequency of history of preterm labour or pPROM before the admission when birth occurred and short cervix, what explains the higher frequency of such interventions. An accurate prediction of which women will have a preterm birth within 7 days when presenting a preterm labour would be very useful to plan which interventions would benefit the most. A systematic review with meta-analysis addressed the performance of placental alpha microglobulin-1 (PAMG-1), fetal fibronectin (fFN) and phosphorylated insulin-like growth factor-binding protein-1 (phIGFBP-1), proteins usually presented in the amniotic fluid and/or choriodecidual interface, in predicting preterm birth (43). The sensitivity and specificity of these biomarkers were 0.76 and 0.97 for PAMG-1, 0.58 and 0.84 for fFN, and 0.93 and 0.73 for phIGFBP-1, respectively. However, the comparison of these biomarkers performance with already established clinical evaluation and procedures had not been already well explored. Another gap to be better explored is the cost-effectiveness when using such biomarkers in an upper-middle income country containing such inequalities and huge area and population as Brazil.

Participants from the Northeast centres were independently associated with APO. Our study was conducted in five referral obstetric facilities placed in four states of Brazil. The HDI in 2010 were 0.783 and 0.746 from the South/Southeast states, and 0.673 and 0.682 from the Northeast states (44). According to a populaion-based study conducted in 2006 in Brazil, the proportion of women with inadequate prenatal care, low schooling and low income is higher in the Northeast when compared to the South/Southeast (18). A more recent study, hospital-based comprising almost 24,000 women in Brazil, reinforced the existence of huge disparities in the different regions of Brazil (10,19). The Northeast lacks of adequate prenatal care and maternities´ human and equipment resources when compared to South/Southeast regions. Secondary analyses of the World Health Organization Multicountry Survey on Maternal and Newborn Health showed that the provision of care and maternal and perinatal outcomes vary according to the human development index (HDI) (4). As the HDI increase, the proportion of adverse perinatal outcomes seems to decrease and pi-PTB, on the contrary, to increase. Also, the accessibility to a preterm resolution of pregnancy when required is limited for younger women and for those with lower schooling (4).

There are strengths and limitations in our study. Early term neonates had 2.6 times more risk for neonatal death and pi-PTB and early term neonates had approximately 11 and 2 times more risk, respectively, for need of intubation. Although remarkable, these findings should be interpreted with caution due to the low number of cases in each group. We only evaluated perinatal and short-term neonatal outcomes, before neonate’s and woman’s discharge. New hospital admissions or complications were not evaluated. Comprehensive multicentre studies evaluating long-term outcomes of preterm birth in low- and middle-income countries are of urge importance. Brazil is a huge country with regional and private/public system inequalities in maternal, perinatal an infant health care (10,19). Low resource settings have the highest rates of maternal and neonatal morbidity and mortality (45), and are the neediest places where improving quality of antenatal care, investments in preterm birth research and implementing maternal and perinatal high evidence-based care will impact the most.

References

1. Vogel JP, Chawanpaiboon S, Moller A-B, Watananirun K, Bonet M, Lumbiganon P. The global epidemiology of preterm birth. Best Pract Res Clin Obstet Gynaecol. 2018 Oct 1;52:3–12.

2. Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: when? Where? Why? Lancet. 2005 Jan;365(9462):891–900.

3. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet. 2012 Jun 9;379(9832):2151–61.

4. Morisaki N, Togoobaatar G, Vogel J, Souza J, Rowland Hogue C, Jayaratne K, et al. Risk factors for spontaneous and provider-initiated preterm delivery in high and low Human Development Index countries: a secondary analysis of the World Health Organization Multicountry Survey on Maternal and Newborn Health. BJOG. 2014 Mar;121 Suppl:101–9.

5. Blencowe H, Cousens S, Chou D, Oestergaard M, Say L, Moller A-B, et al. Born Too Soon: The global epidemiology of 15 million preterm births. Reprod Health. 2013 Nov 15;10(Suppl 1):S2.

6. ACOG Committee Opinion No. 765: Avoidance of Nonmedically Indicated Early-Term Deliveries and Associated Neonatal Morbidities. Obstet Gynecol. 2019 Feb;133(2):e156–63.

7. ACOG Committee Opinion No 579: Definition of term pregnancy. Obstet Gynecol. 2013 Nov;122(5):1139–40.

8. Gyamfi-Bannerman C. The Scope of the Problem: The Epidemiology of Late Preterm and Early-Term Birth. Semin Perinatol. 2011 Oct 1;35(5):246–8.

9. Lansky S, Lima Friche AA de, Silva AAM da, Campos D, Azevedo Bittencourt SD de, Carvalho ML de, et al. Birth in Brazil survey: neonatal mortality, pregnancy and childbirth quality of care. Cad Saude Publica. 2014 Aug;30 Suppl 1:S1-15.

10. de Azevedo Bittencourt SD, da Costa Reis LG, Ramos MM, Rattner D, Rodrigues PL, Neves DCO, et al. Structure in Brazilian maternity hospitals: key characteristics for quality of obstetric and neonatal care. Cad Saude Publica. 2014 Aug;30 Suppl 1:S1-12.

11. Cecatti JG, Souza RT, Sulek K, Costa ML, Kenny LC, McCowan LM, et al. Use of metabolomics for the identification and validation of clinical biomarkers for preterm birth: Preterm SAMBA. BMC Pregnancy Childbirth. 2016 Dec 8;16(1):212.

12. Souza RT, Cecatti JG, Costa ML, Mayrink J, Pacagnella RC, Passini R, et al. Planning, Implementing, and Running a Multicentre Preterm Birth Study with Biobank Resources in Brazil: The Preterm SAMBA Study. Biomed Res Int. 2019 Jan 16;2019:1–8.

13. GROW Birthweight Centiles. Bulk Centile Calculator. [Internet]. 2014 [cited 2015 Jul 7]. Available from: https://www.gestation.net/index.htm

14. Leal M do C, Esteves-Pereira AP, Nakamura-Pereira M, Domingues RMSM, Dias MAB, Moreira ME, et al. Burden of early-term birth on adverse infant outcomes: a population-based cohort study in Brazil. BMJ Open. 2017;7(12):e017789.

15. Delnord M, Zeitlin J. Epidemiology of late preterm and early term births – An international perspective. Semin Fetal Neonatal Med. 2019 Feb 18;24(1):3–10.

16. Federal Council of Medicine. Resolution No 2.144/2016. Brazil. Available from: https://portal.cfm.org.br/images/stories/pdf/res21442016.pdf Accessed on 05 February 2019.

17. Miller S, Abalos E, Chamillard M, Ciapponi A, Colaci D, Comandé D, et al. Beyond too little, too late and too much, too soon: a pathway towards evidence-based, respectful maternity care worldwide. Lancet. 2016 Oct 29;388(10056):2176–92.

18. Ministério da Saúde. Pesquisa Nacional de Demografia e Saúde da Criança e da Mulher – PNDS 2006: dimensões do processo reprodutivo e da saúde da criança. Brasília: Ministério da Saúde; 2009.

19. Viellas EF, Domingues RMSM, Dias MAB, Gama SGN da, Theme Filha MM, Costa JV

da, et al. Prenatal care in Brazil. Cad Saude Publica. 2014 Aug;30:S85–100.

20. Giordano JC, Parpinelli MA, Cecatti JG, Haddad SM, Costa ML, Surita FG, et al. The burden of eclampsia: results from a multicenter study on surveillance of severe maternal morbidity in Brazil. PLoS One. 2014 Jan;9(5):e97401.

21. Zanette E, Parpinelli M, Surita F, Costa M, Haddad S, Sousa M, et al. Maternal near miss and death among women with severe hypertensive disorders: a Brazilian multicenter surveillance study. Reprod Health. 2014;11(1):4.

22. Roberts D, Brown J, Medley N, Dalziel SR. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. Cochrane Database Syst Rev. 2017 Mar 21;3:CD004454.

23. Flenady V, Reinebrant HE, Liley HG, Tambimuttu EG, Papatsonis DN. Oxytocin receptor antagonists for inhibiting preterm labour. Cochrane Database Syst Rev. 2014 Jun 6;(6):CD004452.

24. Neilson JP, West HM, Dowswell T. Betamimetics for inhibiting preterm labour. Cochrane Database Syst Rev. 2014 Feb 5;(2):CD004352.

25. Flenady V, Wojcieszek AM, Papatsonis DN, Stock OM, Murray L, Jardine LA, et al. Calcium channel blockers for inhibiting preterm labour and birth. Cochrane Database Syst Rev. 2014 Jun 5;(6):CD002255.

26. Dias TZ, Passini R, Tedesco RP, Lajos GJ, Rehder PM, Nomura ML, et al. Evaluation of prenatal corticosteroid use in spontaneous preterm labor in the Brazilian Multicenter Study on Preterm Birth (EMIP). Int J Gynecol Obstet. 2017 Nov;139(2):222–9.

27. Gyamfi-Bannerman C, Thom EA, Blackwell SC, Tita ATN, Reddy UM, Saade GR, et al. Antenatal Betamethasone for Women at Risk for Late Preterm Delivery. N Engl J Med. 2016 Apr 7;374(14):1311–20.

28. Vogel JP, Oladapo OT, Pileggi-Castro C, Adejuyigbe EA, Althabe F, Ariff S, et al. Antenatal corticosteroids for women at risk of imminent preterm birth in low-resource countries: the case for equipoise and the need for efficacy trials. BMJ Glob

Heal. 2017;2(3):e000398.

29. Pereira APE, Leal M do C, Gama SGN da, Domingues RMSM, Schilithz AOC, Bastos MH. Determining gestational age based on information from the Birth in Brazil study. Cad Saude Publica. 2014 Aug;30:S59–70.

30. Souza RT, Cecatti JG, Passini R, Tedesco RP, Lajos GJ, Nomura ML, et al. The Burden of Provider-Initiated Preterm Birth and Associated Factors: Evidence from the Brazilian Multicenter Study on Preterm Birth (EMIP). Zakar T, editor. PLoS One. 2016 Feb 5;11(2):e0148244.

31. Broekhuijsen K, van Baaren G-J, van Pampus MG, Ganzevoort W, Sikkema JM, Woiski MD, et al. Immediate delivery versus expectant monitoring for hypertensive disorders of pregnancy between 34 and 37 weeks of gestation (HYPITAT-II): an open-label, randomised controlled trial. Lancet. 2015 Mar 24;

32. Pacagnella RC, Cecatti JG, Parpinelli MA, Sousa MH, Haddad SM, Costa ML, et al. Delays in receiving obstetric care and poor maternal outcomes: results from a national multicentre cross-sectional study. BMC Pregnancy Childbirth. 2014 Jan;14:159.

33. Helou A, Walker S, Stewart K, George J. Management of pregnancies complicated by hypertensive disorders of pregnancy: Could we do better? Aust New Zeal J Obstet Gynaecol. 2017 Jun;57(3):253–9.

34. The National Academies Press. Institute of Medicine (IOM), National Research Council (NRC). Weight Gain During Pregnancy: Reexamining the Guidelines. Washington DC; 2009.

35. Atalah E, Castillo C, Castro R, Aldea A. [Proposal of a new standard for the nutritional assessment of pregnant women]. Rev Med Chil. 1997 Dec;125(12):1429–36.

36. Bodnar LM, Hutcheon JA, Parisi SM, Pugh SJ, Abrams B. Comparison of Gestational Weight Gain z-Scores and Traditional Weight Gain Measures in Relation to Perinatal Outcomes. Paediatr Perinat Epidemiol. 2015 Jan 1;29(1):11–21.

37. Souza JP, Betran AP, Dumont A, de Mucio B, Gibbs Pickens CM, Deneux-Tharaux C, et al. A global reference for caesarean section rates (C-Model): a multicountry cross-sectional study. BJOG. 2016 Feb;123(3):427–36.

38. Chen I, Opiyo N, Tavender E, Mortazhejri S, Rader T, Petkovic J, et al. Non-clinical interventions for reducing unnecessary caesarean section. Cochrane Database Syst Rev. 2018 Sep 28;9:CD005528.

39. Betrán AP, Merialdi M, Lauer JA, Bing-Shun W, Thomas J, Van Look P, et al. Rates of caesarean section: analysis of global, regional and national estimates. Paediatr Perinat Epidemiol. 2007 Mar 1;21(2):98–113.

40. Betrán AP, Vindevoghel N, Souza JP, Gülmezoglu AM, Torloni MR. A Systematic Review of the Robson Classification for Caesarean Section: What Works, Doesn't Work and How to Improve It. Bhattacharya S, editor. PLoS One. 2014 Jun 3;9(6):e97769.

41. Torloni MR, Betran AP, Souza JP, Widmer M, Allen T, Gulmezoglu M, et al. Classifications for Cesarean Section: A Systematic Review. Althabe F, editor. PLoS One. 2011 Jan 20;6(1):e14566.

42. Sandall J, Tribe RM, Avery L, Mola G, Visser GH, Homer CS, et al. Short-term and long-term effects of caesarean section on the health of women and children. Lancet. 2018 Oct 13;392(10155):1349–57.

43. Melchor JC, Khalil A, Wing D, Schleussner E, Surbek D. Prediction of preterm delivery in symptomatic women using PAMG-1, fetal fibronectin and phIGFBP-1 tests: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2018 Oct;52(4):442–51.

44. United Nations Development Programme (UNDP) [Internet]. Human Development Index (HDI). Brazil. 2010. Available from: http://www.pnud.org.br/Atlas.aspx

45. Souza JP, Gülmezoglu AM, Vogel J, Carroli G, Lumbiganon P, Qureshi Z, et al. Moving beyond essential interventions for reduction of maternal mortality (the WHO

Multicountry Survey on Maternal and Newborn Health): a cross-sectional study. Lancet. 2013 May 18;381(9879):1747–55.

Acknowledgements

The Preterm SAMBA study group also included: Mary A. Parpinelli, Karayna G Fernandes, Rafael B Galvão, José Paulo Guida, Danielly S Santana, School of Medical Sciences, University of Campinas, Brazil; Kleber G. Franchini, LNBio, Campinas, Brazil; Bianca F. Cassettari, School of Medicine of Botucatu, UNESP, Brazil; Lucia Pfitscher, Luiza Brust, School of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil; Elias F Melo Junior, Danilo Anacleto, School of Medicine, Federal University of Pernambuco, Recife, Brazil; Daisy de Lucena, Benedita Sousa, School of Medicine, Federal University of Ceará, Fortaleza, Brazil.

Disclosure of Interests

Authors declare no competing interest for the current analysis.

Contribution to authorship

JGC, PNB, LCK and MLC conceived and planned the cohort. JGC, RTS, DFL, FEF, EARF, JV, IMC, JM and RPJr developed all related procedures, implemented and carried out the cohort. RTS and JGC designed and performed the current analysis. RTS, MLC and JGC wrote the manuscript. All author, including those from the Preterm SAMBA study group, read, reviewed and approved the final version of the manuscript.

Ethical Approval

The current study was approved by each local Institutional Review Board (IRB) and amended by the Brazilian National Committee for Ethics in Research (CONEP) - Letter of approval 1.048.565 issued on 28th April 2015. The study complies with national and international regulations for experiments in human beings, including the resolution CNS 466/12 of the Brazilian National Heath Council and the 1989 Declaration of Helsinki.

Funding

This was one of the two big studies selected for sponsoring from the research call “Grand Challenges Brazil: Reducing the burden of preterm birth” number 05/2013 jointly issued by the Brazilian National Research Council (CNPq - Award 401636/2013-5) and the Bill and Melinda Gates Foundation. The funders played no role at all in the study design, writing the manuscript nor in the decision to submit the manuscript for publication.

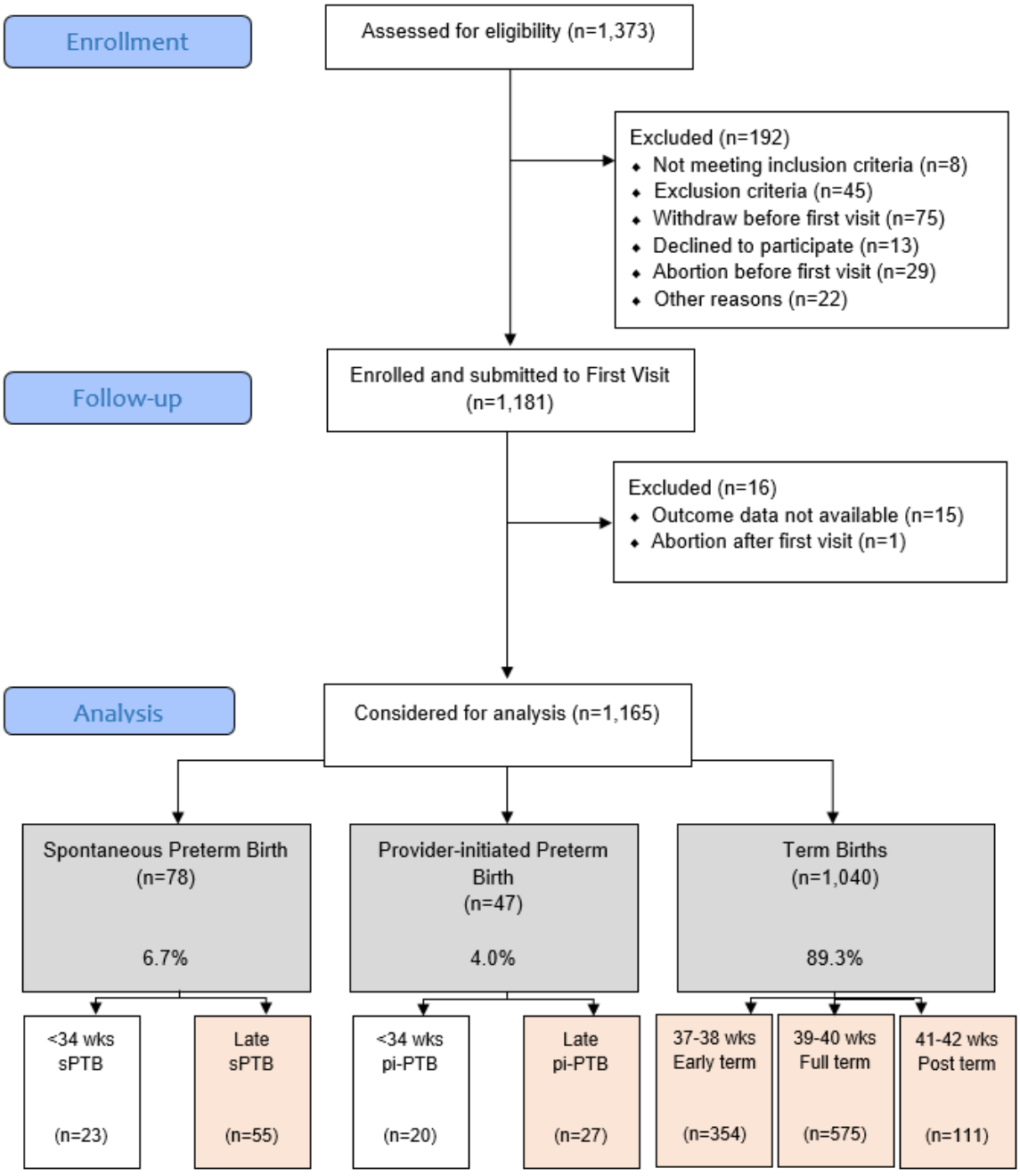


Figure 1. Preterm SAMBA Flowchart – Preterm birth subtypes' analysis

Table 1. Maternal characteristics from women who had sPTB, pi-PTB and term births

Characteristics	sPTB	pi-PTB	Term births	p-value
Region				0.329
Northeast	34 (43.6%)	25 (53.2%)	506 (48.7%)	
South and Southeast	44 (56.4%)	22 (46.8%)	534 (51.3%)	
Maternal age (years)				0.137
≤19	18 (23.1%)	4 (8.5%)	269 (25.9%)	
20-34	54 (69.2%)	37 (78.7%)	705 (67.8%)	
≥35	6 (7.7%)	6 (12.8%)	66 (6.3%)	
Ethnicity				0.429
White	32 (41.0%)	14 (29.8%)	416 (40.0%)	
Non-white	46 (59%.0)	33 (70.2%)	624 (60.0%)	
Marital status				0.127
With partner	52 (66.7%)	39 (83.0%)	762 (73.3%)	
Without partner	26 (33.3%)	8 (17.0%)	278 (26.7%)	
Maternal Occupation				0.085
Paid work	41 (52.6%)	32 (68.1%)	512 (49.2%)	
Housewife	13 (16.7%)	6 (12.8%)	192 (18.5%)	
Not working	24 (30.7%)	9 (19.1%)	360 (32.2%)	
Schooling (years)				0.883
< 12	52 (66.7%)	33 (70.2%)	706 (67.9%)	
≥ 12	26 (33.3%)	14 (29.8%)	334 (32.1%)	
Annual Family Income (US$)				0.511
Up to 3,000	2 (2.6%)	2 (4.3%)	48 (4.6%)	
3,000 to 12,000	41 (52.5%)	29 (61.7%)	563 (54.1%)	
Above 12,000	35 (44.9%)	16 (34.0%)	429 (41.3%)	
Source of prenatal care				0.602
Entirely public	67 (85.9%)	42 (89.4%)	899 (86.4%)	
Private/insurance/mixed	11 (14.1%)	5 (10.6%)	141 (13.6%)	
Total	**78**	**47**	**1,040**	

Table 2. Antenatal and peripartum management characteristics of sPTB, pi-PTB and term births

Characteristics	sPTB	RR (95%CI)	pi-PTB	RR (95%CI)	Term births
Use of vaginal progesterone *[a]					
None	62 (80.5%)	Ref.	42 (91.4%)	Ref.	987 (96%)
1 Trimester only	6 (7.8%)	3.76 [078-18.01]	2 (4.3%)	2.13 [0.24-18.96]	21 (2%)
1st, 2nd and/or 3rd trimesters	9 (11.7%)	5.08 [1.76-14.66]	2 (4.3%)	2.13 [0.42-10.74]	21 (2%)
Cerclage					
Yes	1 (1.3%)	**3.62 [1.07-12.22]**	0 (0%)	-	4 (0.3%)
No	77 (98.7%)	Ref.	47 (100%)	Ref.	1,161 (99.7%)
Pessary					
Yes	3 (3.8%)	**5.55 [3.17-9.71]**	2 (4.3%)	**6.86 [3.39-13.88]**	5 (0.5%)
No	75 (96.2%)	Ref.	45 (95.7%)	Ref.	1,035 (99.5%)
History of preterm labor or pPROM [#]					
Yes	24 (30.8%)	**8.27 [3.70-18.51]**	1 (2.1%)	0.67 [0.02-19.09]	33 (3.2%)
No	54 (69.2%)	Ref.	46 (97.9%)	Ref.	1,007 (96.8%)
Antenatal Steroids [#a]					
Yes	33 (50%)	**9.45 [7.19-12.42]**	31 (73.8%)	**26.53 [7.97-88.32]**	51 (6.3%)
No	33 (50%)	Ref.	11 (26.2%)	Ref.	761 (93.7%)
Tocolysis [#]					
Yes	8 (10.3%)	**6.27 [2.73-14.42]**	2 (4.3%)	3.41 [0.23-51.27]	12 (1.2%)
No	70 (89.7%)	Ref.	45 (95.7%)	Ref.	1,028 (98.8%)

*Initiated until 28 weeks. #before the admission when the birth occurred. Missing information for: a:13.

Table 3. Maternal and neonatal outcomes of sPTB and pi-PTB compared to full term births

Characteristics	sPTB	RR [95% CI]	pi-PTB	RR [95% CI]	Term Births
Onset of labor					
Spontaneous labour	65 (83.3%)	Ref.	0 (0%)	Ref.	617 (59.3%)
PROM + induction	9 (11.5%)	1.66 [0.69-3.95]	0 (0%)	—	48 (4.6%)
Induction intact membranes	0 (0%)	—	6 (12.8%)	—	184 (17.7%)
Elective C-section	4 (5.2%)	0.22 [0.03-1.50]	41 (87.2%)	—	191 (18.4%)
Mode of delivery					
Vaginal	57 (73.1%)	Ref.	4 (8.5%)	Ref.	556 (53.8%)
Intrapartum C-section	5 (6.4%)	0.65 [0.36-1.16]	1 (2.1%)	0.56 [0.02-20.32]	277 (23.8%)
Elective C-section	16 (20.5%)	0.23 [0.05-1.14]	42 (89.4%)	**21.73 [2.60-181.80]**	251 (24.1%)
Length of maternal postpartum hospitalization [a]					
1-3 days	62 (79.5%)	Ref.	28 (59.6%)	Ref.	951 (91.5%)
4-6 days	13 (16.7%)	**2.44 [1.12-5.33]**	13 (27.7%)	**5.22 [1.05-25.89**	74 (7.1%)
≥ 7 days	3 (3.8%)	2.88 [0.64-12.90]	6 (12.8%)	**10.49 [2.34-47.10]**	14 (1.3%)
Preeclampsia	2 (2.6%)	0.40 [0.07-2.18]	18 (38.3%)	**7.32 [3.15-16.99]**	67 (6.4%)
HIP [b]	12 (16.9%)	1.15 [0.39-3.44]	5 (12.5%)	0.83 [0.27-2.51]	133 (14.8%)
Mean (±SD) birthweight (g) [c]	2,253 ±666.9	**1,002 [893.6-1111.0]**[#]	1,824 ±845.7	**1,431 [921.9-1940.0]**[#]	3,255 ±422.3
Adequacy of birthweight to GA [c]					
SGA (p< 10)	8 (10.2%)	0.94 [0.20-4.49]	45 (44.7%)	**5.40 [2.39-12.21]**	117 (11.4%)
AGA (p10-90)	58 (74.4%)	Ref.	23 (48.9%)	Ref.	793 (77.2%)
LGA (p>90)	12 (15.4%)	1.36 [0.48-3.86]	3 (6.4%)	0.89 [0.14-5.65]	117 (11.4%)
Fetal death	1 (1.3%)	**14.51 [10.63-19.79]**	2 (4.3%)	**24.11 [18.88-30.79]**	0 (0%)

Neonatal death	2 (2.6%)	**9.90 [5.08-19.32]**	5 (10.6%)	**22.48 [10.99-45.99]**	1 (0.1%)
Apgar score – 5th minute <7 [d]	5 (6.8%)	**5.47 [2.46-12.15]**	5 (11.1%)	**9.04 [3.51-23.31]**	9 (0.9%)
Need of intubation after birth [e]	12 (15.4%)	**12.42 [8.85-17.43]**	10 (22.2%)	**21.67 [11.79-39.85]**	4 (0.4%)
NICU admission	40(51.3%)	**7.54 [3.65-15.58]**	36 (76.6%)	**23.47 [16.13-34.16]**	97 (9.3%)
Phototherapy for jaundice [e]	47 (61.0%)	**7.08 [3.68-13.60]**	31 (68.9%)	**10.68 [5.66-20.14]**	154 (14.9%)
Length of NICU stay (days)					
1-3 days	4 (10.0%)	Ref.	8 (22.2%)	Ref.	59 (60.8%)
4-6 days	5 (12.5%)	3.58 [0.54-23.81]	3 (8.3%)	1.26 [0.13-12.34]	17 (17.5%)
≥ 7 days	31 (77.5%)	**9.39 [1.73-51.06]**	25 (69.4%)	**4.55 [1.39-14.92]**	21 (21.6%)
Neonatal sepses [f]	14 (18.4%)	**7.17 [4.54-11.33]**	10 (21.7%)	**9.76 [3.88-24.54]**	20 (1.9%)
APO* [g]	37 (50.0%)	**7.17 [3.16-16.28]**	38 (80.9%)	**29.13 [18.07-46.98]**	92 (9.4%)
Total	**78**		**47**		**1,040**

Missing information for: a) 1; b) 157; c) 13; d) 65; e) 11; f) 5; g) 64. #WMD, weighted mean difference [95% CI]. *APO: NICU stay >7 days or intubation at birth or Apgar score<7 at 5 minutes or fetal/neonatal death or discharge home on oxygen or neonatal sepsis or cyanosis or hypoglycaemia or birth asphyxia or respiratory distress or mechanical ventilation.

Table 4. Maternal and neonatal outcomes of late preterm birth and early and post term compared to full term births

Characteristics	Late sPTB 34-36 wks	RR (95% CI) sPTB vs full term	Late pi-PTB 34-36 wks	RR (95% CI) pi-PTB vs full term	Early term 37-38 wks	RR (95% CI) Early term vs full term	Full term 39-40 wks	Post term 41-42 wks	RR (95% CI) Post term vs full term
Onset of labor									
Spontaneous labour	42 (76.4%)	Ref.	0 (0%)	Ref.	189 (53.4%)	Ref.	375 (65.2%)	53 (47.7%)	Ref.
PROM + induction	9 (16.4%)	**3.08 [1.45-6.56]**	0 (0%)	—	24 (6.8%)	**1.63 [1.24-2.14]**	20 (3.5%)	4 (3.6%)	1.35 [0.66-2.74]
Induction intact membranes	0 (0%)	—	4 (6.8%)	—	65 (18.4%)	1.32 [0.73-2.40]	82 (14.3%)	37 (33.3%)	2.51 [0.99-6.36]
Elective C-section	4 (7.2%)	0.39 [0.06-2.50]	23 (85.2%)	—	76 (21.4%)	1.30 [0.81-2.11]	98 (17.0%)	17 (15.4%)	1.19 [0.41-3.43]
Mode of delivery									
Vaginal	39 (70.9%)	Ref.	2 (7.4%)	Ref.	180 (50.8%)	Ref.	330 (57.3%)	49 (44.2%)	Ref.
Intrapartum C-section	11 (20.0%)	0.73 [0.30-1.77]	1 (3.7%)	1.25 [0.02-74.28]	81 (22.9%)	1.08 [0.82-1.41]	132 (23.0%)	38 (34.2%)	**1.73 [1.25-2.40]**
Elective C-section	5 (9.1%)	0.40 [0.09-1.72]	24 (88.9%)	**29.08 [1.34-630.29]**	93 (26.3%)	1.28 [0.91-1.79]	113 (19.7%)	24 (21.6%)	1.35 [0.46-4.01]
Length of maternal postpartum hospitalization [a]									
1-3 days	40 (72.7%)	Ref.	16 (59.3%)	Ref.	318 (90.1%)	Ref.	534 (92.9%)	99 (89.2%)	Ref.
4-6 days	12 (21.8%)	**3.91 [1.57-9.77]**	9 (33.3%)	7.55 [0.86-65.93]	32 (9.1%)	1.34 [0.68-2.64]	32 (5.6%)	10 (9.0%)	1.52 [0.41-5.61]
≥ 7 days	3 (5.5%)	3.59 [0.78-16.40]	2 (7.4%)	**6.25 [1.08-36.13]**	3 (0.8%)	0.67 [0.10-4.46]	9 (1.5%)	2 (1.8%)	1.16 [0.19-7.12]
Preeclampsia	1 (1.8%)	0.45 [0.05-3.69]	8 (29.6%)	**7.50 [2.48-22.67]**	39 (11.0%)	**1.70 [1.36-2.13]**	24 (4.2%)	4 (3.6%)	0.88 [0.13-6.10]
HIP* [b]	9 (18.8%)	1.23 [0.33-4.66]	3 (13.0%)	0.83 [0.35-1.97]	52 (16.4%)	1.04 [0.73-1.47]	76 (15.5%)	5 (5.7%)	0.37 [0.08-1.81]
Mean (SD) birthweight (g) [c]	2,533 ±457	**793.8 [649.7-938.0]#**	2,403 ±520	**924.4 [622.4-1,226.4]#**	3,059 ±390	**268.2 [214.7-321.7]#**	3,327 ±388	3,508 ±438	**-180.9 [(-269.3)- (-92.6)]#**

Adequacy of birthweight to GA [d]									
SGA (p< 10)	6 (10.9%)	1.11 [0.18-6.74]	7 (25.9%)	**2.99 [1.08-8.32]**	43 (12.2%)	1.16 [0.82-1.63]	57 (10.1%)	17 (15.3%)	1.52 [0.73-3.14]
AGA (p10-90)	42 (76.4%)	Ref.	17 (63.0%)	Ref.	265 (75.1%)	Ref.	448 (79.6%)	80 (72.1%)	Ref.
LGA (p>90)	7 (12.7%)	1.26 [0.36-4.36]	3 (11.1%)	1.35 [0.31-5.85]	45 (12.7%)	1.18 [0.81-1.70]	58 (10.3%)	14 (12.6%)	1.28 [0.47-3.54]
Neonatal death	0 (0%)	—	0 (0%)	—	1 (0.3%)	**2.63 [2.25-3.08]**	0 (0%)	0 (0%)	—
Apgar score – 5th minute <7 [e]	1 (2.0%)	1.95 [0.15-24.90]	2 (7.4%)	6.40 [0.67-60.70]	4 (1.2%)	1.16 [0.46-2.97]	5 (0.9%)	0 (0%)	—
Need of intubation after birth [f]	0 (0%)	—	1 (3.7%)	**11.46 [6.60-19.91]**	3 (0.9%)	**1.99 [1.07-3.69]**	1 (0.2%)	0 (0%)	—
NICU admission	19 (34.5%)	**4.16 [1.87-9.23]**	18 (66.7%)	**15.20 [10.80-21.39]**	35 (9.9%)	1.06 [0.89-1.27]	52 (9.0%)	10 (9.0%)	1.00 [0.84-1.18]
Phototherapy for jaundice [g]	29 (52.7%)	**4.85 [2.90-8.13]**	17 (63.0%)	**7.98 [2.85-22.39]**	54 (15.3%)	1.00 [0.87-1.15]	88 (15.4%)	12 (11.0%)	0.72 [0.33-1.58]
Length of neonatal admission (days)									
1-3 days	3 (15.8%)	Ref.	5 (27.8%)	Ref.	22 (62.9%)	Ref.	31 (59.6%)	6 (60.0%)	Ref.
4-6 days	4 (21.1%)	2.83 [0.22-36.24]	2 (11.1%)	1.03 [0.26-4.15]	3 (8.5%)	0.48 [0.08-3.03]	12 (23.1%)	2 (20.0%)	0.88 [0.04-20.09]
≥ 7 days	12 (63.1%)	6.48 [1.00-42.05]	11 (61.1%)	**3.96 [1.29-12.13]**	10 (28.6%)	1.27 [0.94-1.71]	9 (17.3%)	2 (20.0%)	1.12 [0.10-12.30]
Neonatal sepses [h]	3 (5.6%)	**2.58 [1.71-3.90]**	1 (3.7%)	1.89 [0.18-19.88]	6 (1.7%)	0.93 [0.35-2.42]	11 (1.9%)	3 (2.7%)	1.33 [0.32-5.62]
APO* [i]	15 (29.4%)	**3.37 [1.45-7.84]**	18 (66.7%)	**14.65 [9.50-22.57]**	34 (10.2%)	1.07 [0.90-1.26]	50 (9.3%)	8 (7.4%)	0.81 [0.46-1.43]
Total	**55**		**27**		**354**		**575**	**111**	

Missing information for: a) 1; b) 154; c) 1; d) 13; e) 62; f) 9; g) 8; h) 3; i) 64. *Hyperglycemia in pregnancy. #WMD, weighted mean difference [95% CI]. *APO (Any Adverse Perinatal outcome): NICU stay >7 days or intubation at birth or Apgar score<7 at 5 minutes or fetal/neonatal death or discharge home on oxygen or neonatal sepsis or cyanosis or hypoglycaemia or birth asphyxia or respiratory distress or mechanical ventilation.

Table 5: Factors independently associated with APO* among preterm neonates: multiple analyses by non-conditional logistic regression [n=837]

Variables	RR_{adj}	95% CI	p-value
pi-PTB	**8.12**	**2.54–25.93**	**0.007**
Maternal weight gain rate per week 20-27 weeks <p10	**2.04**	**1.23–3.38**	**0.018**
Region (Northeast)	**2.35**	**1.11–4.95**	**0.034**

Variables included in the model: Region; ethnicity; annual family income; source of prenatal care; smoking status; previous maternal condition; cervical length from 18 to 24 weeks <25mm; weight gain rate per week 20-27 weeks <Q1; weight gain rate per week 20-27 weeks <Q2; weight gain rate per week 20-27 weeks <p10; weight gain rate per week 20-27 weeks >p90; sPTB; pi-PTB; gestational age at birth. *APO: NICU stay >7 days or intubation at birth or Apgar score<7 at 5 minutes or fetal/neonatal death or discharge home on oxygen or neonatal sepsis or cyanosis or hypoglycaemia or birth asphyxia or respiratory distress or mechanical ventilation.

S1. Peripartum management characteristics according to spontaneous and provider-initiated preterm birth categories

Characteristics	sPTB			pi-PTB		
	<34w	**34-36w**	**p-value**	**<34w**	**34-36w**	**p-value**
Tocolysis*[a]			**0.001**			-
Yes	10 (43.5%)	7 (14.0%)		0 (0%)	0 (0%)	
No	13 (56.5%)	43 (86.0%)		15 (100%)	17 (100%)	
Antenatal Steroids*[b]			**0.033**			0.378
Yes	15 (65.2%)	16 (29.6%)		14 (73.7%)	14 (56.0%)	
No	8 (34.8%)	37 (70.4%)		5 (26.3%)	11 (44.0%)	

***during admission when birth occurred. Missing information for sPTB and pi-PTB, respectively: a) 5 and 15; b) 2 and 3.

Acknowledgements

The Preterm SAMBA study group also included: Mary A. Parpinelli, School of Medical Sciences, University of Campinas, Brazil; Kleber G. Franchini, LNBio, Campinas, Brazil; Bianca F. Cassettari, School of Medicine of Botucatu, UNESP, Brazil; Lúcia Pfitscher, Luiza Cabreira Brust, School of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil; Daisy de Lucena, School of Medicine, Federal University of Ceará, Fortaleza, Brazil.

Funding

This was one of the two big studies selected for sponsoring from the research call “Grand Challenges Brazil: Reducing the burden of preterm birth” number 05/2013 jointly issued by the Brazilian National Research Council (CNPq) and the Bill and Melinda Gates Foundation (Award 401636/2013-5). The funders played no role at all in the study design, writing the manuscript nor in the decision to submit the manuscript for publication.

5. DISCUSSÃO GERAL

O tópico prematuridade permanece sendo foco de novas pesquisas na área de saúde materna e perinatal ao longo de várias décadas. Isso porque trata-se de uma complicação de desenvolvimento complexo, de etiologia desconhecida, associada com desfechos adversos desde o início da vida e que pode corresponder à perda de qualidade de vida e sequelas a longo prazo, inclusive com possíveis repercussões em condições crônicas na vida adulta. Além disso, está associada a altos custos pela assistência aos indivíduos nascidos prematuros e a suas famílias, ao grande impacto mundial independentemente do nível socioeconômico da população ou de desenvolvimento do país (8,9).

A abordagem aqui apresentada resume-se em tentar explorar alguns fatores clínico-epidemiológicos que, embora não completamente inovadores, foram abordados em contextos e utilizando-se de métodos que podem colaborar para o conhecimento científico. Além dos aspectos clínico-epidemiológicos, foram explorados também o desenvolvimento e implementação de uma abordagem inovadora para a identificação de marcadores de parto prematuro, a metabolômica. Nessa discussão geral, mantenho o foco nos aspectos gerais do desenvolvimento dos estudos e de seus resultados. A discussão mais detalhada dos resultados e seus impactos para o conhecimento e prática clínica foram abordados separadamente em cada artigo. O processo do desenvolvimento dos estudos científicos em prematuridade que compõem essa tese foram baseados em esforços coletivos que merecem uma reflexão contextualizada com a importância do tema, o seu desenvolvimento em nosso contexto no Brasil, o papel da Rede Brasileira de Estudos em Saúde Reprodutiva e Perinatal e as minhas atividades como pesquisador (coordenador nacional do estudo Preterm SAMBA) e como aluno de pós-graduação.

Os dados oficiais sobre prematuridade no Brasil reportavam uma prevalência que não ultrapassava 7,5% de 2002 até 2010 (10). Embora aumentos sucessivos nas taxas e no número absoluto de nascidos prematuros tenham ocorrido nesse período, as estimativas de estudos internacionais apontavam para números quase 50% maiores dos que observados nos dados oficiais para esse período 2010 (9,11). A partir de 2010, com a mudança do formulário de coleta de

dados de nascidos vivos que abastece o SINASC e com o melhor registro dos casos, os números oficiais parecem ter encontrado seu nível real e a prematuridade começa a ter maior destaque no contexto de pesquisa na área de saúde materna e perinatal no Brasil.

A Rede Brasileira de Estudos em Saúde Reprodutiva e Perinatal organiza-se, então, para desenvolver os estudos EMIP (3) em 2011 e Preterm SAMBA (12) e P5 (Pessário e Progesterona para Prevenção de Prematuridade) em 2013. Os três estudos são multicêntricos envolvendo robusta estrutura de pesquisa, múltiplos profissionais da saúde, treinamento de recursos humanos e investimento em infraestrutura de pesquisa. Cabe dizer que, ainda quando residente em Ginecologia e Obstetrícia pela Universidade Federal do Paraná, fui colaborador na coleta de dados do estudo EMIP em todo o período do estudo, participando da reunião final do estudo realizada pelos pesquisadores do centro coordenador (Unicamp) em Campinas. Foi através dessa participação que se oportunizou meu envolvimento em outras atividades em pesquisa com a Unicamp e com a Rede. Em 2014, ingressei no programa de pós-graduação em Tocoginecologia da Faculdade de Ciências Médicas da Unicamp como aluno de mestrado e tive como objetivo da minha dissertação os dados do estudo EMIP. A partir de então, começa meu envolvimento com o tema prematuridade, iniciando uma “história natural” que se desenrolou desde a coleta de dados do EMIP, análise dos dados no mestrado até o desenvolvimento de novos projetos e metodologias nesse tópico. Esse processo de alguns anos retrata, sobretudo, a narrativa do pesquisador desde a elaboração do “problema” ou da pergunta de pesquisa, a escolha da abordagem metodológica, dos instrumentos de coleta de dados, das ferramentas analíticas apropriadas, até, enfim, as reflexões e decisões sobre o desenvolvimento do processo de pesquisa. Essa construção e envolvimento são produtos normalmente não mensurados ou avaliados no decorrer do desenvolvimento de uma tese ou projeto de pesquisa. Entretanto, foi e tem sido fundamental no contexto da formação e manutenção da Rede Brasileira de Estudos em Saúde Reprodutiva e Perinatal, que se baseia fundamentalmente em pessoas, pesquisadores jovens e *seniors*, no contexto da pesquisa em prematuridade, tema que, assim como muitos outros, necessita de *experts* que tenham grande

envolvimento nos processos de desenvolvimento de pesquisa para o advento de transformações e inovações.

Aaron Cicourel (13) e Horward Becker (14), sociólogos americanos do século XX, tratam do desenvolvimento da pesquisa sociológica sob o ponto de vista do papel do indivíduo pesquisador, de suas perspectivas, vivências e percepções. E partir disso, vigora-se o papel do pesquisador a partir da construção de suas percepções do processo e das transformações que esse processo traz para ambos, pesquisador e pesquisa. Os planos de análises, a construção dos métodos e procedimentos de estudo, protocolos operacionais e discussão dos resultados e suas implicações tiveram influência e foram desenvolvidos através do meu envolvimento com a Rede Brasileira, com seus colaboradores e demais projetos em vigência, assim como com outras atividades correlatas em que tive a oportunidade de participar nas linhas de pesquisa de Fisiologia, patologia e epidemiologia das condições obstétricas, perinatais e do ciclo-gravídico-puerperal e de Morbidade Materna Grave (NEAR MISS) e Mortalidade Materna. Durante o curso tive a oportunidade de participar: da consolidação e análise de consistência dos dados de projetos como o COMMAG (15), CIPHER (16); da proposição de análises secundárias do Fetal Growth Study (17), um estudo da Organização Mundial da Saúde, do grupo de trabalho em morbidade materna da OMS (Maternal Morbidity Working Group) (18–20); de reuniões dos programas de pós-graduação da área de Medicina III da Capes, desenvolvendo um tutorial para o preenchimento da Plataforma Sucupira (21), seguindo os preceitos específicos dos avaliadores e do documento de área e de dois artigos sobre as atividades de pesquisa do nosso programa de Tocoginecologia (2,22); do desenvolvimento de um tutorial prático para pesquisadores utilizarem o sistema Importa Fácil Ciência, programa prestado pelos Correios para a importação de itens de pesquisa (23); de realizar seis meses de interstício pelo programa de doutorado sanduíche no exterior financiado pela CAPES, realizado na Universidade de Leicester, Leicester, Reino Unido, desenvolvendo atividades correlatas ao projeto Preterm SAMBA e em outros projetos como o estudo CLIMB (*Complex Lipids In Mother and Babies*), participando de artigos ainda não publicados desse ensaio clínico de suplementação materna de gangliosídeos e do desenvolvimento de projeto de pesquisa e cursos, conforme relatório do orientador no exterior apresentado à

Capes (Anexo 4); da participação por um ano e meio no Programa de Estágio Docente (PED) da Faculdade de Ciências Médicas da Unicamp, em que desenvolvi atividades de ensino supervisionada com os alunos do quarto ano em unidade de atenção primária em saúde; da apresentação de aulas em congressos acadêmicos regionais e estaduais em Recife, Fortaleza, São Paulo; de reuniões com parceiros internacionais como a organizada pela Universidade de Harvard em Uganda para discussão de programas de *mentoring* entre pesquisadores e instituições e um *workshop* da Fundação Bill e Melinda Gates em Nova Déli, para qualificar pesquisadores na implementação de intervenções através da mudança de hábitos. Essa inserção foi muito importante, não só para o desenvolvimento dos projetos apresentados nessa tese, mas também para o desenvolvimento atividades em diferentes etapas do processo de pesquisa e obtenção de produtos que foram fundamentais para a minha qualificação. Esse envolvimento, relatado por Howard e Becker como o papel social do pesquisador na "história natural" da pesquisa e, principalmente, o processo de profissionalização da formação de pesquisadores, são fundamentais para a transposição de barreiras na pesquisa na área de saúde materna e perinatal, especialmente em temas complexos e que envolvem múltiplas abordagens metodológicas (24). Segundo o IOM, a dificuldade na formação/treinamento, na manutenção no mesmo campo de pesquisa, são alguns dos grandes desafios para avançar na pesquisa em prematuridade e suas consequências (24).

O Sistema Único de Saúde (SUS), serviço de acesso universal com princípios de equidade e resolutividade, organiza-se através de diferentes níveis de atenção (primário ao quaternário) para atender as demandas de tratamentos e promoção à saúde (25). Apesar de alguns estudos mostrarem que o Brasil tem assistência pré-natal com boa cobertura, infraestrutura e disponibilidade de serviços como vacinação, consultas e internação (26,27), ainda existem inequidades regionais ou até mesmo interinstitucionais relacionadas principalmente à qualidade do serviço e cuidados clínicos oferecidos (25,28,29). O programa Rede Cegonha, programa lançado pelo Ministério da Saúde em 2011 (30), foi instituído no âmbito do SUS visando garantir o acesso e qualidade a diversos serviços de saúde à mulher. Entretanto, exceto pela proposição de melhoria global ao atendimento pré-natal e do parto, nenhum planejamento

específico para identificação, prevenção ou atendimento ao parto prematuro foi proposto. Não necessariamente por negligência, mas por falta de novos programas com evidência científica de benefício na prevenção. Essa dificuldade e escassez de programas específicos é realidade também de países com outros contextos de assistência à saúde e de renda (alta ou baixa) (31,32).

O investimento em identificação de fatores de risco e modelos preditores se justifica, pois objetivam auxiliar a organização do sistema de saúde e desenvolvimento de intervenções preventivas mais eficazes. Nossos resultados na análise secundária dos dados do EMIP sobre ganho de peso e IMC inicial, por exemplo, não permitem recomendar restrição de ganho de peso a mulheres com categorias específicas de IMC ao início do pré-natal. Entretanto, mostra evidências para novos estudos que objetivem desenvolver intervenções correlatas, já que o IMC inicial e o ganho de peso podem ter diferentes impactos nos diferentes subtipos de prematuridade e, como fatores modificáveis, poderiam ser melhor explorados. Além disso, a análise de *cluster*, também dos dados do EMIP, demonstram que parcela significativa das mulheres com parto prematuro não apresenta condições predisponentes facilmente identificáveis, corroborando a necessidade de investirmos em outros marcadores e modelos de identificação que não clínico-epidemiológicos. Nossos resultados com o estudo em marcadores metabolômicos, por sua vez, traz resultados razoáveis quando mostra um modelo preditor com área de curva ROC acima de 0,70 para predição de parto prematuro espontâneo, por exemplo. Esplin e colaboradores (33) mostraram que a fibronectina fetal e medida do colo uterino por ultrassonografia transvaginal tiveram resultados inferiores na predição do parto prematuro espontâneo em nulíparas (área sob curva ROC de 0,67 [IC 95%, 0,64 - 0,70]). Entretanto, nossos resultados devem ser fruto de cautelosa interpretação, pois não parecem ser resultados de provável reprodutibilidade e validação, uma vez que foram identificados em apenas uma das subpopulações estudadas. De qualquer forma, a expertise adquirida e os marcadores identificados servirão de base para desenvolver novas análises, podendo-se explorar o biobanco contendo milhares de amostras de soro, plasma e cabelo de mulheres brasileiras para estudos de validação ou novas fases de identificação.

De forma geral, a literatura converge na ideia de que dificilmente um único marcador será capaz de compor um modelo preditor para prematuridade com boa performance. Apesar de ainda não bem elucidada, a fisiopatologia do parto prematuro espontâneo parece ser um processo dinâmico ao longo da gestação e que, por isso, estaria associado a múltiplos fatores que interagem por semanas ou dias, e talvez até por poucas horas, para desencadear o processo clínico de esvaecimento do colo, contrações e/ou ruptura de membranas ovulares. Os fatores envolvidos precocemente nesses múltiplos processos têm sido alvo dos estudos de potenciais marcadores.

A predição é um grande desafio do desenvolvimento tecnológico e a interpretação do conceito e método aplicados em diferentes áreas do conhecimento podem ser úteis no avanço das técnicas e abordagens, principalmente na de epidemiologia. Predizer ciclones tropicais, por exemplo, é de grande interesse para a sociedade, principalmente para que se minimizem as catástrofes causadas por esses fenômenos da natureza (34). Modelos complexos e avançados envolvendo ferramentas estatísticas são empregados para obter melhores resultados na predição desse evento, com especial interesse em predizê-lo o mais precocemente possível (35,36). Entretanto, as condições do tempo e suas variáveis envolvidas podem mudar (para melhor ou pior) e o modelo precisa levar em consideração não só essa mudança, mas também a probabilidade de que elas venham a ocorrer. Muitos anos de pesquisa coletando informações sobre essas variáveis como velocidade do vento, altitude, pressão atmosférica, umidade do ar, direção do vento, entre muitas outras, foram necessárias para entender o processo a ponto de produzir modelos preditores de utilidade prática. Algumas reflexões importantes a serem destacadas dessa analogia: um ciclone pode ocorrer com diferentes velocidades do vento, pressões atmosféricas ou graus de umidade do ar; o evento pode ocorrer com diferentes interações dessas variáveis, ou seja, menor grau de umidade do ar com maior pressão atmosférica ou vice e versa, a depender das condições das várias outras. Voltando ao parto prematuro espontâneo, ficam evidentes as limitações do uso de variáveis dicotômicas na avaliação de risco e predição, muito provavelmente porque elas não levam em consideração toda essa possível dinâmica. Nossos resultados mostraram que o parto prematuro espontâneo pode ocorrer havendo

ou não um histórico de parto prematuro anterior, episódios de sangramento na primeira metade da gestação, infecções genitourinárias, história de tabagismo, ou ainda colo curto ao ultrassom transvaginal no segundo trimestre, por exemplo. Provavelmente o risco individual muda na presença ou ausência de cada fator, diferentemente para cada interação. As limitações nos nossos resultados utilizando avaliações clínico-epidemiológicas vão ao encontro da teoria proposta por Menon (37) ou Behrman (38) e seus colaboradores: há que se levar em consideração as interações dos fatores genéticos, epigenéticos, metabólicos, sociais, ambientais, nutricionais, geográficos, comportamentais, étnicos, etc., na avaliação de risco ou predição do parto prematuro espontâneo.

Apesar das limitações na performance da medida do colo uterino na identificação de risco ou predição de parto prematuro, esse se confirma como um importante marcador clínico identificável durante a gestação. E, segundo nossos achados, também está associado a eventos adversos perinatais. Sua importância se dá, sobretudo porque é um dos únicos marcadores que possuem tal força de associação isoladamente e pode ser identificado em qualquer mulher durante o segundo trimestre de gestação. Faço aqui, então, essa importante ressalva para mostrar que há um papel importante da medida do colo uterino no rastreamento de parto prematuro. Há, de fato, algumas limitações na sua utilização prática que incluem o fato de o exame requerer profissional médico ultrassonografista com adequado treinamento das técnicas para avaliação transvaginal do colo uterino, que o exame seja realizado em intervalo específico na gestação (entre 18 a 24 semanas, preferencialmente) e de ainda não haver evidência sobre qual é sua validade em mulheres que foram submetidas a procedimentos cirúrgicos no colo uterino, como, por exemplo, cirurgia de alta frequência (CAF ou LEEP, *Loop electrosurgical excision procedure*). O que se observa na prática é que apesar das limitações e baixa acurácia na identificação de mulheres sob risco de parto prematuro, intervenções como o rastreamento de parto prematuro através da medida do colo durante o pré-natal são adotadas por serem o que “se têm em mãos”. Na ausência de outras boas alternativas, torna-se essa o centro das atenções. A Federação Brasileira das Associações de Ginecologia e Obstetrícia (FEBRASGO), por exemplo, recomenda em seus manuais de Manual de Gestação de Alto Risco (2010) e de Manual de Perinatologia (2013) que sejam

realizados, entre outras medidas, o rastreamento através da medição do colo uterino por ultrassom transvaginal e progesterona vaginal para aquelas com colo curto (39,40).

Entretanto, ainda temos que avançar no conhecimento científico acerca da utilização do rastreamento do colo uterino e medida preventivas correlatas aos achados do rastreamento (progesterona e/ou pessário, por exemplo), sobretudo sobre qual o ponto de corte para mulheres brasileiras, que outros marcadores podem ser associados ao modelo de rastreamento, qual a evidência em mulheres sem parto prematuro anterior, porque ainda temos falso negativos e positivos e o que podemos aprender com esses casos. Alguns autores, experts em prematuridade, alertam para o fato de termos, desde os anos 2000, mais de 790 publicações sobre o tópico colo curto e progesterona (41) e que precisamos avançar urgentemente em alternativas a essa proposta (41,42). Algumas estratégias poderiam encurtar o tempo na busca de evidência científica, com menor uso de recursos em novos ensaios clínicos. A Meta-análises com uso de dados individuais de pacientes (IPD meta-analysis, *individual patient data*), a rede de colaboração de pesquisadores e experts estabelecendo padronização de variáveis e a metanálise em rede (*Network meta-analsyis*) poderiam algumas das estratégias a serem considerada avançar nesse tópico A metanálise em rede é capaz de realizar comparações diretas e indiretas sobre a eficácia de diferentes intervenções em grupos e subgrupos de mulheres de diferentes ensaios clínicos (43,44).

A metabolômica mostra-se como uma das técnicas que é capaz de abordar vários desses aspectos, uma vez que, em teoria, avalia o resultado final dessas interações (45–47). Na avaliação de preditores metabolômicos para o parto prematuro espontâneo, utilizamos amostras provenientes de mulheres de duas subpopulações diferentes. Apesar de termos utilizado o mesmo protocolo de preparo e aquisição de dados no espectrômetro de massas acoplado à cromatografia gasosa, as análises das respectivas subpopulações foram realizadas por equipes e equipamentos diferentes. Além disso, a diferenciação de mulheres com parto prematuro devido a ruptura prematura de membranas ou trabalho de parto prematuro teria sido mais adequada. Não podemos excluir que isso possa ter interferido nos resultados.

Nossa metodologia incluiu a avaliação da razão dos mesmos metabólitos nos dois períodos de coleta no pré-natal. Nossa hipótese era que um aumento ou diminuição dos metabólitos entre a décima quinta e a vigésima semanas poderiam estar envolvidos no dinâmico desenvolvimento do parto prematuro. Entretanto, nenhuma razão de nenhum metabólito foi significativamente associada ao parto prematuro espontâneo. Não temos conhecimento de nenhum estudo publicado na literatura científica que tenha feito essa avaliação “dinâmica” utilizando marcadores metabolômicos. Em analogia, entretanto, há avaliações utilizando mudanças na medida do colo uterino ao longo da gestação, utilizando-se da diminuição em número absoluto (em centímetros ou milímetros) ou proporcional da medida do colo. Uma revisão sistemática incluindo 14 estudos na meta-análise mostra que essas mudanças não são marcadores úteis na predição de parto prematuro. A sensibilidade não ultrapassa 50%. Uma abordagem integrada incluindo as diferentes ciências ômicas (genômica, transcriptômica, protêomica ou metabolômica) possivelmente seja uma alternativa promissora na avaliação do desenvolvimento do parto prematuro e na busca de marcadores biológicos, apesar de complexa e dispendiosa (46).

O parto prematuro espontâneo e, sobretudo, terapêutico foram associados com maior incidência de desfechos maternos e perinatais adversos no estudo de coorte Preterm SAMBA. A taxa de pouco mais de 90% de cesariana nos casos de parto prematuro terapêutico, sendo em torno de 89% eletivas, alerta para a necessidade de uma reflexão sobre as indicações desse procedimento, ainda mais nesse grupo de mulheres – nulíparas de “baixo risco”. As décadas de 80 e 90 foram marcadas pelo aumento das taxas de cesárea e isso ocorreu por uma conjuntura de fatores: modelo de assistência ao parto adotado centrado no médico, desvalorização dos procedimentos médicos relacionados a obstetrícia, “terceirização” da construção do modelo de cuidado à mulher às seguradas de saúde que priorizaram a valorização ao parto cesárea, acesso a parto com analgesia e etc. Os efeitos das políticas públicas implementadas para reduzir a taxa de cesárea no Brasil, como o Pacto para Redução das Taxas de Cesarianas, o Programa de Humanização do Parto e, mais recentemente, o Parto Adequado ainda possuem resultados limitados (48,49).

A associação de pré-eclâmpsia e fetos pequenos para idade gestacional/restritos com prematuridade terapêutica já haviam sido reportados pelo estudo EMIP (50). Complicações maternas e/ou fetais são, por conceito, fatores motivadores da prematuridade terapêutica. Alerta-se, contudo, para o fato de ambos os estudos em prematuridade (EMIP e Preterm SAMBA) terem demonstrados que esses recém-nascidos por prematuridade terapêutica apresentam com mais frequência desfechos perinatais adversos, incluindo maior mortalidade neonatal. Decidir pelo parto prematuro terapêutico é uma medida que visa melhorar os desfechos maternos e perinatais. Para isso, uma decisão baseada em evidência científica de que os riscos em manter a gestação superam os riscos relacionados à intervenção, seja porque se ocasiona prematuridade terapêutica ou seja porque, ainda, a grande maioria das mulheres são submetidas a cesariana. Não só as maternidades e outros serviços de atendimento de referência obstétrica devem possuir médicos e equipe multiprofissional adequadamente treinada e suficiente para atender casos que envolvam complicações obstétricas, mas o sistema de saúde deve funcionar de maneira apropriadamente adequada para regular os casos de maneira a evitar sobrecarga ou falta de vagas. O estudo da Rede de Vigilância Materna Grave, desenvolvido pela Unicamp em 27 serviços de obstetrícia do país em 2009-2010, mostrou que as demoras relacionadas ao atendimento de complicações maternas contribuem para desfechos maternos ainda mais graves (51). As desigualdades de renda, educação e acesso ao cuidado à saúde ainda são algumas das grandes barreiras para a mudança desse cenário da saúde materna e perinatal no Brasil (26,52–54).

6. CONCLUSÃO

Objetivo 1: Avaliar a associação do índice de massa corpórea e ganho de peso gestacional com os diferentes subtipos de parto prematuro e com desfechos perinatais.

A taxa de ganho de peso por semana durante a gestação foi associada com risco de parto prematuro a depender do IMC inicial e da categoria de ganho de peso, se excessivo ou insuficiente. Esse efeito foi diferente para os diferentes subtipos de parto prematuro (parto prematuro espontâneo, RPPMO e terapêutico. Anormalidades na taxa de ganho de peso conforme as recomendações do IOM-2009, categorizado em insuficiente ou excessivo, estão associados com desfechos perinatais adversos. A taxa de ganho de peso, um fator de risco modificável e de fácil aferição durante o pré-natal, já controlada pelo IMC inicial, pode ser uma variável estratégica para ser melhor abordada na prevenção de risco de parto prematuro e eventos adversos em grupos de maior risco.

Objetivo 2: Identificar fenótipos maternos relacionados ao parto prematuro e suas respectivas condições associadas, características maternas e desfechos maternos e perinatais.

A análise por *cluster* dos partos prematuros identificou grupos de mulheres caracterizadas por diferentes condições. Essa abordagem por agrupamento por *cluster* demonstrou que uma parcela significativa de mulheres não possui nenhuma das condições pré-definidas potencialmente associadas com parto prematuro e confirma a associação de pré-eclâmpsia, eclampsia e síndrome HELLP com parto prematuro terapêutico. A investigação de outras condições relacionadas à prematuridade pode melhorar a identificação de mulheres com maior risco de parto prematuro durante o pré-natal e daquelas que terão pior desfecho perinatal.

Objetivo 3: Realizar uma revisão narrativa sobre a aplicação da ciência ômica nos estudos em saúde materna e perinatal, com enfoque na metabolômica e na predição de complicações.

A metabolômica parece ser uma abordagem promissora na investigação de marcadores de predição e na elucidação da fisiopatologia de doenças na área de

saúde materna e perinatal, cuja interação entre metabolismo materno, fetal, placentário e ambiental torna essa investigação desafiadora nas práticas de pesquisa tradicionais, baseadas em hipóteses. Entretanto, validações externas dos resultados e reprodutibilidade são os maiores desafios para que essa técnica seja mais amplamente empregada.

Objetivo 4: Desenvolver um método padronizado para revisar sistematicamente os estudos em predição de prematuridade espontânea através de marcadores metabolômicos.

Essa revisão sistemática verificará a disponibilidade de estudos em predição de parto prematuro utilizando técnicas metabolômicas, buscando evidência científica baseada em estudos com adequado padrão metodológico e que utilizaram definição do desfecho e técnicas padronizadas.

Objetivo 5: Desenvolver o método e procedimentos utilizados em um estudo multicêntrico para investigar a predição de parto prematuro e outras complicações maternas e perinatais.

A adoção de metodologia e técnicas padronizadas são fundamentais para o desenvolvimento do estudo que aborda marcadores biológicos para parto prematuro. A utilização de desfechos com definições precisas e detalhadas, da coleta de dados e amostras seguindo padrões internacionais de boas práticas clínicas, de frequente controle de qualidade e monitoramento dos dados é crucial para atingi os objetivos do estudo. O planejamento inicial, desenvolvendo os métodos e procedimentos, foi uma das partes fundamentais do estudo que objetiva avaliar preditores metabolômicos para parto prematuro e diversas outras abordagens epidemiológicas relacionadas à saúde materna e perinatal.

Objetivo 6: Implementar e desenvolver um estudo para predição de parto prematuro e outras complicações maternas e perinatais, fornecendo subsídios metodológicos e descrevendo detalhes sobre os aspectos práticos dessa implementação e estratégias para a solução de dificuldades encontradas.

O planejamento, implementação e desenvolvimento de um estudo multicêntrico desenhado para abordar diversos desfechos maternos e perinatais em 5 maternidades de referência em 3 regiões diferentes no Brasil foi uma tarefa complexa, com grandes desafios e muitos aprendizados a todos os envolvidos. A organização profissionalizada da Rede e os comprometimentos institucionais de cada centro participante envolvido foram fundamentais para todas as etapas do estudo. A valorização do desenvolvimento de um estudo robusto como esse, no contexto da Rede Brasileira de Estudos em Saúde Reprodutiva e Perinatal, é fundamental para a manutenção de pessoal qualificado e de aproveitamento de recursos tecnológicos e científicos inestimáveis que foram construídos (como o biobanco, por exemplo). A experiência da Rede, através da narrativa apresentada, pode auxiliar no planejamento e desenvolvimento de outros estudos similares em contextos semelhantes.

Objetivo 7: Identificar um conjunto de marcadores metabolômicos, clínicos e/ou sociodemográficos preditivos de parto prematuro.

A combinação de marcadores metabolômicos coletados no sangue materno às 20 semanas de gestação com variáveis clínicas compuseram um modelo preditor com boa performance discriminatória para parto prematuro espontâneo, tanto para partos abaixo de 37 semanas quanto para abaixo de 34 semanas, em mulheres nulíparas de baixo risco. A não identificação de marcadores metabolômicos na outra população estudada seguindo similar abordagem indica que os metabólitos podem ser específicos da população de Cork, na Irlanda. Apesar da boa performance demonstrada pelo modelo desenvolvido em um dos subgrupos do estudo, os achados acarretam mais questionamentos: Provavelmente essa é uma etapa de um processo ainda não concluído. Novas fases de identificação e outras de validação, utilizando outras coortes e populações, serão necessárias para uma contribuição consistente na predição e mecanismos envolvidos no parto prematuro espontâneo.

Objetivo 8: Avaliar a incidência e potenciais fatores de risco associados à ocorrência de parto prematuro espontâneo.

O parto prematuro espontâneo e terapêutico são eventos comuns e sua identificação através de fatores de risco clínico e epidemiológicos é limitada. A medida do colo uterino por ultrassonografia transvaginal confirma-se como um marcador clínico importante. A investigação de prematuridade através de subgrupos fenotípicos de mulheres também se mostra uma estratégia potencialmente útil na identificação de grupos de maior risco, incluindo o rastreamento do uso de álcool. Em ambos os casos, contudo, as estratégias de prevenção devem ser melhor exploradas.

Objetivo 9: Avaliar a ocorrência de desfechos perinatais adversos associados à prematuridade.

Parto prematuro espontâneo e, sobretudo, terapêutico foram associados com maior incidência de desfechos adversos perinatais em mulheres nulíparas de baixo risco. Recém-nascidos cujos partos ocorreram em intervalos de idade gestacional próximas ao termo (prematuridade tardia e termo precoce) representam mais de um terço dos casos e apresentaram desfechos perinatais adversos em maior incidência que os nascidos no termo tardio. Adequado cuidado pré-natal e de assistência ao parto, intervenções obstétricas baseadas em evidência e avanços na prevenção da prematuridade devem ser prioridade na área de saúde materna e perinatal para melhorar os resultados perinatais relacionados ao parto prematuro.

7. REFERÊNCIAS

1. Souza RT, Galvao RBF, Leite DFB, Passini Jr R, Baker PN, Cecatti JG. The use of metabolomics for predicting spontaneous preterm birth in asymptomatic pregnant women: protocol for a systematic review and meta-analysis. PROSPERO: International prospective register of systematic reviews. [Internet]. 2018 [cited 2018 Oct 9]. Available from: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=100172

2. Cecatti JG, Silveira C, Souza RT, Fernandes KG, Surita FG. Experience with the Brazilian Network for Studies in Reproductive and Perinatal Health: the power of collaboration in postgraduate programs. Rev Col Bras Cir. 2015;42(suppl 1):89–93.

3. Passini R, Tedesco RP, Marba ST, Cecatti JG, Guinsburg R, Martinez FE, et al. Brazilian multicenter study on prevalence of preterm birth and associated factors. BMC Pregnancy Childbirth. 2010 Jan;10(1):22.

4. Passini R, Cecatti JG, Lajos GJ, Tedesco RP, Nomura ML, Dias TZ, et al. Brazilian Multicentre Study on Preterm Birth (EMIP): Prevalence and Factors Associated with Spontaneous Preterm Birth. PLoS One. 2014 Jan;9(10):e109069.

5. Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, Lipp R. New Ballard Score, expanded to include extremely premature infants. J Pediatr. 1991 Sep 1;119(3):417–23.

6. McCowan L, North RA, Taylor R. Screening for pregnancy endpoints: preeclampsia, growth restricted baby and spontaneous preterm birth. Actrn12607000551493. Australia New Zealand Clinical Trial Registry.2007. https://www.anzctr.org.au/Trial/Registration/Tri.

7. Kenny LC, Black MA, Poston L, Taylor R, Myers JE, Baker PN, et al. Early pregnancy prediction of preeclampsia in nulliparous women, combining clinical risk and biomarkers: the Screening for Pregnancy Endpoints (SCOPE) international cohort study. Hypertension. 2014 Sep;64(3):644–52.

8. Bick D. Born too soon: the global issue of preterm birth. Midwifery. 2012 Aug;28(4):341–2.

9. Blencowe H, Cousens S, Chou D, Oestergaard M, Say L, Moller A-B, et al. Born Too Soon: The global epidemiology of 15 million preterm births. Reprod Health. 2013 Nov 15;10(Suppl 1):S2.

10. Ministério da Saúde. Brasil [Internet]. SINASC - Sistema de Informações sobre Nascidos Vivos. Available from: http://tabnet.datasus.gov.br/cgi/deftohtm.exe?sinasc/cnv/nvuf.def

11. Tedesco RP, Passini R, Cecatti JG, Camargo RS, Pacagnella RC, Sousa MH. Estimation of preterm birth rate, associated factors and maternal morbidity from a demographic and health survey in Brazil. Matern Child Health J. 2013 Nov;17(9):1638–47.

12. Cecatti JG, Souza RT, Sulek K, Costa ML, Kenny LC, McCowan LM, et al. Use of metabolomics for the identification and validation of clinical biomarkers for preterm birth: Preterm SAMBA. BMC Pregnancy Childbirth. 2016 Aug;16(1):212.

13. Cicourel A. Desvendando Máscaras Sociais. In: Guimarães AZ, editor. Desvendando Máscaras Sociais. Rio de Janeiro: Francisco Alves Editora; 1975. p. 87–121.

14. Becker H. Inferência e prova na observação participante; evidências de trabalho de campo. In: Métodos de Pesquisa nas Ciências Sociais. 4ª ed. São Paulo: Hucitec; 1997. p. 47–99.

15. Pacagnella RC, Cecatti JG, Camargo RP, Silveira C, Zanardi DT, Souza JP, et al. Rationale for a long-term evaluation of the consequences of potentially life-threatening maternal conditions and maternal "near-miss" incidents using a multidimensional approach. J Obstet Gynaecol Can. 2010 Aug;32(8):730–8.

16. Oliveira-Neto AF, Parpinelli MA, Costa ML, Souza RT, Ribeiro do Valle C, Sousa MH, et al. Prediction of Severe Maternal Outcome Among Pregnant and Puerperal Women in Obstetric ICU. Crit Care Med. 2018 Nov 12;1.

17. Kiserud T, Piaggio G, Carroli G, Widmer M, Carvalho J, Neerup Jensen L, et al. The World Health Organization Fetal Growth Charts: A Multinational Longitudinal Study of Ultrasound Biometric Measurements and Estimated Fetal Weight. Myers JE, editor. PLOS Med. 2017 Jan 24;14(1):e1002220.

18. Mayrink J, Souza RT, Silveira C, Guida JP, Costa ML, Parpinelli MA, et al. Reference ranges of the WHO Disability Assessment Schedule (WHODAS 2.0) score and diagnostic validity of its 12-item version in identifying altered functioning in healthy postpartum women. Int J Gynaecol Obstet. 2018 May;141 Suppl 1:48–54.

19. Silveira C, Souza RT, Costa ML, Parpinelli MA, Pacagnella RC, Ferreira EC, et al. Validation of the WHO Disability Assessment Schedule (WHODAS 2.0) 12-item tool against the 36-item version for measuring functioning and disability associated with pregnancy and history of severe maternal morbidity. Int J Gynaecol Obstet. 2018 May;141 Suppl 1:39–47.

20. Guida JP, Costa ML, Parpinelli MA, Pacagnella RC, Ferreira EC, Mayrink J, et al. The impact of hypertension, hemorrhage, and other maternal morbidities on functioning in the postpartum period as assessed by the WHODAS 2.0 36-item tool. Int J Gynaecol Obstet. 2018 May;141 Suppl 1:55–60.

21. Souza RT, Cecatti JG, Surita FG, Souza MCF, Ferreira LM, Calderon I de M, et al. Tutorial Prático da Plataforma Sucupira. Versão 1.2 [Internet]. Ministério da Educação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). 2016 [cited 2018 Nov 30]. p. 1–61. Available from: https://www.fcm.unicamp.br/fcm/pos-graduacao-em-tocoginecologia/tutoriais

22. Cecatti JG, Fernandes KG, Souza RT, Silveira C, Surita FG. STRATEGIES IN SEARCHING HOMOGENEITY IN A FACULTY OF A POSTGRADUATE PROGRAM. Rev Col Bras Cir. 2015;42(suppl 1):83–6.

23. Souza RT. Manual Prático – Importa Fácil Ciência, do pesquisador para o pesquisador... Versão 1.0 [Internet]. Programa de Pós-Graduação em Tocoginecologia. Faculdade de Ciências Médicas. Universidade Estadual de Campinas. 2016 [cited 2018 Nov 30]. p. 1–43. Available from: https://www.fcm.unicamp.br/fcm/pos-graduacao-em-tocoginecologia/tutoriais

24. Behrman R, Butler A, editors. Barriers to Clinical Research on Preterm Birth and Outcomes for Preterm Infants. In: Preterm Birth: Causes, Consequences, and Prevention. Washington (DC): Institute of Medicine (US) Committee on Understanding Premature Birth and Assuring Healthy Outcomes. National Academies Press (US); 2007.

25. Paim J, Travassos C, Almeida C, Bahia L, Macinko J. The Brazilian health system: history, advances, and challenges. Lancet. 2011 May 21;377(9779):1778–97.

26. Viellas EF, Domingues RMSM, Dias MAB, Gama SGN da, Theme Filha MM, Costa JV da, et al. Prenatal care in Brazil. Cad Saude Publica. 2014 Aug;30:S85–100.

27. Domingues RMSM, Viellas EF, Dias MAB, Torres JA, Theme-Filha MM, Gama SGN da, et al. Adequacy of prenatal care according to maternal characteristics in Brazil. Rev Panam Salud Pública. 2015;37(3):140–7.

28. Tomasi E, Fernandes PAA, Fischer T, Siqueira FCV, Silveira DS da, Thumé E, et al. [Quality of prenatal services in primary healthcare in Brazil: indicators and social inequalities]. Cad Saude Publica. 2017 Apr 3;33(3):e00195815.

29. Guimarães WSG, Parente RCP, Guimarães TLF, Garnelo L. [Access to prenatal care and quality of care in the Family Health Strategy: infrastructure, care, and management]. Cad Saude Publica. 2018;34(5):e00110417.

30. Ministério da Saúde. Secretaria de Atenção à Saúde. Departamento de Ações Programáticas Estratégicas. Portaria nº 1.459, 24 de junho de 2011. Institui, no âmbito do Sistema Único de Saúde, a Rede Cegonha. Diário Oficial da União, Brasília, 2011e. Seção 1. [Internet]. [cited 2018 Nov 26]. Available from: http://bvsms.saude.gov.br/bvs/saudelegis/gm/2011/prt1459_24_06_2011.html

31. Dean S V, Mason E, Howson CP, Lassi ZS, Imam AM, Bhutta ZA. Born Too Soon: Care before and between pregnancy to prevent preterm births: from evidence to action. Reprod Health. 2013 Jan;10 Suppl 1(Suppl 1):S3.

32. Requejo J, Merialdi M, Althabe F, Keller M, Katz J, Menon R. Born Too Soon: Care during pregnancy and childbirth to reduce preterm deliveries and improve health outcomes of the preterm baby. Reprod Health. 2013 Jan;10 Suppl 1(Suppl 1):S4.

33. Esplin MS, Elovitz MA, Iams JD, Parker CB, Wapner RJ, Grobman WA, et al. Predictive Accuracy of Serial Transvaginal Cervical Lengths and Quantitative Vaginal Fetal Fibronectin Levels for Spontaneous Preterm Birth Among Nulliparous Women. JAMA. 2017 Mar 14;317(10):1047.

34. Emanuel K. Tropical cyclones. Annu Rev Earth Planet Sci. 2003 May 28;31(1):75–104.

35. Wu C-C, Chou K-H, Wang Y, Kuo Y-H, Wu C-C, Chou K-H, et al. Tropical Cyclone Initialization and Prediction Based on Four-Dimensional Variational Data Assimilation. J Atmos Sci. 2006 Sep 1;63(9):2383–95.

36. Yu H, Chen P, Li Q, Tang B, Yu H, Chen P, et al. Current Capability of Operational Numerical Models in Predicting Tropical Cyclone Intensity in the Western North Pacific. Weather Forecast. 2013 Apr 12;28(2):353–67.

37. Menon R. Oxidative stress damage as a detrimental factor in preterm birth pathology. Front Immunol. 2014;5(NOV).

38. Behrman R, ButlerAS, editors. Institute of Medicine (IOM). Preterm Birth: Causes, Consequences, and Prevention. Washington, D.C.: National Academies Press; 2007.

39. Nascimento DJ, editor. Manual de Gestação de Alto Risco. Federação Brasileira das Associações de Ginecologia e Obstetrícia; 2011. 220 p.

40. da Fonseca EB, editor. Manual de Perinatologia. São Paulo: Federação Brasileira das

Associações de Ginecologia e Obstetrícia; 2013. 118 p.

41. Norman JE, Bennett P. Preterm birth prevention—Time to PROGRESS beyond progesterone. PLOS Med. 2017 Sep 26;14(9):e1002391.

42. Kyvernitakis I, Maul H, Bahlmann F. Controversies about the Secondary Prevention of Spontaneous Preterm Birth. Geburtshilfe Frauenheilkd. 2018 Jun;78(6):585–95.

43. Santos E, Ferreira R, Marques A. How to Perform and Interpret a Network Meta-Analysis for Indirect and Mixed Comparisons: Key Methodological Strategies. Rev Enferm Ref. 2016 Mar 29;IV Série(Nº8):133–40.

44. Rouse B, Chaimani A, Li T. Network meta-analysis: an introduction for clinicians. Intern Emerg Med. 2017 Feb 2;12(1):103–11.

45. Dessì A, Marincola FC, Fanos V. Metabolomics and the great obstetrical syndromes - GDM, PET, and IUGR. Best Pract Res Clin Obstet Gynaecol. 2014 Aug 21;29(2):156–64.

46. Horgan RP, Clancy OH, Myers JE, Baker PN. An overview of proteomic and metabolomic technologies and their application to pregnancy research. BJOG. 2009 Jan;116(2):173–81.

47. Romero R, Espinoza J, Gotsch F, Kusanovic J, Friel L, Erez O, et al. The use of high-dimensional biology (genomics, transcriptomics, proteomics, and metabolomics) to understand the preterm parturition syndrome. BJOG An Int J Obstet Gynaecol. 2006 Dec 19;113:118–35.

48. Victora CG, Aquino EML, do Carmo Leal M, Monteiro CA, Barros FC, Szwarcwald CL. Maternal and child health in Brazil: progress and challenges. Lancet (London, England). 2011 May 28;377(9780):1863–76.

49. Occhi GM, de Lamare Franco Netto T, Neri MA, Rodrigues EAB, de Lourdes Vieira Fernandes A. Strategic measures to reduce the caesarean section rate in Brazil. Lancet. 2018 Oct 13;392(10155):1290–1.

50. Souza RT, Cecatti JG, Passini R, Tedesco RP, Lajos GJ, Nomura ML, et al. The Burden of Provider-Initiated Preterm Birth and Associated Factors: Evidence from the Brazilian Multicenter Study on Preterm Birth (EMIP). PLoS One. 2016 Jan 2;11(2):e0148244.

51. Pacagnella RC, Cecatti JG, Parpinelli MA, Sousa MH, Haddad SM, Costa ML, et al. Delays in receiving obstetric care and poor maternal outcomes: results from a national multicentre cross-sectional study. BMC Pregnancy Childbirth. 2014 Jan;14(1):159.

52. Ministério da Saúde. Pesquisa Nacional de Demografia e Saúde da Criança e da Mulher – PNDS 2006: dimensões do processo reprodutivo e da saúde da criança. Brasília: Ministério da Saúde; 2009.

53. de Azevedo Bittencourt SD, da Costa Reis LG, Ramos MM, Rattner D, Rodrigues PL, Neves DCO, et al. Structure in Brazilian maternity hospitals: key characteristics for quality of obstetric and neonatal care. Cad Saude Publica. 2014 Aug;30 Suppl 1:S1-12.

54. Kleinert S, Horton R. Brazil: towards sustainability and equity in health. Lancet (London, England). 2011 May 21;377(9779):1721–2.

8. Anexo 1 – Formulário de coleta de dados EMIP

emip

Coleta encerrada □ Checado □ Digitado □

INVESTIGAÇÃO EM PREMATURIDADE

UNICAMP

NOME: _____ REGISTRO HOSPITALAR □□□□□□□

ENDEREÇO: _____ TEL: (____) _____

MUNICÍPIO: _____ CEP: □□□□□-□□□ ESTADO: _____

IDENTIFICAÇÃO: (Faça um círculo sobre as variáveis anotadas ou preencha o espaço reservado)

1. INSTITUIÇÃO □□	2. CASO □□□□	3. CONTROLE □□□□

4. IDADE (anos): _____; NÃO SABE
5. ZONA: RURAL; URBANA; NÃO SABE
6. COR DA PELE: BRANCA; PARDA; PRETA; AMARELA; OUTRA
7. ESTADO CIVIL: CASADA; SOLTEIRA; UNIÃO MARITAL; SEPARADA; VIÚVA
8. ESCOLARIDADE (anos): _____; NÃO SABE

CONDIÇÕES SOCIODEMOGRÁFICAS: (Faça um círculo sobre as variáveis anotadas ou preencha o espaço reservado)

1. RENDA FAMILIAR (R$): NENHUMA; ATÉ 500; 501-1.000; 1.001-2.000; > 2.000; NÃO SABE
2. VOCÊ FAZ ALGUM TRABALHO REMUNERADO? SIM; NÃO (passe para questão 10)
3. TRABALHO REMUNERADO DURANTE A GRAVIDEZ? SIM; NÃO (passe para questão 9)
4. SE SIM, ATÉ QUE MÊS DA GESTAÇÃO? _____; NÃO TRABALHOU
5. SEU ESFORÇO FÍSICO NO TRABALHO REMUNERADO DURANTE A GRAVIDEZ FOI INTENSO? SIM; NÃO; ÀS VEZES
6. SEU TRABALHO DURANTE A GESTAÇÃO FOI NA MAIOR PARTE FEITO EM PÉ? SIM; NÃO; ÀS VEZES
7. QUANTAS HORAS POR DIA A SRA. TRABALHA NO SEU EMPREGO? _____; NÃO SABE
8. EXERCEU TRABALHO NOTURNO NA GRAVIDEZ? SIM; NÃO; ÀS VEZES
9. VOCÊ É A PRINCIPAL FONTE DE RENDA DA CASA? SIM; NÃO; NÃO SABE
10. VOCÊ REALIZA O TRABALHO DOMÉSTICO NA SUA CASA? SIM TOTALMENTE; SIM COM AJUDA; NÃO
11. MORA EM DOMICÍLIO PRÓPRIO? SIM; NÃO; MORADORA DE RUA (passe para questão 16)
12. MORA EM RUA PAVIMENTADA? SIM; NÃO; NÃO SABE
13. ONDE VOCÊ MORA EXISTE ÁGUA ENCANADA? SIM; NÃO; NÃO SABE
14. EXISTE REDE DE ESGOTO? SIM; NÃO; NÃO SABE
15. QUANTAS PESSOAS MORAM COM VOCÊ? _____
16. Nº DE FILHOS COM MENOS DE 5 ANOS? (COM EXCEÇÃO DO ATUAL): NÃO TEM; 1; 2; 3 OU MAIS
17. IDADE DO SEU FILHO MAIS NOVO (anos completos)? NÃO TEM OUTROS FILHOS; ATÉ 1; 2; 3 a 5; > 5

DADOS PÔNDERO-ESTATURAIS: (Preencha o espaço reservado)

1. QUAL É O SEU PESO HABITUAL (Kg) (anotar o da 1ª consulta, desde que < 20 sem)? _____;	NÃO LEMBRA
2. QUAL É A SUA ESTATURA (m) (medir a paciente)? _____;	NÃO LEMBRA
3. PESO NO FINAL DA GESTAÇÃO (Kg) (anotar o da última consulta ou da internação): _____;	NÃO CONSTA

(Anotação de cartão/prontuário) (Deixar em branco, pois serão calculados durante a digitação dos dados)

4. GANHO DE PESO NA GESTAÇÃO (Kg): □□	5. IMC INÍCIO DA GESTAÇÃO: □□	6. IMC FINAL DA GESTAÇÃO: □□

ANTECEDENTES OBSTÉTRICOS: (Faça um círculo sobre as variáveis anotadas ou preencha o espaço reservado)

1. NÚMERO DE GESTAÇÕES: _____;	NÃO LEMBRA
2. NÚMERO DE PARTOS VAGINAIS (sem contar o atual): _____;	NÃO LEMBRA
3. NÚMERO DE CESÁREAS (sem contar a atual): _____;	NÃO LEMBRA
4. NÚMERO DE ABORTOS: _____;	NÃO LEMBRA
5. TEVE QUE FAZER QUANTAS CURETAGENS UTERINAS? _____;	NÃO LEMBRA
6. O ÚLTIMO PARTO OU ABORTO FOI HÁ QUANTO TEMPO (meses)? PRIMIGESTA; ATÉ 12; 13 – 24; 25 – 36; > 36;	NÃO SABE
7. FOI SUBMETIDA À CIRCLAGEM DO COLO DO ÚTERO? SIM; NÃO;	NÃO SABE
8. Nº PARTOS OCORRIDOS COM MENOS DE 9 MESES (sem contar o atual)? 0; 1; 2; 3 OU MAIS;	NÃO SABE
9. Nº PARTOS GEMELARES OCORRIDOS COM MENOS DE 9 MESES (em gestações anteriores)? 0; 1; 2; 3 OU MAIS;	NÃO SABE
10. PRECISOU FICAR INTERNADA POR CONTRAÇÕES ANTES DOS 9 MESES EM GESTAÇÕES ANTERIORES? SIM; NÃO;	NÃO SABE
11. EM OUTRA GRAVIDEZ SUA BOLSA ROMPEU ANTES DOS 9 MESES? SIM; NÃO;	NÃO SABE
12. ALGUM PARTO TEVE QUE SER FEITO ANTES DOS 9 MESES, POR ALGUM PROBLEMA NA SUA SAÚDE OU DO BEBÊ? SIM; NÃO; NÃO SABE	
13. ALGUM FILHO PESOU MENOS DE DOIS QUILOS E MEIO QUANDO NASCEU? SIM; NÃO;	NÃO SABE

DOENÇAS MATERNAS PRÉVIAS À GRAVIDEZ ATUAL: (Faça um círculo sobre as variáveis anotadas)

1. ANTES DA GRAVIDEZ VOCÊ TINHA QUAL OU QUAIS DESTAS DOENÇAS? (pode assinalar mais de uma)

PRESSÃO ALTA	DIABETES	DOENÇA DA TIREÓIDE	DOENÇA DO CORAÇÃO
DOENÇA DOS PULMÕES	DOENÇA DOS RINS	DOENÇA APARELHO DIGESTIVO	DOENÇA DO SANGUE
DOENÇA NEUROLÓGICA	DOENÇA PSIQUIÁTRICA	NENHUMA	HIV
OUTRAS			

2. SE OUTRA (S), QUAL (IS) DOENÇA? _____

GESTAÇÃO ATUAL: (Faça um círculo sobre as variáveis anotadas ou preencha o espaço reservado)

1. ONDE FEZ O PRÉ-NATAL DESTA GESTAÇÃO? (pode assinalar mais de uma) UBS; HOSPITAL; SERVIÇO PRIVADO; OUTRO; NÃO FEZ (Neste caso, passe para a questão 8)	
2. FOI ATENDIDA POR QUEM NO PRÉ-NATAL? (pode assinalar mais de uma) MÉDICO; ENFERMEIRO; OUTRO	
3. EM QUE MÊS INICIOU O PRÉ-NATAL? _____;	NÃO LEMBRA
4. MÊS DE INÍCIO: (anotação de cartão/prontuário) _____;	NÃO CONSTA
5. QUAL FOI O NÚMERO DE CONSULTAS REALIZADAS DURANTE O SEU PRÉ-NATAL? _____;	NÃO LEMBRA
6. Nº CONSULTAS REALIZADAS DURANTE O PRÉ-NATAL: (anotação de cartão/prontuário) _____;	NÃO CONSTA
7. FOI REALIZADA ULTRA-SONOGRAFIA DURANTE O SEU PRÉ-NATAL? SIM; NÃO;	NÃO LEMBRA
8. VOCÊ FEZ ESFORÇO FÍSICO INTENSO NA GRAVIDEZ? POUCAS VEZES; MUITAS VEZES; NÃO FEZ;	NÃO LEMBRA
9. ACHA QUE TEVE PERÍODOS DE DEPRESSÃO NESTA GESTAÇÃO? POUCAS VEZES; MUITAS VEZES; NÃO TEVE;	NÃO LEMBRA
10. TEVE PERÍODOS DE MUITA ANSIEDADE NA GRAVIDEZ? POUCAS VEZES; MUITAS VEZES; NÃO TEVE;	NÃO LEMBRA
11. SE VOCÊ FUMOU NA GRAVIDEZ, QUANTOS CIGARROS, EM MÉDIA, FUMOU POR DIA? _____;	NÃO FUMOU
12. SE VOCÊ FUMOU NA GESTAÇÃO, ATÉ QUE MÊS FUMOU? _____; NÃO FUMOU NA GESTAÇÃO;	NUNCA FUMOU
13. ALGUÉM QUE MORA COM VOCÊ FUMA? SIM; NÃO;	NÃO SABE
14. VOCÊ TOMOU BEBIDA ALCOÓLICA NA GRAVIDEZ? POUCAS VEZES; MUITAS VEZES; NÃO TOMOU;	NÃO LEMBRA
15. EM RELAÇÃO AO USO DE DROGAS: NUNCA USOU; USAVA ANTES DA GRAVIDEZ E PAROU; USOU DURANTE A GRAVIDEZ	
16. USOU QUAIS DROGAS NA GRAVIDEZ? (pode anotar mais que uma) NÃO USOU; MACONHA; COCAÍNA; CRACK; ECSTASY; ANFETAMINA; OUTRA	
17. TEVE QUE TRATAR CORRIMENTO VAGINAL NESTA GRAVIDEZ? SIM; NÃO;	NÃO LEMBRA
18. VULVOVAGINITE NA GESTAÇÃO: (anotação de cartão/prontuário, pode assinalar mais de uma) VAGINOSE; CANDIDÍASE; TRICOMONÍASE; OUTRA; NÃO;	NÃO CONSTA

19. TRATAMENTO DE VULVOVAGINITE: (anotação de cartão/prontuário) NÃO TEVE VULVOVAGINITE; SIM; NÃO; NÃO CONSTA
20. TEVE QUE TRATAR INFECÇÃO URINÁRIA NESTA GESTAÇÃO? SIM, 1 VEZ ; SIM, MAIS DE 1 VEZ; NÃO; NÃO LEMBRA
21. INFECÇÃO URINÁRIA NA GESTAÇÃO: (anotação cartão/prontuário) SIM; NÃO; NÃO CONSTA
22. QUAL? (anotação de cartão/prontuário, pode assinalar mais de uma) NÃO TEVE; BACTERIÚRIA ASSINTOMÁTICA; CISTITE; PIELONEFRITE; NÃO CONSTA
23. TRATAMENTO DE INFECÇÃO URINÁRIA? (anotação cartão/prontuário) SIM; NÃO; NÃO TEVE INFECÇÃO; NÃO CONSTA
24. TEVE INFLAMAÇÃO OU INFECÇÃO NOS DENTES OU GENGIVA NA GRAVIDEZ ATUAL? SIM; NÃO; NÃO LEMBRA
25. TEVE OUTRA INFECÇÃO DURANTE ESTA GESTAÇÃO ALÉM DAS QUE FORAM PERGUNTADAS? SIM; NÃO; NÃO LEMBRA
26. SE SIM, QUAL FOI? (pode assinalar mais de uma) NÃO TEVE; FEBRE SEM CAUSA APARENTE; DIARRÉIA FEBRIL; HIV; PNEUMONIA; TUBERCULOSE; SINUSITE/AMIGDALITE; HEPATITE; HERPES GENITAL; TOXOPLASMOSE; OUTRA
27. TEVE ANEMIA NESTA GRAVIDEZ? SIM; NÃO; NÃO SABE
28. TOMOU REMÉDIO PARA TRATAMENTO OU PREVENÇÃO DE ANEMIA NA GRAVIDEZ? SIM; NÃO; NÃO SABE
29. TEVE SANGRAMENTO PELA VAGINA DURANTE A GESTAÇÃO? SIM; NÃO; NÃO SABE
30. EM QUAL ÉPOCA DA GESTAÇÃO? (pode assinalar mais de uma) NÃO SANGROU; ENTRE O 1º E 3º MÊS; DO 4º ao 6º MÊS; DO 7º ao 9º MÊS; NÃO SABE
31. PRECISOU FICAR INTERNADA NESTA GRAVIDEZ, SEM CONTAR COM ESTA INTERNAÇÃO ATUAL? SIM; NÃO; NÃO SABE
32. SE SIM, QUAL O MOTIVO? (pode assinalar mais de uma) NÃO FICOU INTERNADA; NÁUSEAS E VÔMITOS; CONTRAÇÃO ANTES DO TEMPO; PERDA DE LÍQUIDO; SANGRAMENTO PELA VAGINA; DOENÇA MATERNA; COMPLICAÇÃO DO BEBÊ; OUTRA; NÃO LEMBRA

(Anotações de prontuário):

33. SÍFILIS NESTA GESTAÇÃO: NÃO; SEM SOROLOGIA; SIM-TRATADA ADEQUADAMENTE; SIM-TRATADA INADEQUADAMENTE; SIM-TRATAMENTO NÃO ESPECIFICADO
34. OUTRA INFECÇÃO NA GESTAÇÃO: (pode assinalar mais de uma) NÃO TEVE; FEBRE SEM CAUSA APARENTE; HIV; HEPATITE; HPV; DIARRÉIA FEBRIL; PNEUMONIA; TUBERCULOSE; SINUSITE/AMIGDALITE; HERPES GENITAL; TOXOPLASMOSE; OUTRA
35. ANEMIA NESTA GESTAÇÃO: SIM; NÃO; NÃO CONSTA
36. QUAL O MENOR VALOR DE HEMOGLOBINA ENCONTRADO: _____; NÃO CONSTA
37. TRATAMENTO PARA ANEMIA? SIM; NÃO; NÃO CONSTA
38. COLO UTERINO CURTO (< 25 mm) AO ULTRA-SOM TRANSVAGINAL: SIM; NÃO; NÃO FEZ US TRANSVAGINAL; NÃO CONSTA (Se não houver colo curto, passe para a questão 41)
39. IDADE GESTACIONAL DO DIAGNÓSTICO DE COLO CURTO (semanas): _____
40. QUAL A MENOR MEDIDA CERVICAL ENCONTRADA (mm): _____
41. SUSPEITA CLÍNICA E/OU ULTRA-SONOGRÁFICA DE INSUFICIÊNCIA CERVICAL: SIM; NÃO; NÃO CONSTA
42. CIRCLAGEM: SIM-NESTA GESTAÇÃO; SIM-PRÉ-GESTACIONAL; NÃO; NÃO CONSTA (Se NÃO/NÃO CONSTA, passe para 44)
43. IDADE GESTACIONAL EM QUE FOI FEITA A CIRCLAGEM (semanas): _____;
44. MALFORMAÇÃO UTERINA DIAGNOSTICADA POR ULTRA-SOM OU OUTRO EXAME: NÃO; UNICORNO; BICORNO; SEPTADO; OUTRAS MALFORMAÇÕES; NÃO CONSTA
45. MIOMA UTERINO DIAGNOSTICADO POR ULTRA-SOM OU OUTRO EXAME DE IMAGEM: SIM; NÃO; NÃO CONSTA (se não houver, passe para a questão 49)
46. QUAL O NÚMERO DE MIOMAS: _____ NÃO CONSTA
47. QUAL O TAMANHO DO MAIOR MIOMA (mm): _____ NÃO CONSTA
48. LOCALIZAÇÃO DOS MIOMAS: (pode assinalar mais de uma) SUBMUCOSO; INTRAMURAL; SUBSEROSO; NÃO CONSTA
49. SANGRAMENTO VAGINAL DURANTE A GESTAÇÃO: (pode assinalar mais de uma) NÃO TEVE; AMEAÇA DE ABORTAMENTO; PLACENTA PRÉVIA; DESCOLAMENTO DE PLACENTA; OUTRO; NÃO CONSTA
50. ALTERAÇÃO DO VOLUME DE LÍQUIDO AMNIÓTICO: NÃO; OLIGOÂMNIO; POLIHIDRÂMNIO; NÃO CONSTA

51. MORBIDADE MATERNA CRÔNICA OU INTERCORRENTE: (pode assinalar mais de uma) NENHUMA; DIABETES; HIV; HIPERTENSÃO GESTACIONAL; PRÉ-ECLÂMPSIA/ECLÂMPSIA/HELLP; HIPERTENSÃO CRÔNICA; OUTRA INFECÇÃO CRÔNICA; HIPO/HIPERTIREOIDISMO; NEFROPATIA; ANEMIA FALCIFORME; OUTRA ANEMIA CRÔNICA; CARDIOPATIA; PNEUMOPATIA; EPILEPSIA; LÚPUS ERITEMATOSO SISTÊMICO; OUTRA COLAGENOSE; DOENÇA DO TUBO DIGESTIVO; CIRURGIA BARIÁTRICA; DOENÇA PSIQUIÁTRICA; OUTRA DOENÇA NEUROLÓGICA; DOENÇA ORTOPÉDICA; NEOPLASIA MALIGNA; TROMBOSE OU TROMBOFILIA; OUTRA; NÃO CONSTA
52. MORBIDADE FETAL: (pode assinalar mais de uma) MALFORMAÇÃO; RCIU; OUTRA; NÃO; NÃO CONSTA

GESTAÇÃO MÚLTIPLA (Se a gestação não foi múltipla, passe para a próxima seção)

1. SUA GESTAÇÃO FOI DE QUANTOS BEBÊS? 2; 3, 4 OU MAIS
2. VOCÊ TEVE QUE SE SUBMETER A ALGUM TRATAMENTO PARA ENGRAVIDAR? SIM; NÃO

(Anotações de prontuário):

3. EM RELAÇÃO AO NÚMERO DE PLACENTAS, A GESTAÇÃO FOI: MONOCORIÔNICA; DICORIÔNICA; OUTRA; NÃO CONSTA
4. QUANTO AO Nº DE BOLSAS AMNIÓTICAS, A GESTAÇÃO FOI: MONOAMNIÓTICA; DIAMNIÓTICA; OUTRA; NÃO CONSTA
5. DIAGNÓSTICO ULTRA-SONOGRÁFICO DE TRANSFUSÃO FETO-FETAL: SIM; NÃO; NÃO CONSTA

CONDIÇÕES CAUSAIS DE PARTO PRÉ-TERMO (Se o parto não foi PRÉ-TERMO, passe para próxima seção)

1. O SEU TRABALHO DE PARTO TEVE INÍCIO ESPONTÂNEO? SIM; NÃO; NÃO SABE REFERIR
2. A BOLSA SE ROMPEU ANTES DO TRABALHO DE PARTO COMEÇAR? SIM; NÃO; NÃO SABE REFERIR
3. O SEU PARTO TEVE QUE SER FEITO ANTES DA HORA POR ALGUM PROBLEMA COM VOCÊ OU COM O BEBÊ? SIM-COMIGO; SIM-COM BEBÊ; SIM-COM OS DOIS; NÃO; NÃO SABE REFERIR

(Anotações de prontuário):

4. TRABALHO DE PARTO PRÉ-TERMO ESPONTÂNEO: SIM; NÃO; NÃO CONSTA
5. RUPTURA PREMATURA PRÉ-TERMO DE MEMBRANAS: SIM; NÃO; NÃO CONSTA
6. PARTO PREMATURO TERAPÊUTICO OU ELETIVO: SIM; NÃO; NÃO CONSTA

DADOS DE PARTO (Somente anotações de prontuário)

1. FORMA DE INÍCIO DO TRABALHO DE PARTO: ESPONTÂNEA; PARTO INDUZIDO; CESÁREA ELETIVA
2. FOI ADMINISTRADO ANTIBIÓTICO DURANTE O TRABALHO DE PARTO: SIM; NÃO; NÃO CONSTA
3. MOTIVO DO USO DE ANTIBIÓTICO: NÃO USOU; FEBRE; CULTURA POSITIVA PARA EGB; OUTRO FATOR DE RISCO PARA EGB; OUTRO MOTIVO; NÃO CONSTA
4. FOI REALIZADA ANALGESIA NO TRABALHO DE PARTO COM MÉTODOS FARMACOLÓGICOS: (pode assinalar mais de uma) NÃO; PERIDURAL; COMBINADA; RAQUIDIANA; MEPERIDINA, TRAMADOL, BENZODIAZEPÍNICOS; ANTI-ESPASMÓDICOS; ANALGÉSICOS POR VIA ORAL; OUTROS; NÃO CONSTA
5. QUAL FOI A FORMA DE PARTO: VAGINAL NORMAL; FÓRCIPE/VÁCUO; CESÁREA; VAGINAL+CESÁREA (se CESÁREA, passe para a questão 8)
6. FOI REALIZADA EPISIOTOMIA? SIM; NÃO; NÃO CONSTA
7. SE UTILIZOU FÓRCIPE/VÁCUO NO PARTO, QUAL FOI A INDICAÇÃO? NÃO UTILIZOU; ALÍVIO; ROTAÇÃO; PÉLVICO; OUTRA; NÃO CONSTA (se PARTO VAGINAL, passe para a próxima seção)

8. QUAL FOI A INDICAÇÃO DA CESÁREA? (pode assinalar mais que uma): SOFRIMENTO FETAL AGUDO/CRÔNICO; DCP; ITERATIVIDADE; PÉLVICO OU OUTRA APRESENTAÇÃO ANÔMALA; DISTÓCIA FUNCIONAL; DISTÓCIA DE PARTES MOLES; DIABETES; FALHA DE INDUÇÃO; HIPERTENSÃO ARTERIAL; CARDIOPATIA; HIV; PLACENTA PRÉVIA; DPP; RUPTURA UTERINA; MALFORMAÇÃO FETAL; MACROSSOMIA FETAL; OPÇÃO MATERNA; OUTRA; NÃO CONSTA
9. TIPO DE INCISÃO UTERINA REALIZADA? SEGMENTAR TRANSVERSA; SEGMENTO CORPORAL; CORPORAL; NÃO CONSTA

DADOS DO RECÉM-NASCIDO (Somente anotações de prontuário)

1. IDADE GESTACIONAL CALCULADA AO NASCIMENTO (em semanas): _____ (em gemelar, anotar a do primeiro RN)
2. MÉTODO UTILIZADO PARA DETERMINAR A IDADE GESTACIONAL: DATA DA ÚLTIMA MENSTRUAÇÃO; ULTRA-SONOGRAFIA; NEW BALLARD
3. PESO AO NASCIMENTO (em gramas): _____; NÃO CONSTA (Se gravidez única passe para a questão 7)
4. PESO DO 2º GEMELAR (em gramas): _____; NÃO CONSTA
5. PESO DO 3º GEMELAR: (em gramas) _____; NÃO CONSTA
6. PESO DO 4º GEMELAR (em gramas): _____; NÃO CONSTA
7. ÍNDICE DE APGAR DO 1º MINUTO: _____; NÃO CONSTA . (Se gravidez única passe para a questão 11)
8. ÍNDICE DE APGAR DO 1º MINUTO DO 2º GEMELAR: _____; NÃO CONSTA
9. ÍNDICE DE APGAR DO 1º MINUTO DO 3º GEMELAR: _____; NÃO CONSTA
10. ÍNDICE DE APGAR DO 1º MINUTO DO 4º GEMELAR: _____; NÃO CONSTA
11. ÍNDICE DE APGAR DO QUINTO MINUTO: _____; NÃO CONSTA . (Se gravidez única passe para a questão 15)
12. ÍNDICE DE APGAR DO QUINTO MINUTO DO 2º GEMELAR: _____; NÃO CONSTA
13. ÍNDICE DE APGAR DO QUINTO MINUTO DO 3º GEMELAR: _____; NÃO CONSTA
14. ÍNDICE DE APGAR DO QUINTO MINUTO DO 4º GEMELAR: _____; NÃO CONSTA
15. PERÍMETRO CEFÁLICO (cm): _____; NÃO CONSTA (Se gravidez única passe para a questão 19)
16. PERÍMETRO CEFÁLICO DO 2º GEMELAR (cm): _____; NÃO CONSTA
17. PERÍMETRO CEFÁLICO DO 3º GEMELAR (cm): _____; NÃO CONSTA
18. PERÍMETRO CEFÁLICO DO 4º GEMELAR (cm): _____; NÃO CONSTA
19. COMPRIMENTO (cm): _____; NÃO CONSTA (Se gravidez única passe para a questão 23)
20. COMPRIMENTO DO 2º GEMELAR (cm): _____; NÃO CONSTA
21. COMPRIMENTO DO 3º GEMELAR (cm): _____; NÃO CONSTA
22. COMPRIMENTO DO 4º GEMELAR (cm): _____; NÃO CONSTA

MORBIDADE E MORTALIDADE NEONATAIS
(Somente anotações de prontuário. Se gemelar, somar todas as intercorrências positivas e anotá-las)

23. ÓBITO FETAL (se gemelar, pode haver mais de uma opção): NÃO; SIM-NA ADMISSÃO; SIM-APÓS A ADMISSÃO (Se SIM, passe para a próxima seção se for CASO, ou encerre o questionário se for CONTROLE)
24. pH DE CORDÃO UMBILICAL AO NASCIMENTO: _____; NÃO CONSTA
25. NECESSIDADE DE INTUBAÇÃO OROTRAQUEAL AO NASCIMENTO: SIM; NÃO; NÃO CONSTA
26. UTILIZAÇÃO DE SURFACTANTE: SIM; NÃO; NÃO HÁ SURFACTANTE NO HOSPITAL; NÃO CONSTA
27. MALFORMAÇÃO DIAGNOSTICADA AO NASCIMENTO: SIM; NÃO; NÃO CONSTA (Se NÃO/NÃO CONSTA, passe para 29)
28. QUAL MALFORMAÇÃO: _____
29. TEMPO DE INTERNAÇÃO EM UTI NEONATAL (dias): _____; NÃO CONSTA
30. QUANTOS DIAS FICOU INTERNADO NO TOTAL (número de dias completos): _____; NÃO CONSTA
31. NECESSIDADE DE SUPORTE VENTILATÓRIO: SIM; NÃO; NÃO CONSTA
32. MORBIDADE NEONATAL: SIM; NÃO; NÃO CONSTA (Se NÃO/NÃO CONSTA, passe para a questão 53)

33. SEPSE NEONATAL: SIM-CLÍNICO; SIM-HEMOCULTURA; NÃO; NÃO CONSTA (Se NÃO/NÃO CONSTA, passe para questão 35)	
34. IDADE DO NEONATO POR OCASIÃO DO DIAGNÓSTICO DA SEPSE: _____;	NÃO CONSTA
35. DESCONFORTO RESPIRATÓRIO: SIM; NÃO;	NÃO CONSTA
36. ESCAPE DE AR: SIM; NÃO;	NÃO CONSTA
37. GRAU DE HEMORRAGIA CEREBRAL: _____; (anotar "0" se não teve hemorragia)	NÃO CONSTA
38. HEMORRAGIA PUMONAR: SIM; NÃO;	NÃO CONSTA
39. DISFUNÇÃO HEMATOLÓGICA: SIM; NÃO;	NÃO CONSTA
40. DISUNÇÃO ENDÓCRINA: SIM; NÃO;	NÃO CONSTA
41. DISFUNÇÃO RENAL: SIM; NÃO;	NÃO CONSTA
42. DISFUNÇÃO IMUNOLÓGICA: SIM; NÃO;	NÃO CONSTA
43. MORBIDADE MÚSCULO-ESQUELÉTICA: SIM; NÃO;	NÃO CONSTA
44. DISFUNÇÃO GASTROINTESTINAL/FALÊNCIA HEPÁTICA: SIM; NÃO;	NÃO CONSTA
45. HIPOVOLEMIA: SIM; NÃO;	NÃO CONSTA
46. ENTEROCOLITE NECROSANTE: SIM; NÃO;	NÃO CONSTA
47. CONVULSÃO/USO DE ANTI-CONVULSIVANTES: SIM; NÃO;	NÃO CONSTA
48. USO DE AMINAS VASOATIVAS: SIM; NÃO;	NÃO CONSTA
49. PNEUMONIA: SIM; NÃO;	NÃO CONSTA
50. OXIGENIOTERAPIA COM 28 DIAS: SIM; NÃO;	NÃO CONSTA
51. OXIGENIOTERAPIA COM 56 DIAS: SIM; NÃO;	NÃO CONSTA
52. GRAU DE RETINOPATIA DA PREMATURIDADE: _____;	NÃO CONSTA
53. CONDIÇÃO DO NEONATO NA ALTA/TRANSFERÊNCIA: VIVO; ÓBITO INTRAPARTO; ÓBITO NEONATAL; NÃO TEVE ALTA (Se VIVO, passe para a próxima seção)	
54. IDADE DO NEONATO POR OCASIÃO DO ÓBITO (dias): _____;	NÃO CONSTA

- Se o caso for de trabalho de parto prematuro espontâneo, passe para questão 1 da FOLHA A
- Se o caso for ruptura prematura de membranas em gestação pré-termo, passe para questão 1 da FOLHA B
- Se o caso for parto prematuro terapêutico ou eletivo, passe para questão 1 da FOLHA C

- SE ESTA FOI UMA GESTAÇÃO DE TERMO, ENCERRE AQUI O QUESTIONÁRIO

PARTE ESPECÍFICA (Somente Para os Casos)

FOLHA A: TRABALHO DE PARTO PRÉ-TERMO ESPONTÂNEO (TPP)

EPISÓDIO DE TPP NESTA GRAVIDEZ, ANTES DO EPISÓDIO ATUAL

1. FICOU INTERNADA ALGUMA VEZ NESTA GRAVIDEZ POR TRABALHO DE PARTO ANTES DO TEMPO, SEM SER A INTERNAÇÃO ATUAL? SIM; NÃO; NÃO LEMBRA (Se NÃO/NÃO LEMBRA, passe para as anotações de prontuário - questão 9)
2. DEPOIS DA ALTA HOSPITALAR, ONDE FEZ O PRÉ-NATAL? (pode assinalar mais de uma) POSTO SAÚDE; HOSPITAL; PRIVADO; OUTRO; NÃO FEZ
3. DEPOIS DA ALTA HOSPITALAR, RECEBEU ORIENTAÇÃO PARA FAZER USO DE PROGESTERONA? SIM; NÃO; NÃO LEMBRA
4. DEPOIS DA ALTA VOCÊ FICOU DE REPOUSO? SIM; NÃO; NÃO LEMBRA
5. DEPOIS DA ALTA VOCÊ TEVE RELAÇÕES SEXUAIS? DO MESMO JEITO; AUMENTOU; DIMINUIU; NÃO TEVE; NÃO LEMBRA
6. FOI AFASTADA DO TRABALHO DEPOIS DA ALTA? SIM PARCIALMENTE; SIM TOTALMENTE; NÃO FOI; NÃO TRABALHA
7. USOU DE MEDICAMENTOS EM CASA PARA NÃO TER CONTRAÇÃO? SIM; NÃO; NÃO LEMBRA
8. TOMOU INJEÇÃO PARA "AMADURECER" O PULMÃO DO BEBÊ DEPOIS DA ALTA? SIM; NÃO; NÃO SABE

(Anotações de prontuário):

9. HÁ INFORMAÇÃO NO PRONTUÁRIO SOBRE O EPISÓDIO DE TPP ANTERIOR: SIM; NÃO (Se NÃO passe para a questão 16)
10. FOI UTILIZADO TOCOLÍTICO TERAPÊUTICO NAQUELE EPISÓDIO: (pode assinalar mais de uma) NÃO; BETA-AGONISTA; INIBIDOR DA SÍNTESE DE PROSTAGLANDINA; BLOQUEADOR DE CANAL DE CÁLCIO; ANTAGONISTA DA OCITOCINA; OUTRO; NÃO CONSTA
11. FOI UTILIZADO CORTICOSTERÓIDE PARA MATURAÇÃO PULMONAR FETAL NAQUELE EPISÓDIO: SIM; NÃO; NÃO CONSTA
12. FOI UTILIZADO ANTIBIOTICOTERAPIA PROFILÁTICA NAQUELE EPISÓDIO: SIM; NÃO; NÃO CONSTA
13. USO DE PROGESTERONA APÓS O EPISÓDIO: SIM; NÃO; NÃO CONSTA
14. USO DE TOCOLÍTICOS PARA USO EM CASA APÓS O EPISÓDIO: (pode assinalar mais de uma) NÃO USOU; BETA-AGONISTA; INIBIDOR DA SÍNTESE DE PROSTAGLANDINA; BLOQUEADOR DE CANAL DE CÁLCIO; OUTRO; NÃO CONSTA
15. USO DE CORTICOSTERÓIDE PARA USO FORA DO HOSPITAL APÓS O EPISÓDIO: SIM; NÃO; NÃO CONSTA

CONDIÇÃO OBSTÉTRICA DA GESTANTE NO MOMENTO DA INTERNAÇÃO ATUAL

(Anotações de prontuário):

16. Nº DE CONTRAÇÕES UTERINAS NO MOMENTO DA INTERNAÇÃO ATUAL (em dez minutos): _____; NÃO CONSTA
17. DURAÇÃO MÉDIA DAS CONTRAÇÕES (em segundos): _____; NÃO CONSTA
18. DILATAÇÃO CERVICAL NO MOMENTO DA INTERNAÇÃO ATUAL (em centímetros): _____; NÃO CONSTA
19. GRAU DE ESVAECIMENTO NO MOMENTO DA INTERNAÇÃO ATUAL (em porcentagem): _____; NÃO CONSTA
20. ÍNDICE DE BISHOP NO MOMENTO DA INTERNAÇÃO ATUAL: < 6; 6-8; > 8; NÃO CONSTA
21. INTEGRIDADE DAS MEMBRANAS NO MOMENTO DA INTERNAÇÃO: ÍNTEGRAS (passe para 23); ROTAS; NÃO CONSTA
22. TEMPO ENTRE O INÍCIO DO TPP ATUAL E A RUPTURA DE MEMBRANAS: (Nº de horas completas) _____; NÃO CONSTA
23. HAVIA SANGRAMENTO VAGINAL NO MOMENTO DA INTERNAÇÃO ATUAL; SIM; NÃO; NÃO CONSTA

USO DE CORTICOSTERÓIDES PARA INDUÇÃO DE MATURIDADE PULMONAR FETAL

(Anotações de prontuário):

24. HOUVE UTILIZAÇÃO DE CORTICOSTERÓIDE PARA A INDUÇÃO DE MATURIDADE PULMONAR FETAL DURANTE A INTERNAÇÃO ATUAL: SIM; NÃO; NÃO CONSTA (Se NÃO/NÃO CONSTA, passe para questão 31)
25. QUAL FOI O MEDICAMENTO UTILIZADO: BETAMETASONA; DEXAMETASONA; OUTRO; NÃO CONSTA
26. QUAL FOI A VIA DE ADMINISTRAÇÃO UTILIZADA: VO; IV; IM; NÃO CONSTA
27. DOSE TOTAL ADMINISTRADA (some, anote o total prescrito e administrado até o parto, em mg): _____; NÃO CONSTA
28. Nº DE DOSES ADMINISTRADAS (some e anote quantas vezes foi administrado até o parto): _____; NÃO CONSTA
29. QUAL FOI O INTERVALO ENTRE AS DOSES (em horas):: _____; NÃO CONSTA
30. EM QUE IDADE GESTACIONAL FOI UTILIZADO (episódio atual, em semanas): _____; NÃO CONSTA

USO DE AGENTES TOCOLÍTICOS

(Anotações de prontuário):

31. UTILIZAÇÃO DE TOCOLÍTICOS NA INTERNAÇÃO ATUAL: SIM; NÃO; NÃO CONSTA (Se NÃO/NÃO CONSTA, passe para 39)
32. MEDICAÇÃO UTILIZADA INICIALMENTE: BETA-AGONISTA; INIBIDOR DA SÍNTESE DE PROSTAGLANDINA; BLOQUEADOR DE CANAL DE CÁLCIO; ANTAGONISTA DA OCITOCINA; OUTRO; NÃO CONSTA
33. VIA DE ADMINISTRAÇÃO UTILIZADA: VO; IV; IM; SC; NÃO CONSTA
34. QUAL FOI O TEMPO DE UTILIZAÇÃO (em horas): _____; NÃO CONSTA
35. HOUVE ASSOCIAÇÃO DE TOCOLÍTICOS COMO PRIMEIRA OPÇÃO: SIM; NÃO; NÃO CONSTA
36. QUAL FOI A ASSOCIAÇÃO? NÃO ASSOCIOU; BETA-AGONISTA; INIBIDOR DA SÍNTESE DE PROSTAGLANDINA; BLOQUEADOR DE CANAL DE CÁLCIO; ANTAGONISTA DA OCITOCINA; OUTRO; NÃO CONSTA
37. HOUVE NECESSIDADE DE TROCA DE TOCOLÍTICO POR FALHA TERAPÊUTICA: SIM; NÃO; NÃO CONSTA
38. QUAL FOI O OUTRO TOCOLÍTICO UTILIZADO: NÃO USOU; BETA-AGONISTA; INIBIDOR DA SÍNTESE DE PROSTAGLANDINA; BLOQUEADOR DE CANAL DE CÁLCIO; ANTAGONISTA DA OCITOCINA; OUTRO; NÃO CONSTA
39. USO DE SULFATO DE MAGNÉSIO COMO NEUROPROTETOR FETAL: SIM; NÃO; NÃO CONSTA

USO DE ANTIBIÓTICOS

(Anotações de prontuário):

40. UTILIZAÇÃO DE ANTIBIÓTICO NA INTERNAÇÃO ATUAL: SIM; NÃO; NÃO CONSTA (Se NÃO/NÃO CONSTA, passe para 48)
41. QUAL FOI A MEDICAÇÃO UTILIZADA: _____
42. VIA DE ADMINISTRAÇÃO UTILIZADA: VO; IV; IM; NÃO CONSTA
43. QUAL FOI O TEMPO DE UTILIZAÇÃO (em dias): _____; NÃO CONSTA
44. ASSOCIAÇÃO DE ANTIBIÓTICO COMO PRIMEIRA OPÇÃO: SIM; NÃO; NÃO CONSTA (Se NÃO/NÃO CONSTA, passe para 46)
45. SE SIM, QUAL FOI A ASSOCIAÇÃO: _____
46. UTILIZAÇÃO DE OUTRO ANTIB. POR FALHA TERAPÊUTICA: SIM; NÃO; NÃO CONSTA (Se NÃO/NÃO CONSTA, passe para 48)
47. SE SIM, QUAL FOI O OUTRO ANTIBIÓTICO UTILIZADO: _____

OUTRAS INFORMAÇÕES

(Anotações de prontuário):

48. FOI REALIZADA PESQUISA PARA ESTREPTOCOCO DO GRUPO B: NÃO-NÃO É ROTINA; NÃO REALIZADA-É ROTINA; SIM-COM CULTURA SIMPLES; SIM-COM CULTURA SELETIVA; SIM-COM PCR; NÃO CONSTA
49. FORAM UTILIZADAS OUTRAS MEDICAÇÕES, ALÉM DAS AVALIADAS, NESTA INTERNAÇÃO: SIM; NÃO; NÃO CONSTA

COMPLICAÇÕES MATERNAS/FETAIS DURANTE O TRATAMENTO DO TPP

(Anotações de prontuário):

50. COMPLICAÇÕES OCORRIDAS: (pode assinalar mais de uma) NENHUMA DETECTADA; HEMORRAGIA GENITAL; CORIOAMNIONITE; SEPSE MATERNA; EFEITO COLATERAL GRAVE MATERNO/FETAL COM A MEDICAÇÃO UTILIZADA; NECESSIDADE DE INTERROMPER O TOCOLÍTICO DEVIDO A EFEITO COLATERAL; DESCOMPENSAÇÃO CARDÍACA MATERNA; OLIGOÂMNIO; OUTRA; ÓBITO MATERNO; NÃO CONSTA

PARTE ESPECÍFICA (Somente Para os Casos)

FOLHA B: RUPTURA PREMATURA DE MEMBRANAS NO PRÉ-TERMO

ÉPOCA DA OCORRÊNCIA E MÉTODO DE DIAGNÓSTICO

(Anotações de prontuário):	
1. IDADE GESTACIONAL EM QUE OCORREU A RUPTURA (semanas): _____;	NÃO CONSTA
2. TEMPO TRANSCORRIDO ENTRE A RUPTURA E O DIAGNÓSTICO (em horas): _____;	NÃO CONSTA
3. O DIAGNÓSTICO DA RUPTURA DAS MEMBRANAS FOI FEITO NESTE HOSPITAL: SIM; NÃO;	NÃO CONSTA
4. COMO FOI FEITO O DIAGNÓSTICO: (pode anotar mais de um) HISTÓRIA CLÍNICA; VISUALIZAÇÃO DE SAÍDA DE LA; CRISTALIZAÇÃO EM LÂMINA; pH VAGINAL; PROVA DO FORRO; ULTRA-SOM; OUTRO;	NÃO CONSTA
5. ALTERAÇÃO DO VOLUME DE LÍQUIDO AMNIÓTICO PELO ULTRA-SOM: VOLUME NORMAL; OLIGOAMNIO ACENTUADO; OLIGOAMNIO; POLIHIDRAMNIO; SEM ULTRA-SOM;	NÃO CONSTA
6. FOI REALIZADO ALGUM PROCEDIMENTO INVASIVO: (pode ser mais de um) NÃO; BIÓPSIA DE VILO CORIAL; AMNIOCENTESE; CORDOCENTESE; TRANSFUSÃO FETAL; DERIVAÇÃO FETAL; FETOSCOPIA; OUTRO;	NÃO CONSTA

CONDUTA ADOTADA NA MESMA GESTANTE, SE JÁ ESTEVE INTERNADA POR RUPTURA DE MEMBRANAS NESTA GESTAÇÃO

7. VOCÊ JÁ ESTEVE INTERNADA NESTA GRAVIDEZ, ANTES DA INTERNAÇÃO ATUAL, PORQUE A BOLSA ROMPEU? SIM-NESTE HOSPITAL; SIM-EM OUTRO HOSPITAL; SIM-EM OUTRO HOSPITAL E NESTE; A BOLSA ROMPEU E NÃO FIQUEI INTERNADA; ROMPEU SÓ AGORA (neste caso, passar para questão 25)	
8. FEZ ACOMPANHAMENTO PRÉ-NATAL DEPOIS QUE TEVE ALTA DO HOSPITAL COM A BOLSA ROTA? (pode anotar mais de uma) NÃO TEVE ALTA; NÃO FEZ PRÉ-NATAL; FEZ NO POSTO; FEZ EM HOSPITAL; FEZ PARTICULAR; FEZ EM OUTRO LOCAL	
9. RECEBEU ALGUMA ORIENTAÇÃO DEPOIS QUE TEVE ALTA DO HOSPITAL COM A BOLSA ROTA? SIM; NÃO;	NÃO LEMBRA
10. RECEBEU ORIENTAÇÃO PARA FICAR EM REPOUSO? SIM; NÃO;	NÃO LEMBRA
11. NÃO TER RELAÇÃO SEXUAL? SIM; NÃO;	NÃO LEMBRA
12. AFASTAMENTO DO TRABALHO? SIM; NÃO;	NÃO LEMBRA
13. TRATAMENTO DE CORRIMENTO? SIM; NÃO;	NÃO LEMBRA
14. AUMENTO DA INGESTÃO DE LÍQUIDO? SIM; NÃO;	NÃO LEMBRA
15. TOMAR INJEÇÃO PARA "AMADURECER" O PULMÃO DO BEBÊ? SIM; NÃO;	NÃO LEMBRA
16. TOMAR REMÉDIO PARA NÃO DEIXAR NASCER O BEBÊ? SIM; NÃO;	NÃO LEMBRA

(Anotações de prontuário):	
17. TRATAMENTO INICIAL PROPOSTO NO MOMENTO DA RUPTURA: INTERRUPÇÃO IMEDIATA DA GESTAÇÃO (passe para questão 59); CONDUTA CONSERVADORA	
18. TRATAMENTO DE VULVOVAGINITES APÓS A RUPTURA: SIM; NÃO;	NÃO CONSTA
19. USO DE ANTIESPASMÓDICO APÓS A RUPTURA: SIM; NÃO;	NÃO CONSTA
20. PROGESTERONA APÓS A RUPTURA: SIM; NÃO;	NÃO CONSTA
21. HIPERHIDRATAÇÃO APÓS A RUPTURA: SIM; NÃO;	NÃO CONSTA
22. CORTICOSTERÓIDE APÓS A RUPTURA: SIM; NÃO;	NÃO CONSTA
23. ANTIBIÓTICO APÓS A RUPTURA: SIM; NÃO; NÃO CONSTA (Se NÃO/NÃO CONSTA, passe para 26)	
24. QUAL A INDICAÇÃO DO ANTIBIÓTICO APÓS A RUPTURA: PROFILAXIA DE INFECÇÃO POR EGB; PROLONGAMENTO DA GESTAÇÃO; CORIOAMNIONITE; INFECÇÃO URINÁRIA; OUTRA;	NÃO CONSTA
25. QUAL ANTIBIÓTICO FOI UTILIZADO: (pode ser mais de um) _____	
26. TOCOLÍTICO APÓS A RUPTURA: SIM; NÃO;	NÃO CONSTA

HIPERHIDRATAÇÃO MATERNA

(Anotações de prontuário):	
27. SOLUÇÃO UTILIZADA: NÃO UTILIZOU; SORO FISIOLÓGICO; SORO GLICOSADO; RINGER; OUTRA; (Se NÃO USOU/NÃO CONSTA, passe para 31)	NÃO CONSTA
28. VOLUME POR 24 HORAS (ml): _____;	NÃO CONSTA
29. VIA DE ADMINISTRAÇÃO: VO; IV; AMBOS;	NÃO CONSTA
30. TEMPO DE ADMINISTRAÇÃO (dias): _____;	NÃO CONSTA

USO DE CORTICOSTERÓIDES PARA INDUÇÃO DE MATURIDADE PULMONAR FETAL

31. HOUVE UTILIZAÇÃO DE CORTICOSTERÓIDE PARA A INDUÇÃO DE MATURIDADE PULMONAR FETAL DURANTE A INTERNAÇÃO ATUAL: SIM; NÃO; NÃO CONSTA (Se NÃO/NÃO CONSTA, passe para a questão 41)
32. QUAL FOI O MEDICAMENTO UTILIZADO: BETAMETASONA; DEXAMETASONA; OUTRO; NÃO CONSTA
33. QUAL FOI A VIA DE ADMINISTRAÇÃO UTILIZADA: VO; IV; IM; NÃO CONSTA
34. DOSE TOTAL ADMINISTRADA (some, anote o total prescrito e administrado até o parto, em mg): _____; NÃO CONSTA
35. Nº DE DOSES ADMINISTRADAS (some e anote quantas vezes foi administrado até o parto): _____; NÃO CONSTA
36. QUAL FOI O INTERVALO ENTRE AS DOSES (em horas): _____; NÃO CONSTA
37. HOUVE USO DESTA MEDICAÇÃO ANTERIORMENTE NA GESTAÇÃO: SIM; NÃO; NÃO CONSTA
38. APÓS A RUPTURA, EM QUAL IDADE GESTACIONAL FOI FEITA A 1ª UTILZAÇÃO (em semanas): _____; NÃO CONSTA
39. FORAM REALIZADAS DOSES SEMANAIS APÓS A RUPTURA: SIM; NÃO; NÃO CONSTA
40. FOI REALIZADA ALGUMA OUTRA DOSE EM INTERVALO MAIOR QUE 15 DIAS: SIM; NÃO; NÃO CONSTA

USO DE AGENTES TOCOLÍTICOS

41. UTILIZAÇÃO DE TOCOLÍTICOS NA INTERNAÇÃO ATUAL: SIM; NÃO; NÃO CONSTA (Se NÃO/NÃO CONSTA, passe para 50)
42. MEDICAÇÃO UTILIZADA INICIALMENTE: INIBIDOR DA SÍNTESE DE PROSTAGLANDINA; BLOQUEADOR DE CANAL DE CÁLCIO; BETA-AGONISTA; ANTAGONISTA DA OCITOCINA; OUTRO
43. VIA DE ADMINISTRAÇÃO UTILIZADA: VO; IV; IM; NÃO CONSTA
44. MOTIVO DA UTILIZAÇÃO: PARA PREVENIR CONTRAÇÕES; DEVIDO À OCORRÊNCIA DE TPP; NÃO CONSTA
45. QUAL FOI O TEMPO DE UTILIZAÇÃO (em horas): _____; NÃO CONSTA
46. HOUVE ASSOCIAÇÃO DE TOCOLÍTICOS COMO PRIMEIRA OPÇÃO: SIM; NÃO; NÃO CONSTA
47. QUAL FOI A ASSOCIAÇÃO? (pode anotar mais de uma) NÃO ASSOCIOU; INIBIDOR DA SÍNTESE DE PROSTAGLANDINA; BETA-AGONISTA; BLOQUEADOR DE CANAL DE CÁLCIO; ANTAGONISTA DA OCITOCINA; OUTRO NÃO CONSTA
48. HOUVE NECESSIDADE DE TROCA DE TOCOLÍTICO POR FALHA TERAPÊUTICA: SIM; NÃO; NÃO CONSTA
49. SE SIM, QUAL FOI O OUTRO UTILIZADO: (pode anotar mais de um) NÃO USOU; BETA-AGONISTA; NÃO CONSTA; INIBIDOR DA SÍNTESE DE PROSTAGLANDINA; BLOQUEADOR DE CANAL DE CÁLCIO; ANTAGONISTA DA OCITOCINA; OUTRO

USO DE ANTIBIÓTICOS

50. UTILIZAÇÃO DE ANTIBIÓTICO NA INTERNAÇÃO ATUAL: SIM; NÃO; NÃO CONSTA (Se NÃO/NÃO CONSTA, passe para 58)
51. QUAL FOI A MEDICAÇÃO UTILIZADA: _____
52. VIA DE ADMINISTRAÇÃO UTILIZADA: VO; IV; IM; NÃO CONSTA
53. QUAL FOI O TEMPO DE UTILIZAÇÃO (em dias): _____; NÃO CONSTA
54. ASSOCIAÇÃO DE ANTIBIÓTICOS COMO PRIMEIRA OPÇÃO: SIM; NÃO; NÃO CONSTA (Se NÃO/NÃO CONSTA, passe para 56)
55. SE SIM, QUAL FOI A ASSOCIAÇÃO? _____
56. HOUVE NECESSIDADE DE UTILIZAÇÃO DE OUTRO ANTIBIÓTICO POR FALHA TERAPÊUTICA: SIM; NÃO; NÃO CONSTA (Se NÃO/NÃO CONSTA, passe para 58)
57. SE SIM, QUAL FOI O OUTRO ANTIBIÓTICO UTILIZADO: _____

PESQUISA DE INFECÇÃO NA GESTANTE

58. EXAMES LABORATORIAIS REALIZADOS: (pode anotar mais de um) NENHUM; HEMOGRAMA; SEDIMENTO URINÁRIO; CULTURA DE URINA; HEMOCULTURA; PROTEÍNA C REATIVA; VHS; CULTURA DE LÍQUIDO AMNIÓTICO; GLICOSE NO LÍQUIDO AMNIÓTICO; OUTRO; NÃO CONSTA
59. PESQUISA COM CULTURA PARA ESTREPTOCOCO DO GRUPO B: NÃO É ROTINA NO HOSPITAL; É ROTINA-NÃO FOI FEITA; SIM-POR CULTURA SIMPLES; SIM-POR CULTURA SELETIVA; SIM-POR PCR; NÃO CONSTA

OUTRAS CONDIÇÕES

60. OUTRAS MEDICAÇÕES UTILIZADAS NESTA INTERNAÇÃO: SIM; NÃO; NÃO CONSTA
61. COMPLICAÇÕES MATERNO/FETAIS: (pode anotar mais de uma) NENHUMA; CORIOAMNIONITE; EFEITO COLATERAL GRAVE MATERNO/FETAL; HIPOPLASIA PULMÃO FETAL; SEPSE (MÃE); HEMORRAGIA (MÃE); DEFEITOS ANATÔMICOS FETAIS; OUTRA; NÃO CONSTA
62. TEMPO TRANSCORRIDO ENTRE RUPTURA E O NASCIMENTO (em horas): _____; NÃO CONSTA

PARTE ESPECÍFICA (Somente Para os Casos)

FOLHA C: PARTO PREMATURO TERAPÊUTICO OU ELETIVO

INDICAÇÃO DA INTERRUPÇÃO

(Anotações de prontuário):

1. CONDIÇÃO CLÍNICA PRINCIPAL QUE MOTIVOU A INTERRUPÇÃO DA GESTAÇÃO: MATERNA; FETAL (passe para 3); AMBAS
2. CONDIÇÃO CLÍNICA MATERNA QUE MOTIVOU A INTERRUPÇÃO DA GESTAÇÃO: (pode assinalar mais de uma) DIABETES; HIPERTENSÃO GESTACIONAL; HIPERTENSÃO CRÔNICA; PRÉ-ECLÂMPSIA; ECLÂMPSIA; SÍNDROME HELLP; CARDIOPATIA; HIPERTENSÃO PULMONAR; DOENÇA AUTO-IMUNE; DESCOLAMENTO PREMATURO DE PLACENTA; PLACENTA PRÉVIA; INFECÇÃO AMNIÓTICA; TRAUMA MECÂNICO; INFECÇÃO NÃO OBSTÉTRICA; ERRO DE AVALIAÇÃO DE IDADE GESTACIONAL; PEDIDO DA GESTANTE; INSUFICIÊNCIA PLACENTÁRIA; OUTRA
3. CONDIÇÃO CLÍNICA FETAL QUE MOTIVOU A INTERRUPÇÃO DA GESTAÇÃO: (pode assinalar mais de uma) CAUSA MATERNA EXCLUSIVA; SOFRIMENTO FETAL; RESTRIÇÃO DE CRESCIMENTO; MALFORMAÇÃO; OUTRA
4. QUAL FOI A OUTRA INDICAÇÃO MATERNA E/OU FETAL: (se não houve, deixe em branco) _____
5. QUAIS MÉTODOS DIAGNÓSTICOS FORAM UTILIZADOS PARA AVALIAÇÃO DE CONDIÇÕES FETAIS: (pode ser mais de um) CARDIOTOCOGRAFIA; DOPPLERFLUXOMETRIA; PERFIL BIOFÍSICO FETAL; MOBILOGRAMA; OUTRO; NÃO CONSTA
6. PRINCIPAIS EXAMES SUBSIDIÁRIOS QUE EMBASARAM A INTERRUPÇÃO (pode assinalar mais de um): NENHUM; CARDIOTOCOGRAFIA; DOPPLERVELOCIMETRIA; PERFIL BIOFÍSICO FETAL; ECOCARDIO FETAL; FUNÇÃO HEPÁTICA MATERNA; FUNÇÃO RENAL MATERNA; ECOCARDIO MATERNO; ULTRA-SONOGRAFIA MATERNA; ALTERAÇÕES HEMATOLÓGICAS MATERNAS (incluindo coagulograma); OUTROS; NÃO CONSTA
7. HOUVE TENTATIVA DE TRATAMENTO DA CONDIÇÃO CLÍNICA NA INTERNAÇÃO ANTES DE REALIZADA A INTERRUPÇÃO DA GESTAÇÃO: SIM; NÃO; NÃO CONSTA (Se NÃO/NÃO CONSTA, passe para 9)
8. SE SIM, QUAL A DURAÇÃO DO TRATAMENTO (em dias)? _____; NÃO CONSTA

USO DE CORTICOSTERÓIDES PARA INDUÇÃO DE MATURIDADE PULMONAR FETAL

Anotações de prontuário):

9. UTILIZAÇÃO DE CORTICOSTERÓIDE PARA A INDUÇÃO DE MATURIDADE PULMONAR FETAL NA INTERNAÇÃO ATUAL: SIM; NÃO; NÃO CONSTA (Se NÃO/NÃO CONSTA, passe para 21)
10. QUAL FOI O MEDICAMENTO UTILIZADO: BETAMETASONA; DEXAMETASONA; OUTRO; NÃO CONSTA
11. QUAL FOI A VIA DE ADMINISTRAÇÃO UTILIZADA: VO; IV; IM; NÃO CONSTA
12. DOSE TOTAL ADMINISTRADA (some, anote o total prescrito e administrado até o parto, em mg): _____; NÃO CONSTA
13. Nº DE DOSES ADMINISTRADAS (some e anote quantas vezes foi administrado até o parto): _____; NÃO CONSTA
14. QUAL FOI O INTERVALO ENTRE AS DOSES (em horas): _____; NÃO CONSTA
15. HOUVE USO DESTA MEDICAÇÃO ANTERIORMENTE NA GESTAÇÃO: SIM; NÃO; NÃO CONSTA
16. EM QUAL IDADE GESTACIONAL FOI FEITA A 1ª UTILIZAÇÃO (em semanas): _____; NÃO CONSTA
17. FORAM REALIZADAS DOSES SEMANAIS APÓS O DIAGNÓSTICO DA CONDIÇÃO MÓRBIDA: SIM; NÃO; NÃO CONSTA (Se NÃO/NÃO CONSTA, passe para 19)
18. SE SIM, QUANTAS DOSES: _____; NÃO CONSTA
19. FORAM REALIZADAS DOSES EM INTERVALO > QUE 15 DIAS: SIM; NÃO; NÃO CONSTA (NÃO/NÃO CONSTA, passe para 21)
20. SE SIM, QUANTAS DOSES: _____; NÃO CONSTA

OUTRAS CONDIÇÕES

Anotações de prontuário):

21. CONDIÇÃO CLÍNICA MATERNA 48 HORAS APÓS A INTERRUPÇÃO: CURADA; INALTERADA; MELHORA PARCIAL; MELHORA ACENTUADA; PIORA; ÓBITO; NÃO CONSTA

9. Anexo 2 – Aprovação ética EMIP

FACULDADE DE CIÊNCIAS MÉDICAS
COMITÊ DE ÉTICA EM PESQUISA

www.fcm.unicamp.br/pesquisa/etica/index.html

CEP, 08/09/09.
(Grupo III)

PARECER CEP: N° 704/2009 (Este nº deve ser citado nas correspondências referente a este projeto)
CAAE: 0564.1.146.000-09

I - IDENTIFICAÇÃO:

PROJETO: **"ESTUDO MULTICÊNTRICO SOBRE A PREMATURIDADE NO BRASIL".**
PESQUISADOR RESPONSÁVEL: Renato Passini Júnior.
INSTITUIÇÃO: CAISM/UNICAMP
APRESENTAÇÃO AO CEP: 07/08/2009
APRESENTAR RELATÓRIO EM: 08/09/10 (O formulário encontra-se no *site* acima)

II - OBJETIVOS

Avaliar a prevalência de partos pré-termo numa Rede de Instituições Hospitalares do Brasil, aferindo suas principais condições causais, fatores de risco associados, normas de atendimento e morbimortalidade perinatal.

III - SUMÁRIO

Pesquisa composta por um estudo de prevalência, de corte transversal multicêntrico e um estudo de caso-controle aninhado, a serem implementados em 27 unidades obstétricas de referência nas diversas regiões geográficas do Brasil (Região Norte – 1; Nordeste – 10; Centro-Oeste – 1; Sudeste – 13; Sul – 2). Para o estudo de prevalência os pesquisadores principais e os pesquisadores locais deverão realizar vigilância prospectiva, durante um período de seis meses, de todas as mulheres internadas nessas unidades para parto, para a identificação dos casos de parto pré-termo e suas principais causas. Nos primeiros três meses do estudo, além da avaliação da prevalência do parto prematuro e de suas causas, será feita uma análise de eventuais fatores associados ao parto prematuro, comparando mulheres que tiveram o parto pré-termo com aquelas que tiveram recém-nascidos de termo. Para o estudo de prevalência serão avaliados 37.000 partos (termo e pré-termo), correspondendo a aproximadamente metade dos partos ocorridos no total das instituições participantes em doze meses. Para o estudo de caso-controle foi estimado um tamanho amostral de 1.055 mulheres em cada grupo (casos e controles). O total de partos pré-termo avaliados, incluindo o estudo de prevalência e o caso-controle, corresponderá a 3.600. Os dados serão coletados através de questionário aplicado após o parto, codificados em formulário eletrônico e enviados a um banco de dados central. Análise de dados: A análise dos dados será feita por sub-grupos de acordo com a época da ocorrência do parto pré-termo, suas causas prováveis, as opções de terapêuticas adotadas e resultados neonatais obtidos, estimando-se as respectivas taxas, razões e riscos relativos para os possíveis preditores. Com os resultados encontrados, pretende-se conhecer melhor o nascimento pré-termo no Brasil, seus principais fatores de risco sociais e biológicos, bem como fundamentar ações de política de saúde e dar início a ensaios clínicos abordando as estratégias de prevenção e tratamento das condições causais de partos pré-termo, que tantos agravos físicos e emocionais traz para essas crianças e suas famílias.

IV - COMENTÁRIOS DOS RELATORES

Após respostas às pendências, o projeto encontra-se adequadamente redigido e de acordo com a Resolução CNS/MS 196/96 e suas complementares, bem como o Termo de Consentimento Livre e Esclarecido.

V - PARECER DO CEP

O Comitê de Ética em Pesquisa da Faculdade de Ciências Médicas da UNICAMP, após acatar os pareceres dos membros-relatores previamente designados para o presente caso e atendendo todos os dispositivos das Resoluções 196/96 e complementares, resolve aprovar sem restrições o Protocolo de Pesquisa, bem como ter aprovado o Termo do Consentimento Livre e Esclarecido, assim como todos os anexos incluídos na Pesquisa supracitada.

O conteúdo e as conclusões aqui apresentados são de responsabilidade exclusiva do CEP/FCM/UNICAMP e não representam a opinião da Universidade Estadual de Campinas nem a comprometem.

VI - INFORMAÇÕES COMPLEMENTARES

O sujeito da pesquisa tem a liberdade de recusar-se a participar ou de retirar seu consentimento em qualquer fase da pesquisa, sem penalização alguma e sem prejuízo ao seu cuidado (Res. CNS 196/96 – Item IV.1.f) e deve receber uma cópia do Termo de Consentimento Livre e Esclarecido, na íntegra, por ele assinado (Item IV.2.d).

Pesquisador deve desenvolver a pesquisa conforme delineada no protocolo aprovado e descontinuar o estudo somente após análise das razões da descontinuidade pelo CEP que o aprovou (Res. CNS Item III.1.z), exceto quando perceber risco ou dano não previsto ao sujeito participante ou quando constatar a superioridade do regime oferecido a um dos grupos de pesquisa (Item V.3.).

O CEP deve ser informado de todos os efeitos adversos ou fatos relevantes que alterem o curso normal do estudo (Res. CNS Item V.4.). É papel do pesquisador assegurar medidas imediatas adequadas frente a evento adverso grave ocorrido (mesmo que tenha sido em outro centro) e enviar notificação ao CEP e à Agência Nacional de Vigilância Sanitária – ANVISA – junto com seu posicionamento.

Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas. Em caso de projeto do Grupo I ou II apresentados anteriormente à ANVISA, o pesquisador ou patrocinador deve enviá-las também à mesma junto com o parecer aprovatório do CEP, para serem juntadas ao protocolo inicial (Res. 251/97, Item III.2.e)

Relatórios parciais e final devem ser apresentados ao CEP, de acordo com os prazos estabelecidos na Resolução CNS-MS 196/96.

VII- DATA DA REUNIÃO

Homologado na VIII Reunião Ordinária do CEP/FCM, em 25 de agosto de 2009.

Prof. Dr. Carlos Eduardo Steiner
PRESIDENTE DO COMITÊ DE ÉTICA EM PESQUISA
FCM/UNICAMP

10. Anexo 3 – Aprovação ética Preterm SAMBA

COMITÊ DE ÉTICA EM PESQUISA DA UNICAMP - CAMPUS CAMPINAS

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Utilização da metabolômica para identificação e validação de biomarcadores para parto pré-termo

Pesquisador: Jose Guilherme Cecatti

Área Temática:

Versão: 2

CAAE: 38522214.8.1001.5404

Instituição Proponente: Hospital da Mulher Prof. Dr. José Aristodemo Pinotti - CAISM

Patrocinador Principal: MINISTERIO DA CIENCIA, TECNOLOGIA E INOVACAO
Bill & Melinda Gates Foundation

DADOS DO PARECER

Número do Parecer: 912.714

Data da Relatoria: 14/12/2014

Apresentação do Projeto:

O objetivo principal deste estudo é o desenvolvimento de um teste de rastreamento com biomarcadores para parto pré-termo, incluindo os componentes de desenvolvimento e validação, com relevante aplicabilidade clínica. Visa-se identificar, no início da gestação, mulheres sob risco de apresentar trabalho de parto pré-termo, o que poderia colaborar para a realização de intervenções precisas e oportunas capazes de reduzir a ocorrência de desfechos maternos e perinatais adversos relacionados à prematuridade. Esse tema tem aumentado sua importância no cenário brasileiro e mundial na atualidade devido as impactantes consequências da prematuridade. Método: O estudo será composto por 2 componentes: um componente de desenvolvimento, contemplado por um estudo de caso-controle utilizando mulheres que participaram do estudo SCOPE, uma coorte internacional que coletou amostras às 15 semanas de gestação de 5690 mulheres nulíparas, analisando dois grupos: Grupo Caso, composto com dados e amostras de mulheres que tiveram parto prematuro espontâneo antes de 34 semanas, e Grupo Controle, composto por mulheres que evoluíram para parto a termo. O perfil metabolômico será analisado juntamente com dados sociodemográficos para o desenvolvimento de um modelo preditor de parto pré-termo. O outro componente, de validação do modelo preditor, será um estudo de coorte com mulheres brasileiras de cinco centros participantes. Coletar-se-ão amostras de sangue e

Endereço: Rua Tessália Vieira de Camargo, 126
Bairro: Barão Geraldo **CEP:** 13.083-887
UF: SP **Município:** CAMPINAS
Telefone: (19)3521-8936 **Fax:** (19)3521-7187 **E-mail:** cep@fcm.unicamp.br

cabelo (às 20 semanas de gestação) para análise metabolômica, além de dados sociodemográficos, dados relativos à gestação, parto, puerpério e dados relativos a desfechos perinatais maternos e neonatais. As duas fases ocorrerão simultaneamente. Assim, os resultados do componente de desenvolvimento não estarão disponíveis antes do término do estudo de coorte. Portanto, a avaliação dos desfechos maternos e perinatais do estudo de coorte com o modelo preditor gerado pela fase de desenvolvimento (estudo caso-controle) será retrospectiva. A metabolômica, ciência de alta tecnologia para análise de bioamostras, será inicialmente realizada na Universidade de Auckland, Nova Zelândia. O estudo prevê um consórcio internacional envolvendo o centro coordenador, a Universidade de Auckland e o laboratório brasileiro LNBio para a transferência de tecnologia, para propiciar a realização da análise metabolômica das amostras do estudo em laboratório brasileiro. Para isso serão incluídas na coorte 1150 nulíparas de baixo risco, aproximadamente 230 em cada centro participante. Análise de dados: A análise do primeiro componente será realizada através de sofisticados processos estatísticos utilizando a plataforma MetaboAnalyst®. A análise do segundo componente será basicamente a análise de validação diagnóstica do modelo preditor utilizando estimativas de sensibilidade, especificidade, valores preditivos e razões de verossimilhança.

Objetivo da Pesquisa:

Objetivo Primário:

Desenvolver e validar um algoritmo de predição para identificar as gestantes com maior risco de parto pré-termo.

Objetivo Secundário:

1. Identificar um conjunto de marcadores metabolômicos relacionados ao parto pré-termo em nulíparas. 2.Construir um algoritmo preditivo de parto pré-termo incluindo marcadores metabolômicos, clínicos e/ou sociodemográficos. 3. Validar a predição obtida pelo algoritmo com desfechos maternos e neonatais em outro grupo de nulíparas.

Avaliação dos Riscos e Benefícios:

Riscos:

Vale ressaltar que o estudo não realizará nenhum tipo de intervenção, preconizando, no componente do estudo de coorte, apenas coleta de material biológico (uma amostra de sangue e fios de cabelo) às 20 semanas e coleta de informações clínicas e de prontuário conforme protocolos estabelecidos. Os riscos potenciais mínimos se referem à própria coleta de sangue e cabelo. Será garantida a confidencialidade sobre a fonte das informações.

Benefícios:O estudo não traz benefícios imediatos às participantes. Entretanto, a implementação de um eficaz algoritmo preditor de parto pré-termo em idade gestacional precoce traria grandes benefícios na sistematização da assistência obstétrica e neonatal. A identificação da população de risco na idade gestacional ora proposta
(vinte semanas) proporcionaria uma janela de intervenção ampla, começando no início do segundo trimestre. Novas perspectivas de enfoque em futuros estudos poderão ser geradas, caso os resultados obtidos com essa coorte não sejam capazes de efetivamente predizer qual a população de risco para PPT.

Comentários e Considerações sobre a Pesquisa:

Trata-se de um projeto de pesquisa multicêntrico da Faculdade de Ciências Médicas da UNICAMP que será realizado no CAISM/UNICAMP. Este estudo terá duas etapas, uma retrospectiva, a qual contempla um estudo de caso-controle utilizando mulheres que participaram do estudo SCOPE, uma coorte internacional que coletou amostras às 15 semanas de gestação de 5690 mulheres nulíparas, com o objetivo de desenvolver um modelo preditor de parto pré-termo, e outra de validação do modelo preditor, a qual será um estudo de coorte com mulheres brasileiras de cinco centros participantes, onde serão coletadas amostras de sangue e de cabelo (n=230) e feita uma entrevista. O projeto é patrocinado pelo MINISTERIO DA CIENCIA, TECNOLOGIA E INOVACAO e pela Bill & Melinda Gates Foundation. Os riscos potenciais mínimos se referem à própria coleta de sangue e cabelo e não trás benefícios diretos aos participantes da pesquisa. Haverá envio de amostras para o exterior.
Consideramos a pesquisa pertinente, de grande relevância social e embasada na literatura.

Considerações sobre os Termos de apresentação obrigatória:

Já haviam sido apresentados Projeto de Pesquisa, TCLE, Folha de rosto e parecer da Comissão de Pesquisa do CAISM. O pesquisador respondeu às pendências colocadas no parecer inicial deste CEP, a saber:
1)TCLE:
1.1) Os pesquisadores devem ser localizados não apenas pelo telefone ou e-mail de contato, mas em seu endereço profissional, salientando o departamento ou unidade em que poderão serlocalizados. Readequar. PENDÊNCIA RESPONDIDA.
1.2) Deixar claro que o contato do CEP serve para eventuais reclamações e/ou denúncias referentes aos aspectos éticos da pesquisa. PENDÊNCIA RESPONDIDA.

1.3) Informar endereço e e-mail do CEP, não somente o telefone. PENDÊNCIA RESPONDIDA.
1.4) Deixar claro que o participante não terá nenhum benefício financeiro.PENDÊNCIA RESPONDIDA.
2) Carta de autorização e/ou anuência das outras instituições participantes. PENDÊNCIA RESPONDIDA.
4) Anexar regras que regem o biobanco para as novas amostras. PENDÊNCIA RESPONDIDA.

Reavaliação da pendência 3) colocada no parecer anterior ("Como haverá envio de amostras para o exterior, o projeto deve ser enviado diretamente à CONEP.")". De acordo com a resolução 466, a necessidadde de avaliaçõ peloa CONEP se dá quando há "envio para o exterior de material genético ou qualquer material biológico humano para obtenção de material genético, salvo nos casos em que houver cooperação com o Governo Brasileiro". Neste projeto, o material enviado não é genético e tampouco para obtenção de material genético, além de ser um projeto de cooperação com o Governo Brasileiro, inclusive com financiamento aprovado.

Recomendações:

Não há.

Conclusões ou Pendências e Lista de Inadequações:

Aprovado.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

- A pesquisa só deve ser iniciada após o parecer de aprovação deste CEP.
- O sujeito de pesquisa deve receber uma via do Termo de Consentimento Livre e Esclarecido, na íntegra, devidamente assinado.
- O sujeito da pesquisa tem a liberdade de recusar-se a participar ou de retirar seu consentimento em qualquer fase da pesquisa, sem penalização alguma e sem prejuízo ao seu cuidado.
- O pesquisador deve desenvolver a pesquisa conforme delineada no protocolo aprovado. Se o pesquisador considerar a descontinuação do estudo, esta deve ser justificada e somente ser realizada após análise das razões da descontinuidade pelo CEP que o aprovou. O pesquisador deve

aguardar o parecer do CEP quanto à descontinuação, exceto quando perceber risco ou dano não previsto ao sujeito participante ou quando constatar a superioridade de uma estratégia diagnóstica ou terapêutica oferecida a um dos grupos da pesquisa, isto é, somente em caso de necessidade de ação imediata com intuito de proteger os participantes.

- O CEP deve ser informado de todos os efeitos adversos ou fatos relevantes que alterem o curso normal do estudo. É papel do pesquisador assegurar medidas imediatas adequadas frente a evento adverso grave ocorrido (mesmo que tenha sido em outro centro) e enviar notificação ao CEP e à Agência Nacional de Vigilância Sanitária – ANVISA – junto com seu posicionamento.
- Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas. Em caso de projetos do Grupo I ou II apresentados anteriormente à ANVISA, o pesquisador ou patrocinador deve enviá-las também à mesma, junto com o parecer aprovatório do CEP, para serem juntadas ao protocolo inicial.
- Relatórios parciais e final devem ser apresentados ao CEP, inicialmente seis meses após a data deste parecer de aprovação e ao término do estudo.

CAMPINAS, 13 de Dezembro de 2014

Assinado por:
Monica Jacques de Moraes
(Coordenador)

Endereço: Rua Tessália Vieira de Camargo, 126
Bairro: Barão Geraldo **CEP:** 13.083-887
UF: SP **Município:** CAMPINAS
Telefone: (19)3521-8936 **Fax:** (19)3521-7187 **E-mail:** cep@fcm.unicamp.br

11. Anexo 4 – Carta do orientador no exterior – Programa PDSE Capes

UNIVERSITY OF LEICESTER

College of Life Sciences

University of Leicester
Maurice Shock Medical Sciences Building
University Road
Leicester LE1 9HN, UK

Pro-Vice-Chancellor, Head of College
& Dean of Medicine
Professor Philip N Baker
BMedSci, BM,BS, DM, FRCOG, FMedSci

t: +44 (0)116 252 2962
f: +44 (0)116 252 5001
e: philip.baker@le.ac.uk

21th March, 2018

Dear Capes,

Re: PhD "sandwich" training programme of Renato T Souza – 6-month Internship in University of Leicester

Dr Souza worked under my supervision from late August 2017 to late February 2018 for his training period in University of Leicester, where he was able to conduct different academic and research activities.

Dr Souza accomplished the tasks that we expected from his internship as he:

1. attended classes on basic and intermediate statistics;
2. participated in journal clubs/scientific discussions of the Department of Health Science, with a focus on epidemiology and biostatistics;
3. completed a course of metabolomics analysis that gave him basics expertise in metabolomic data acquisition, metabolomic study design and metabolomic data normalization and analysis;
4. participated in other projects in maternal and perinatal health research; he was co-author of manuscripts related to several analyses;
5. will shortly complete the main manuscript related to the Preterm SAMBA Project, the main topic of his thesis; he was very active in writing and discussing this manuscript;
6. helped to implement a research project related with preterm birth (premature rupture of membranes) together with international collaborators;
7. was a co-applicant of a new research proposal submitted to the MRC-RCUK – FAPESP joint call; this provided an excellent opportunity to contribute to the whole process of writing a "big grant" proposal, following all steps from conception to application;
8. visited a metabolomics laboratory in Ireland which is a partner of Preterm SAMBA Project. This provided him with the opportunity to appreciate the basic science involved in metabolomic data acquisition (sample analysis) and also to discuss and manage the next steps of Preterm SAMBA Project (validation of metabolomics biomarkers using Brazilian biosamples).

Considering all these achievements, I have no hesitation in concluding that his 6-month period in University of Leicester was helpful for his early career, for his PhD programme training and to his thesis completion. In addition, his participation in our group was key to completing the activities of the Preterm SAMBA Project and for future collaborations.

Yours sincerely,

Professor Philip Baker BMedSci, BM, BS, DM, FRCOG, FRANZCOG, FMedSci
Pro-Vice-Chancellor and Head of the College of Life Sciences, Dean of Medicine
(Distinguished National Professor, Chongqing Medical University, China & Professor of Maternal and Fetal Health, University of Auckland, New Zealand)

Athena SWAN Bronze Award
Athena SWAN Silver Award
The Queen's Anniversary Prizes For Higher and Further Education 2013