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DEBORA FARIAS BATISTA LEITE

EXPLORANDO A OCORRÊNCIA, PREDIÇÃO E IMPACTO DA RESTRIÇÃO DE CRESCIMENTO FETAL

EXPLORING THE OCCURRENCE, PREDICTON AND IMPACT OF FETAL GROWTH RESTRICTION

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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 Doutora em Ciências da Saúde, na Área de Concentração de Saúde Materna e Perinatal.

Thesis presented to the School of Medical Sciences of the University of Campinas, Postgraduation Program of Obstetrics and Gynecology, as part of the requirements for obtaining the title of Ph.D. in Health Sciences, in the concentration area of Maternal and Perinatal Health.

ORIENTADOR: JOSÉ GUILHERME CECATTI

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DA TESE DEFENDIDA PELA ALUNA DEBORA FARIAS BATISTA LEITE, E ORIENTADA PELO PROF. DR. JOSÉ GUILHERME CECATTI.

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> “Queria agradecer a meus pais por seu amor incondicional. Agradecer a meu pai por não cortar minhas asas e me deixar voar. Obrigada, mamãe, por me inspirar a ser paciente e falar sempre a verdade.”

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RESUMO

Introdução: A restrição de crescimento fetal (RCF) é uma condição heterogênea; avaliação de risco e predição ainda são estratégias desafiadoras para a Obstetrícia moderna. Os fatores clínicos podem orientar na seleção de mulheres que se beneficiam de métodos complementares para vigilância fetal. Biomarcadores laboratoriais podem ser úteis na abordagem da RCF.

Objetivos: Analisar a RCF de uma forma ampla, incluindo avaliação de risco, predição e diagnóstico. Objetivos específicos: analisar os recém-nascidos pequenos para a idade gestacional (PIG) como desfecho secundário do estudo Preterm-SAMBA quanto aos fatores clínicos de risco; realizar revisão narrativa da literatura sobre o rastreio de RCF e aplicação da metabolômica no seu estudo; e desenvolver uma revisão sistemática da literatura sobre a acurácia da metabolômica na predição dos recém-nascidos PIG, e a identificação de biomarcadores.

Métodos: Para o Preterm-SAMBA, nulíparas de risco obstétrico habitual foram incluídas entre 19^{+0}-20^{+6} semanas de uma gestação única, e acompanhadas até o parto. Dados sociodemográficos, clínicos e reprodutivos foram obtidos na 1ª visita; dados perinatais foram acessados nos prontuários médicos. O peso ao nascer abaixo do percentil 10 da curva customizada de peso foi considerado como *proxy* para RCF. Recém-nascidos com peso entre os percentis 10 e 90 foram considerados adequados para a idade gestacional (AIG). Para a revisão sistemática, dois pesquisadores independentes pesquisaram onze bases de dados eletrônicas, selecionaram os estudos e extraíram os dados. Um terceiro revisor dirimiu dúvidas. A pesquisa foi realizada em fevereiro 2018 e novembro 2018, sem restrições de idiomas ou limites.

Resultados: A prevalência de PIG na amostra do SAMBA foi 12,8%. Os grupos PIG e AIG foram semelhantes em relação às características maternas, exceto pela assistência pré-natal pública (*p* 0,012) e a presença de qualquer infecção na primeira metade da gestação (*p* 0,016). Essas características se associaram a maior risco para PIG na análise multivariada (RR 2,02; 95%CI 1,23-3,33; e RR 1,36; 95%CI 1,10-1,68, respectivamente). Na revisão sistemática da literatura, foram incluídos 15 estudos. Meta-análise não foi realizada devido à heterogeneidade na seleção dos participantes e métodos empregados. Análise do sangue ou cabelo materno no 2º trimestre da gestação apresentou alta capacidade preditiva em estudos individuais do tipo *untargeted*. Os metabólitos preditivos compreendem onze classes químicas, e a subclasse mais prevalente foi a dos ácidos graxos.

Conclusão: A prevalência de PIG numa população é um marcador do seu desenvolvimento socioeconômico. Avaliação de risco clínico deve ser amplamente oferecida durante a gravidez. Os achados de biomarcadores envolvidos com o metabolismo dos lipídios no 2º trimestre da gestação são promissores; a validação destes achados é encorajada.

Palavras-chave: Retardo do crescimento fetal. Recém-nascido pequeno para a idade gestacional. Metabolômica. Indicadores de morbimortalidade. Cuidado Pré-natal.

ABSTRACT

Background: Fetal growth restriction (FGR) is a heterogeneous condition; risk assessment and prediction are still challenging for modern obstetrics. Clinical factors may guide selection of women who benefit from additional methods for fetal surveillance. Laboratory biomarkers may be useful in addressing FGR.

Objectives: To comprehensively analyze the FGR condition, including risk assessment, prediction and diagnosis. Secondary objectives: to analyze clinical risk factors for small for gestational age (SGA) infants as a secondary outcome of the Preterm-SAMBA study; to carry out narrative reviews of literature on the screening for FGR and the use of metabolomics for its evaluation; and to develop a systematic review of the literature on the accuracy of metabolomics in the prediction of SGA infants, and the identification of biomarkers.

Methods: For the Preterm-SAMBA study, nulliparous low-risk women were enrolled between 19+0 - 20+6 weeks of a single pregnancy and were followed up until delivery. Sociodemographic, clinical and reproductive data were obtained at the first visit; perinatal data were accessed on medical records. SGA, defined as having customized birth weight below the 10th centile, was considered proxy for FGR. Newborns with birthweight 10th - 90th centiles were adequate for gestational age (AGA). For the systematic review, two independent researchers assessed eleven electronic databases, selected studies, and extracted data. A third reviewer has helped to resolve discrepancies. The literature search was performed in February 2018 and November 2018, with no limits or language restrictions.

Results: SGA prevalence in the Preterm-SAMBA was 12.8%. SGA and AGA groups were similar regarding maternal characteristics, except for public prenatal care (p

0.012) and the presence of any infection in the first half of gestation (p 0.016). These characteristics were associated with an increased risk for SGA in the multivariate analysis (RR 2.02, 95% CI 1.23-3.33 and RR 1.36, 95% CI 1.10-1.68, respectively). In the systematic review, 15 studies were included. Meta-analysis was not performed due to heterogeneity in the selection of participants and methods employed by the original studies. Analysis of maternal blood or hair in the second trimester of pregnancy presented high predictive accuracy in untargeted studies. Predictive metabolites comprise eleven chemical classes, and the most prevalent subclass was fatty acids.

Conclusions: SGA prevalence is a marker of socioeconomic development. Clinical risk assessment should be widely offered during pregnancy. The findings of biomarkers involved with lipid metabolism in the second trimester of gestation are promising; the validation of these results is encouraged.

Keywords: Fetal growth retardation. Infant, small for gestational age. Metabolomics. Indicators of morbidity and mortality. Prenatal care.

LISTA DE ABREVIATURAS E SIGLAS

ACOG	*American College of Obstetrics and Gynecology*
AGA	*Adequate for gestational age*
AIG	Adequado para a idade gestacional
aOR	*Adjusted odds ratio*
ARIF	*Aggressive Research Intelligence Facility*
aRR	*Adjusted Risk Ratio*
AUC	*Area under the curve*
BMI	*Body mass index*
CAISM	Centro de Atenção Integral à Saúde da Mulher
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CEP	Comitê de Ética em Pesquisa
CI	*Confidence interval*
CINAHL	*Cumulative Index of Nursing and Allied Health Literature*
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
DARE	*Database of Abstracts of Reviews of Effects*
DBP	*Diastolic blood pressure*
DCNT	Doenças crônicas não transmissíveis
DOHaD	*Developmental origins of health and disease*
EFW	*Estimated fetal weight*
EMBASE	*Excerpta Medica dataBASE*
FGR	*Fetal growth restriction*
GIG	Grande para a Idade Gestacional
GRN	*Growth restriction of the newborn*
HCPA	Hospital de Clínicas de Porto Alegre
HC-UFPE	Hospital das Clínicas da Universidade Federal de Pernambuco
HOMA-IR	Índice de resistência à insulina
HMDB	*Human Metabolome Database*
HSROC	*Hierarchical summary receiver characteristic operating curve*
HTA	*Health Technology Assessment*
IC	Intervalo de confiança
INFANT	*The Irish Centre for Fetal and Neonatal Translational Research*

ISSHP	*International Society for the Study of Hypertension in Pregnancy*
IMC	Índice de massa corpórea
IP	Índice de pulsatilidade
IRB	*Institutional Review Board*
INTERGROWTH-21st	*International Fetal and Newborn Growth Consortium for the 21st Century*
IUGR	*Intrauterine growth restriction (or retardation)*
KEGG	*Kyoto Encyclopedia of Genes and Genomes*
LGA	*Large for gestational age*
LILACS	*Latin American and Caribbean Health Sciences Literature*
MAP	*Mean arterial pressure*
MEAC	Maternidade Escola Assis Chateaubriand
MIDIRS	*Maternity and Infant Care*
NICU	*Neonatal intensive care unit*
OR	*Odds ratio*
OMS	Organização Mundial da Saúde
PAPP-A	Proteína placentária A
PAD	Pressão arterial diastólica
PAS	Pressão arterial sistólica
PAM	Pressão arterial média
PDSE	Programa de Doutorado Sanduíche no Exterior
PFE	Peso fetal estimado
PI	*Pulsatility Index*
PIG	Pequeno para a idade gestacional
PlGF	*Placental Growth Factor*
Preterm-SAMBA	*Preterm Screening and Metabolomics in Brazil and Auckland*
PRISMA	*Preferred Reporting Items for Systematic Reviews and Meta-Analysis*
PRISMA-P	*Preferred Reporting Items for Systematic Reviews and Meta-Analysis – protocol*
PROSPERO	*International prospective register of systematic reviews*
PSU	*Primary sampling unit*
QUADAS-2	*Quality Assessment of Diagnostic Accuracy Studies*
RCF	Restrição do Crescimento Fetal

RCOG	*Royal College of Obstetricians and Gynecologists*
RI	*Resistance index*
ROC curve	*Receiver operating characteristic curve*
RR	*Risk ratio*
RS	Revisão sistemática
SBP	*Systolic blood pressure*
Scielo	*Scientific Electronic Library Online*
SCOPE	*Screening for Pregnancy Endpoints*
SGA	*Small for gestational age*
SPSS	*Social Package for Social Sciences*
SR	*Systematic Review*
TCLE	Termo de Consentimento Livre e Esclarecido
UCC	*University College Cork*
UFC	Universidade Federal do Ceará
UNESP	Universidade Estadual Paulista Júlio de Mesquita Filho
UNICAMP	Universidade Estadual de Campinas

Sumário

1. APRESENTAÇÃO

" 'Ass!' said the Director, breaking a long silence. 'Hasn't it occurred to you that an Epsilon embryo must have an Epsilon environment as well as an Epsilon heredity?'

It evidently hadn't occurred to him. He was covered with confusion.

'The lower the caste,' said Mr. Foster, 'the shorter the oxygen.' The first organ affected was the brain. After that the skeleton. At seventy per cent of normal oxygen you got dwarfs. At less than seventy eyeless monsters.

'Who are no use at all,' concluded Mr. Foster.

'And that,' put in the Director sententiously, 'that is the secret of happiness and virtue-liking what you've got to do. All conditioning aims at that: making people like their unescapable social destiny'."

Admirável Mundo Novo (*Brave New World*), Aldous Huxley, 1932

A influência que o ambiente intrauterino exerce sobre o feto é um conhecido determinante de saúde e qualidade de vida do indivíduo. Neste trecho do livro Admirável Mundo Novo (1), escrito na primeira metade do século XX, Aldous Huxley já idealizava (e antecipava) algumas das mais intrigantes teorias científicas dos últimos anos: as origens desenvolvimentistas da saúde e da doença (*DOHad*, *Developmental Origins of Health and Disease)*. Ou seja, o suporte de oxigênio e de nutrientes ao feto não apenas interfere com as medidas antropométricas do recém-nascido, mas modula sua resposta aos estímulos ambientais vivenciados durante toda a vida.

De fato, a epigenética tem demonstrado alterações pós-translacionais em recém-nascidos pequenos para a idade gestacional (PIG) (2), e que embasam a 'Hipótese do Fenótipo Econômico' (*Thrifty Phenotype Hypothesis*) (3). Segundo Hales & Barker, o déficit nutricional ao feto, especialmente de aminoácidos,

diminuiria a função das células beta pancreáticas e induziria a mudanças no funcionamento dos sistemas musculares, hepático e tecido adiposo, por exemplo (3). Ao mesmo tempo, o estado de saúde materno – não apenas durante a gravidez, mas também ao nascer - também surgiu como um relevante fator contributivo nesta cascata fisiopatológica (3). Como o fato de a mãe ter nascido PIG aumenta em quase três vezes o risco de que sua prole seja PIG, há autores que consideram que o perfil PIG é transgeracional (4,5).

Portanto, a predição oportuna, na gravidez, e o correto diagnóstico dos recém-nascidos PIG é de relevância não apenas para a Obstetrícia: trará consequências na dimensão de Saúde Pública. A suspeita de um recém-nascido PIG propiciará o manejo adequado da mãe e do recém-nascido, incluindo cuidado pré-natal especializado, parto em serviço de atenção terciária, e seguimento individualizado na infância, adolescência e vida adulta. Assim, estratégias de predição, na gravidez, devem ser testadas, e os critérios diagnósticos, validados em diferentes populações.

Dados clínicos e ultrassonográficos têm sido estudados em várias coortes, mas com acurácia moderada para predição (6). É possível que, em parte, isso seja devido à heterogeneidade fenotípica destes fetos e neonatos, submetidos a insultos diferentes no ambiente intrauterino, de intensidades distintas e por tempos díspares. Assim, novos biomarcadores preditivos devem ser determinados. Por outro lado, é também possível que precisemos elaborar um novo ‘padrão ouro’ para definição da restrição de crescimento.

Neste contexto, a minha principal motivação para esta tese foi a de aprofundar a investigação sobre a restrição de crescimento fetal (RCF), por considerar que o papel do obstetra não se limita ao pré-natal ou ao parto. Durante o

período do doutorado, tive a oportunidade de me aproximar do tema mediante duas estratégias principais: atuar como coordenadora local (no Hospital das Clínicas da Universidade Federal de Pernambuco, HC-UFPE) do estudo *Preterm Screening and Metabolomics Brazil and Auckland* (Preterm-SAMBA) (7); e de participar do Programa de Doutorado Sanduíche no Exterior (PDSE) promovido pela CAPES. Neste último caso, fui estudante visitante na Universidade de Cork, na República da Irlanda (*University College Cork, UCC*).

Apesar de ser uma obstetra por natureza e por paixão, decidimos usar, nesta tese, o peso ao nascer como uma medida do desenvolvimento fetal. Compreendemos que possivelmente são condições distintas, e que RCF e PIG não são termos intercambiáveis. Porém, e infelizmente, as definições e critérios para identificação de um feto aquém do seu desenvolvimento não são consensuais. Além disso, (i) o potencial ótimo de crescimento fetal não pode ser previsto apenas pelo seu padrão genético, uma vez que o ambiente intrauterino exerce influência direta sobre o desenvolvimento do concepto, e (ii) exames ultrassonográficos não estavam previstos de forma sistemática na coorte do Preterm-SAMBA. Desse modo, concordamos com a literatura que o peso ao nascer é o melhor modelo para estudarmos o crescimento fetal intrauterino. Nesta tese, os recém-nascidos PIG são usados como *proxy* para a RCF, e maior atenção foi dada ao rastreio e ao diagnóstico.

O Preterm-SAMBA foi desenhado para validar biomarcadores precoces para desfechos gestacionais adversos em gestantes nulíparas de risco obstétrico habitual. Especificamente previa a utilização de uma tecnologia translacional, a metabolômica, para a identificação de novos marcadores para parto prematuro, síndromes hipertensivas, diabetes mellitus gestacional e RCF. Seguiu um protocolo

de pesquisa semelhante ao estudo SCOPE (*Screening for Pregnancy Endpoints),* coordenado por nossos colaboradores internacionais. Neste sentido, realizamos uma ampla busca sistematizada da literatura, para identificarmos a acurácia da metabolômica em predizer os recém-nascidos PIG, e quais os metabólitos que podem ser usados como biomarcadores. A revisão sistemática seguiu as recomendações internacionais de transparência em pesquisa e método científico. O protocolo está publicado no *British Medical Journal Open* (Leite e colaboradores, http:// dx. doi.org/ 10. 1136/ bmjopen- 2018-022743; *Metabolomics for predicting fetal growth restriction: protocol for a systematic review and meta-analysis*) (8). O artigo com a revisão sistemática também está no Capítulo Resultados, e logo será enviado a publicação.

Ainda em relação à coorte longitudinal do estudo Preterm-SAMBA, avaliamos as características das participantes no momento da inclusão na pesquisa (entre 19 e 20 semanas) e estabelecemos a incidência e fatores de risco clínico para recém-nascidos PIG (*Assessing clinical risk factors for small for gestational age infants in a cohort of low-risk nulliparous pregnant women*). Este manuscrito está no Capítulo de Resultados, e será brevemente submetido para publicação. Outras análises estão propostas para a coorte do Preterm-SAMBA, e serão investigadas no pós-doutorado. Pretendemos analisar outros biomarcadores preditores de PIG, tanto os ultrassonográficos (quando disponíveis) tanto os metabolômicos. Intencionamos, ainda, estudar os desfechos perinatais de acordo com (i) diferentes percentis de peso ao nascer; (ii) o momento do diagnóstico do *status* PIG: se houve suspeita durante a gravidez ou se foi identificado apenas ao nascimento; e (iii) a classificação para restrição de crescimento no recém-nascido (9).

A oportunidade de fazer o PDSE permitiu abranger os horizontes de pesquisa na área da Obstetrícia, trabalhando em uma pesquisa ‘de bancada’. Sob orientação da Prof. Louise Kenny e de sua equipe do Centro Irlandês para Pesquisa Translacional Fetal e Neonatal (*INFANT Centre*), escrevemos uma proposta de análise metabolômica para as participantes do SCOPE. Dosamos biomarcadores em amostras de sangue materno com 20 semanas de gestação (*discovery phase study*), o que permitirá futura validação destes resultados no estudo Preterm-SAMBA. Os resultados desta investigação estão em análise estatística pelos pesquisadores do INFANT, e deverão ser publicados em um futuro próximo.

A experiência como estudante numa universidade estrangeira possibilitou, ainda, a reflexão de minha prática como discente e docente. Ao cumprir créditos acadêmicos na *University College Cork*, no módulo *Getting Started with Graduate Research and Generic Skills,* pude escrever um ensaio sobre a centralidade da revisão de literatura em dissertações e teses. Esta avaliação foi adaptada para submissão ao periódico *Higher Education* (*Approaching literature review for academic purposes: The Literature Review Checklist*), e consta como Apêndice (A) nesta tese. Esta exploração conceitual sobre a revisão da literatura foi também fundamental para a realização das respectivas revisões constantes da introdução e de parte dos objetivos e resultados da tese, abordando especificamente a restrição do crescimento fetal e a ocorrência, predição e diagnóstico de fetos e recém-nascidos pequenos para a idade gestacional.

Assim, por fim, do ponto de vista teórico, a contínua reflexão sobre o tema permitiu a elaboração de três artigos. A partir da adaptação do capítulo de Introdução, dois artigos de revisão narrativa de literatura foram construídos e submetidos para publicação. Um deles trata sobre o rastreio da RCF durante a

gestação (*Fetal growth restriction prediction: how to move beyond?*), submetido ao *The Scientific World Journal*. O segundo discute os mais recentes avanços na avaliação de mães e recém-nascidos com restrição de crescimento com o uso da metabolômica (*New approaches for fetal growth restriction: it is time for metabolomics*), submetido à Revista Brasileira de Ginecologia e Obstetrícia. Ambos artigos estão no Capítulo Resultados desta tese. Quanto ao diagnóstico, houve a publicação recente de novos critérios para a identificação de restrição de crescimento no recém-nascido (9). Uma reflexão sobre estes critérios foi publicada no *Journal of Pediatrics* (Leite e colaboradores, https://doi.org/10.1016/j.jpeds.2018.07.094; *Fetal and neonatal growth restriction: new criteria, renew challenges*) (10). Esta Carta ao Editor encontra-se como Apêndice (B) nesta tese, juntamente com a resposta dos autores do artigo original.

Na gestação, a maioria das condições apresenta modelos de predição e diagnóstico baseados em múltiplos fatores. A restrição de crescimento talvez seja a mais complexa e intrigante delas, ao afetar ambos, mãe e feto, por mais de uma geração. Os estudos desenvolvidos nesta tese são inéditos, e aspiramos que os nossos resultados auxiliem profissionais de saúde e pesquisadores a interpretar a restrição de crescimento.

Campinas, fevereiro de 2019.

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2. INTRODUÇÃO

2.1. Caracterização do problema

O século XX presenciou uma mudança de paradigmas em relação às condições de saúde da população. O avanço no enfrentamento das doenças infecciosas foi acompanhado por um rápido crescimento das doenças crônicas não transmissíveis (DCNT). Estas se apresentam, atualmente, como a principal causa de morte em todo o mundo (11). A partir da década de 1980, Prof. D. Barker e colaboradores chamaram a atenção para a importância do ambiente intrauterino e sua relação com a mortalidade por doença cardíaca isquêmica na vida adulta (3,12,13). De fato, a proporcionalidade do crescimento fetal já demonstrava ser um parâmetro independente para a identificação de pessoas sob maior risco cardiovascular (13).

A elaboração de curvas de normalidade para o peso ao nascer (14) e, em seguida, para o peso fetal estimado por ultrassonografia (15), representou, graficamente, a observação de que o crescimento intrauterino não é uniforme ao longo da gestação. Assim, o feto que não atinge seu potencial ótimo de crescimento passou a definir a restrição de crescimento fetal (RCF), quantificada por um peso estimado inferior ao percentil 10 de uma determinada curva de normalidade (16). Tais fetos apresentam maior risco para desfechos negativos no período perinatal e na infância, como morte intrauterina e neonatal (17–19), admissão em unidades de cuidados intensivos neonatais (20), e atraso do desenvolvimento cognitivo (21), especialmente quando houve redistribuição do fluxo sanguíneo cerebral (22).

O peso fetal ou ao nascer, como dado isolado, traduz pouca informação a respeito do bem-estar intrauterino (23), de modo que outros fatores devem ser considerados ao se avaliar a proporcionalidade do recém-nascido. Caso o peso ao

nascer seja menor do que o percentil 10 para idade gestacional e sexo, de uma determinada curva de normalidade (16,24), define-se como recém-nascido pequeno para a idade gestacional (PIG). Infelizmente, existem dificuldades ainda patentes para a mensuração adequada do crescimento fetal e, consequentemente, para a identificação de fetos restritos (25). Portanto, apesar de RCF e PIG não serem termos ou conceitos intercambiáveis, os recém-nascidos PIG são relatados em vários estudos como *proxy* do crescimento intrauterino restrito (20,26–29).

Em paralelo, evidências recentes têm considerado o papel singular das características maternas e ambientais para um desenvolvimento intrauterino saudável (30–32). Vários fatores de risco têm sido descritos para os recém-nascidos PIG (26,27), mas a sua predição permanece um desafio para a Obstetrícia moderna. Fatores clínicos, como a medida da altura do fundo uterino (33); ultrassonográficos, como o peso fetal estimado (PFE) no terceiro trimestre (20); e os relacionados à função placentária, como o fator de crescimento placentário (PlGF) (34), têm demonstrado limitada aplicabilidade clínica. Portanto, a necessidade de investigação de novos fatores preditores e, em especial, com a utilização de novas tecnologias, é urgente. A predição deste distúrbio de crescimento fetal oferecerá a possibilidade de uma mudança da trajetória de saúde do indivíduo, tanto a curto prazo, reduzindo a morbimortalidade perinatal, como a longo prazo, ao impactar na sua suscetibilidade às DCNT.

2.2. Crescimento fetal

O desenvolvimento fetal é heterogêneo ao longo da gestação, e reflete a complexa interação entre fatores maternos, placentários e do próprio feto. Até o início do 2º trimestre, há principalmente hiperplasia celular fetal. Entre a 16ª semana

e a 32ª, ocorrem hiperplasia e hipertrofia celulares. A partir da 32ª, finalmente, o processo dominante para o crescimento e desenvolvimento fetal é a hipertrofia (35).

A placenta é um órgão singular; comporta tecidos maternos e fetais, e se modifica, ao longo da gravidez, para equilibrar as ofertas maternas ao potencial de crescimento do feto (35,36). Assim, a repercussão que possíveis eventos adversos terão sobre o concepto dependerá da idade gestacional em que houve o dano. É possível que anomalias cromossômicas e infecções maternas, por exemplo, interfiram com o adequado desenvolvimento fetal. De fato, condições placentárias patológicas podem ser identificadas em até 65% dos casos de óbito intrauterino (37). A adequada invasão placentária do leito miometrial oferece tensão de oxigênio suficiente para que as vilosidades placentárias se estabeleçam, inicialmente com várias ramificações (‘*branched angiogenesis*’) e, em seguida, apenas por alongamento dos vilos (‘*non branched angiogenesis*’) (38). Esta última fase de desenvolvimento angiogênico ocorre em concomitância ao acúmulo de gordura e desenvolvimento de adipócitos fetais.

Parâmetros de referência para acompanhamento do que seria um crescimento fetal ótimo têm se modificado ao longo dos anos. O conceito de baixo peso ao nascer (i.e., <2500g) teve sua importância histórica ao considerar recém-nascidos sob maior risco de morte perinatal (23,39). Este ponto de corte ainda permanece como um dado absoluto facilmente comparável entre os estudos e como uma medida de saúde de uma população (40). Entretanto, as evidências mais recentes demonstram que o desenvolvimento fetal se adapta diante de uma multiplicidade de fatores, sejam constitucionais maternos ou ambientais.

A influência da alimentação materna sobre o peso ao nascer é longamente conhecida, e ainda mais evidente a partir dos estudos sobre a ‘Fome

Holandesa'. Entre 1944 e 1945, a Holanda foi invadida pelo exército alemão; o embargo ao transporte de alimentos permitia o suprimento apenas através dos rios ou canais (41). Estes, porém, congelaram durante um inverno particularmente rigoroso, de modo que os estoques alimentícios precisaram ser racionalizados na região norte do país (41). Apesar de gestantes, lactantes e crianças terem quantidade extra de comida ofertada (41,42), a dieta das gestantes alcançou um nadir de cerca de 731 quilocalorias/dia em fevereiro de 1945 (43). Estima-se que os principais déficits ocorreram em relação aos aportes de proteínas, cálcio e vitaminas A, B2 (riboflavina) e B3 (niacina) (42). As mulheres que já estavam grávidas no período, i.e. em que houve subnutrição no 2º ou 3º trimestres da gravidez, tiveram filhos com aproximadamente 240g a menos em comparação àquelas cujos partos ocorreram no início do período (42,43).

É interessante observar, porém, que a interrupção intermitente de aporte nutricional pode não impactar sobremaneira o peso ao nascer. Uma recente revisão sistemática avaliou o efeito do jejum durante o mês do Ramadã, costume típico de culturas muçulmanas, sobre os desfechos perinatais. Houve marcante heterogeneidade entre os estudos e ausência de dados sobre mortalidade perinatal, tempo total de jejum diário e idade gestacional em que houve tal restrição alimentar. Apesar de apenas um estudo identificar diminuição do peso placentário, a meta-análise não encontrou diferenças no peso dos recém-nascidos cujas mães fizeram ou não o jejum (44). Tal fato pode indicar que, diante de uma dieta materna balanceada, o feto consegue manter sua homeostase e padrão de crescimento.

O entendimento de que cada feto tem um padrão ótimo de crescimento a ser alcançado ainda dentro do útero alude à interação entre os fatores genéticos (não apenas ao nível individual, mas também populacional) e ambientais. A real

interferência da etnia materna (32,45,46), da altitude de residência (14,47) ou da suplementação alimentar durante a gravidez é ainda bastante controversa. Uma vez que as definições para RCF e PIG diferem entre os estudos, é possível que tais características tenham importâncias diferentes em contextos também díspares.

No tocante à suplementação, por exemplo, a Organização Mundial da Saúde (OMS) orienta a ingesta diária de ferro e ácido fólico durante a gestação (48) para reduzir os riscos de baixo peso ao nascer e anemia materna. Iniciar o uso de folato antes mesmo da concepção tem, inclusive, efeito protetor para o peso ao nascer menor que o percentil 10 (P10) (razão de chances ajustada, aOR, 0,80; IC 95% 0,71-0,90) ou o percentil 5 (aRR 0,78; IC 95% 0,66-0,91). Esta recomendação parece ser ainda mais importante para países em desenvolvimento, em que várias deficiências nutricionais podem coexistir. O uso de polivitamínicos contendo ferro e ácido fólico também mostrou efeito protetor para recém-nascidos PIG na revisão sistemática da Biblioteca Cochrane (49).

Vários microelementos já foram estudados quanto à participação no ganho de peso fetal. Os ácidos graxos são peças chave do metabolismo humano, pois exercem funções energéticas e estruturais, além de serem precursores metabólitos das prostaglandinas e compostos relacionados (50). A gordura total livre pode ter relação linear com o peso fetal, independente do trimestre gestacional (51). Na gravidez, o consumo de frutos do mar, ricos em vitamina D, microelementos e ácidos graxos poli-insaturados tem demonstrado resultados conflitantes em relação ao risco de recém-nascido PIG, e talvez o tipo e quantidade de peixe consumido influencie nos achados (52). Ao mesmo tempo, os níveis eritrocitários maternos de ácido graxo docosahexaenóico, ômega 3, parecem ter relação positiva com o peso fetal a termo acima de 2500g (53). A partir de estudos de intervenção, porém, ainda

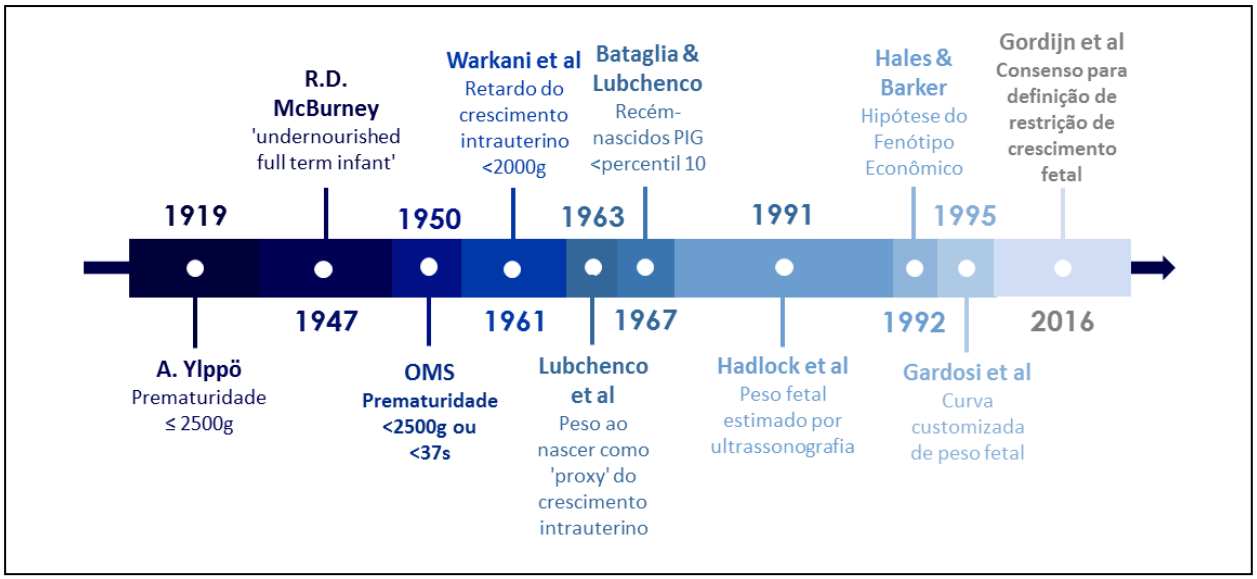
não há evidências que suportem a suplementação adicional de ácidos graxos para diminuir a prevalência de PIG (54,55), ou para evitar a recorrência de restrição de crescimento (56).

2.3. Restrição de crescimento fetal

Breve histórico da restrição de crescimento fetal

A identificação da restrição de crescimento fetal como uma entidade fisiopatológica distinta confunde-se com a história das síndromes hipertensivas na gestação e da prematuridade (Figura 1). De fato, na primeira metade do século XX, os conceitos de idade gestacional ao nascer e peso ao nascer se sobrepunham e, de certa forma, os estudiosos e clínicos da época já reconheciam que os recém-nascidos de gestantes hipertensas estavam mais propensos a apresentarem menor peso (57,58).

Figura 1. Fatos históricos marcantes na identificação e conceito da RCF.



Até 1919, vários critérios eram utilizados para definir prematuridade, como o comprimento ao nascer <46cm ou o peso ao nascer <2275g ou <2750g (23). Neste ano, Prof. Arvo Ylppö propôs o ponto de corte de <2500g para caracterizar o parto pré-termo a partir de um estudo longitudinal com 2168 recém-nascidos vivos (23,59). A partir de então, vários autores adotaram esta descrição. Em 1950, no Relatório Final do '*Expert Group on Prematurity'*, a Organização Mundial de Saúde (OMS) endossava essa recomendação (23,39). Havia a ressalva, porém, de que fossem também considerados 'prematuros' (ou 'imaturos') os neonatos cujo peso ao nascer fosse desconhecido, mas que a idade gestacional fosse estimada em 37 semanas ou menos (39).

Entretanto, a subnutrição intrauterina chamava a atenção de vários autores, incomodados com recém-nascidos 'pseudoprematuros' e em crônico sofrimento devido a possível insuficiência da placenta (57,60–62). Os autores descrevem o constrangimento dos colegas Obstetras ao assistirem a estes partos, principalmente quando não existia síndrome hipertensiva materna associada (58). Os primeiros casos relatados datam de 1947, denominados por McBurney de '*small undernourished full term infants*' (58). Outras nomenclaturas na época eram *'small full term infant'* (57,62) ou ainda '*light for dates'* (63), numa clara alusão à estimativa adequada da idade gestacional e de que o peso esperado não havia sido alcançado.

Na série de Rumbolz & McGoogan (57,62), tais recém-nascidos foram caracterizados por peso ao nascer abaixo de 2.050g. Tais autores descreveram uma alta taxa de mortalidade intrauterina (40%), e sugeriram que a interrupção da gestação seria uma conduta salvadora para estes fetos, ao livrá-los da hipóxia. Curiosamente, já consideravam a possibilidade de existir uma insuficiência placentária 'pura', ou seja, não associada a outras doenças maternas (57,62).

Em 1961, foi proposto o termo ‘retardo do crescimento intrauterino’ (*intrauterine growth retardation*, IUGR) (58). Warkany e colaboradores ponderaram que o IUGR compreende ‘todas as condições no período intrauterino que culminam em uma expressiva redução em tamanho’ ao nascer (58). A quase totalidade dos casos descritos pelos autores era de crianças a termo com peso inferior a 2000g, que apresentaram fenótipos variados ao nascer e durante a infância. Há descrição detalhada do heredograma e do seguimento pediátrico de muitos desses casos; algumas mães já haviam apresentado outros recém-nascidos com peso semelhante ou inferior, e outras crianças descritas apresentavam malformações congênitas (possivelmente cromossomopatias). Apesar de não definirem claramente o conceito de IUGR, e de o ponto de corte de 2000g ser reconhecidamente arbitrário, consideraram a importância da relação entre o peso observado e o esperado para a idade gestacional.

O uso do peso ao nascer para avaliação do crescimento intrauterino não é incomum. Na primeira curva de peso ao nascer proposta por Lubchenco e colaboradores (64), em 1963, essa assunção já era ponderada, uma vez que o crescimento intrauterino seria a fase inicial do crescimento pós-natal (63). Em 1967, esses autores sugeriram o termo ‘pequeno para a idade gestacional’ como os recém-nascidos com peso inferior ao percentil 10 para idade gestacional e sexo (24). Este grupo de recém-nascidos seria caracterizado por taxas de mortalidade duas vezes maior quando comparados aos que nasceram entre os percentis 25 e 75. Além disso, os autores justificaram que havia certa concordância, entre as curvas disponíveis à época, para o percentil 10 de peso ao nascer (63).

Um marco importante na Obstetrícia foi a introdução da ultrassonografia. A avaliação fetal em tempo real permitiu a identificação pré-natal de gestações

múltiplas e da estimativa de peso, por exemplo. O cálculo proposto por Hadlock e colaboradores (15) é um dos mais utilizados mundialmente, e quando comparado à curva do *International Fetal and Newborn Growth Consortium for the 21st Century* (INTERGROWTH-21st), por exemplo, mostra melhor acurácia para identificação dos recém-nascidos PIG (65). Na década de 1990, o termo ‘restrição de crescimento fetal’ já se referia ao feto que não atingiu seu potencial ótimo de crescimento (66), e esse conceito ainda é seguido pelo *American College of Obstetricians and Gynecologists* (ACOG) e outras sociedades internacionais (16,67).

Infelizmente, esse permanece um conceito difícil de ser mensurado, mesmo entre especialistas (68). É comum que o potencial de crescimento seja traduzido matematicamente como um ponto de corte numa curva de peso fetal por idade gestacional. O ACOG (67) e o *Royal College of Obstetricians and Gynecologists* (RCOG) (69), por exemplo, identificam o feto como restrito quando seu peso estimado pela ultrassonografia está abaixo do percentil 10 para curvas de peso fetal (16). No Quadro 1, os valores correspondentes para os percentis 3, 5 e 10 de algumas curvas de peso ao nascer estão comparadas aos pesos fetais estimados com 37 semanas.

Entretanto, a definição matemática baseada na expectativa de crescimento fetal ótimo não reflete a interpretação clínica dessa população. Um recém-nascido com peso no percentil 25 pode ter sofrido alguma restrição ao crescimento quando o seu potencial seria de nascer no percentil 50, por exemplo. Por outro lado, ter um peso fetal abaixo do percentil 10 pode ser constitucional em até 70% dos casos (35), e esses fetos estariam suscetíveis a intervenções obstétricas iatrogênicas. Além disso, o percentil 10 de adequação do peso não necessariamente identifica todos os fetos sob maior risco de morbimortalidade

perinatal. Mesmo recém-nascidos classificados como adequados para a idade gestacional (AIG) apresentam desfechos negativos no período perinatal (p.ex., parto operatório) quando houve decréscimo da velocidade ou do padrão de crescimento (70). A chance de morte perinatal é nitidamente maior nos recém-nascidos abaixo do percentil 10, e tem prevalência decrescente até o percentil 93 (71–73). Porém, as menores taxas de morbidade neonatal parecem ocorrer entre os percentis 75 e 90 (71–73).

Portanto, curvas customizadas de peso fetal e neonatal foram desenvolvidas a partir de 1995 (30,74). Estas também levam em consideração as características maternas que podem interferir com o peso ao nascer, tais como paridade, antropometria (altura e peso), ordem de nascimento e etnia. Na análise dos desfechos adversos perinatais, os percentis customizados de peso têm melhor performance para identificar os recém-nascidos sob risco (75,76).

Diagnóstico da RCF

A partir da série de casos de Warkani e colaboradores (58), o conceito de IUGR foi ainda mais discutido na literatura. Em 1967, foi usado o termo ‘retardo do crescimento fetal’ (‘*fetal growth retardation’,* FGR) (77), que só foi adicionado ao vocabulário controlado da base de dados PubMed em 1978. Nas últimas duas décadas, porém, tem sido preferido o termo ‘restrição do crescimento fetal’ (RCF) (16,78). Segundo o Dicionário Michaellis da língua portuguesa, o vocábulo ‘retardo’ significa ‘ato ou efeito de retardar’, ‘tornar mais lento; atrasar; demorar’, ou ‘fazer chegar mais tarde’. Ao mesmo tempo, define ‘restrição’ como ‘limitação imposta à realização de algo’ (79). Neste último caso, no idioma inglês, a ideia de constrição e impedimento é a mesma (80), e está de acordo com a observação de que estes

fetos e recém-nascidos apresentam um impedimento patológico ao crescimento. ‘Retardo’ pode dar a falsa ideia de que a condição – seja genética ou ambiental - que interferiu com o ganho de peso fetal é reversível (78). E as evidências recentes reforçam que tais interferências modulam a resposta do indivíduo a curto, médio e longo prazos.

Quadro 1. Percentis 3, 5 e 10 para o peso fetal e o peso ao nascer com 37 semanas de idade gestacional em diferentes curvas populacionais.

	Percentil 3		**Percentil 5**		**Percentil 10**	
	Feminino	**Masculino**	**Feminino**	**Masculino**	**Feminino**	**Masculino**
Curvas de peso ao nascer						
Lubchenco & cols, 1963 (14)					2220g	2330g
Gruenwald, 1966 (81)			2220g		2280g	
Alexander & cols, 1996 (82)			2357g		2484g	2596g
Pedreira & cols, 2011 (83)	2042g	2113g	2171g	2247g	2361g	2436g
INTERGROWTH, 2014 (32)	2110g	2130g			2330g	2380g
OMS, 2017 (84)			1968g	2062g		
Curvas de peso fetal estimado por ultrassonografia						
Hadlock & cols, 1991 (15)	2271g				2513g	
INTERGROWTH, 2014 (32)	2016g		2190g		2321g	
OMS, 2017 (84)			2372g		2537g	

De fato, na década de 1990, a classificação da RCF em simétrica e assimétrica já chamava a atenção para a necessidade de seguimento longitudinal do

crescimento fetal (66,85). Por se basear na razão entre a circunferência cefálica e a abdominal, relacionava a proporcionalidade fetal ao momento em que houve danos às fases de hiperplasia ou hipertrofia celulares (66,85). O RCF simétrico ocorreria principalmente em função de infecções congênitas e anomalias cromossômicas, ao passo que a insuficiência placentária estaria associada ao tipo assimétrico (35,66,85).

Com o avanço da ultrassonografia em Obstetrícia, especialmente com a incorporação do estudo Doppler, a identificação e o manejo de fetos fenotipicamente distintos quanto aos desvios do crescimento, submetidos a graus diferentes de disfunção placentária, foi possível (86). Atrasos no desenvolvimento intrauterino em fetos morfologicamente normais têm sido classicamente atribuídos à insuficiência da placenta prover nutrientes e oxigênio de acordo com as demandas fetais (67). Tal disfunção placentária é um conceito vago, e difícil de ser avaliada de forma objetiva. Dentro de um largo espectro de gravidade, inclui alterações de vascularização materno-fetal (p.ex., incisuras protodiastólicas nas artérias uterinas) e culmina com a morte fetal anteparto (87). Desse modo, compreende-se atualmente que os fetos com anomalias genéticas têm trajetórias de crescimento próprios, e que a classificação de fetos restritos e morfologicamente normais de acordo com a idade gestacional em que é identificada tem maior aplicabilidade clínica.

A RCF precoce é identificada antes da 32ª semana de idade gestacional e, a princípio, está relacionada a um quadro clínico fetal e neonatal mais grave (86). Coexiste com as síndromes hipertensivas da gestação em até 30% dos casos (35,86) e sua fisiopatogenia envolve uma placentação inadequada (avaliada, por exemplo, pelo índice de pulsatilidade, IP, aumentado das artérias uterinas). Evolui com crescente hipóxia (aumento da impedância da artéria umbilical), centralização

da circulação fetal (dilatação da artéria cerebral média) e acidose fetal (ducto venoso e istmo aórtico com pulsatilidades aumentadas) (86,88). A RCF tardia é diagnosticada a partir de 32 semanas, e seu principal achado é a diminuição da razão cérebro-placentária (IP da artéria cerebral média/ IP da artéria umbilical). Também há progressão para hipóxia e acidose fetais, e a descompensação fetal (ex., desacelerações cardiotocográficas, ou até mesmo óbito intrauterino) pode ocorrer diante de pequenos estímulos, como contrações uterinas (86).

Assim, a RCF passou a ser vista como uma síndrome, e o peso fetal abaixo de um determinado ponto de corte é apenas uma de suas manifestações. De fato, outras medidas biométricas fetais (ex., circunferência abdominal) e parâmetros funcionais (ex., Doppler de vasos maternos, placentários e fetais) passaram a fazer parte dos critérios definidores de RCF em vários estudos nos últimos quinze anos (89–91). Recente consenso em obstetrícia (Figura 1), que reuniu a opinião de 45 especialistas (92), concordou com a distinção da restrição de crescimento entre precoce (<32 semanas) e tardia (≥32 semanas), e propôs critérios isolados e contributivos (92) (Quadro 2). Aguarda-se a validação deste promissor consenso em um cenário clínico.

Epidemiologia da restrição de crescimento fetal

A RCF tem prevalência díspar ao redor do mundo, e difícil de ser estimada. Primeiramente, um dos principais complicadores é a heterogeneidade de conceitos nos diferentes estudos (93) e as múltiplas curvas de peso fetal ou neonatal empregadas (76,94–96). Em segundo lugar, a ultrassonografia não é um exame amplamente disponível no mundo, especialmente em países em desenvolvimento. Por último, as medidas biométricas fetais e o cálculo do peso são apenas uma

estimativa, sujeitas a erros sistemáticos de medida, por parte dos observadores (25). Portanto, é ainda comum observar, na literatura, o uso do peso ao nascer como '*proxy'* para o desenvolvimento fetal, e o recém-nascido PIG como representação da RCF (7,20,27,97,98).

Quadro 2. Critérios diagnósticos para restrição de crescimento fetal à ultrassonografia (adaptado de Gordijn e colaboradores, 2016) (92).

Critérios	**RCF precoce** **<32 semanas**	**RCF tardia** **≥32 semanas**
Isolados	- PFE <P3; ou - CA <P3; ou - Fluxo umbilical diastólico ausente	- PFE <P3; ou - CA <P3
Contributivos	- PFE <P10 ou - CA <P10 Associados a um dos seguintes: - IP artéria umbilical >P95; ou - IP artérias uterinas >P95	Pelo menos dois dos seguintes: - PFE <P10 ou CA <P10; - RCP <P5 ou IP artéria umbilical >P95; - IP artéria umbilical >P95; - Mudança de dois quartis nas curvas de PFE ou AC

RCF: restrição de crescimento fetal. PFE: Peso fetal estimado; CA: Circunferência abdominal; IP: índice de pulsatilidade; RCP: relação cérebro-placentária.

A prevalência de neonatos PIG varia amplamente de acordo com a referência empregada, e tende a ser maior com o uso de curvas customizadas (74,99). Em um estudo multicêntrico europeu e australiano, houve 11,3% de fetos PIG (27). Nos Estados Unidos, a prevalência de recém-nascidos PIG aumentou de 9,4% para 11,7% com o uso de curva customizada (100), e de 10% para 15% em outro estudo (76). Na Austrália, houve 50% mais neonatos PIG com o uso da customização, e prevalência atingiu 11,6% (75,96). Nestes estudos, os recém-nascidos classificados como PIG apenas pela curva populacional não apresentaram eventos neonatais adversos, ao passo que os identificados pela curva customizada

estiveram relacionados com maior morbimortalidade neonatal, como asfixia (escore Apgar <7 no 5º minuto de vida), admissão em unidade de terapia intensiva (UTI) neonatal, suporte ventilatório e morte intrauterina. Além disso, curvas populacionais subestimaram a prevalência de recém-nascidos PIG em mães obesas (101).

Os países de baixa e média renda são heterogêneos quanto aos seus sistemas de saúde e às populações. Em 2010, estima-se que 27% dos recém-nascidos foram classificados como PIG (curva de Alexander e colaboradores (82)), mas a maior prevalência foi vista no sul asiático: dos cerca de 39 milhões de nascidos vivos, 45% foram PIG (102). Em 2012, calcula-se que 19,1% dos nascidos vivos (i.e., 23,3 milhões de neonatos em 2012) tenham apresentado peso ao nascer abaixo do 10º percentil da curva do INTERGROWTH-21st (40). Os valores de referência propostos pelo estudo INTERGROWTH-21st classificam menor número de recém-nascidos como PIG, de modo que sua utilização pode não identificar corretamente os bebês sob maior risco perinatal (76,96). Considerando-se os países individualmente, as taxas podem ser tão baixas quanto 10%, no Nepal, quando se aplica uma curva africana de referência, ou tão altas quanto 78%, na Índia, quando são usadas curvas propostas originalmente para populações americanas ou europeias (102,103).

No Brasil, um estudo numa maternidade terciária em São Paulo identificou 17,9% dos recém-nascidos como PIG a partir da curva de Alexander e colaboradores (82), e 9,3% foram PIG pela curva do INTERGROWTH-21st no Rio de Janeiro (104). Porém, a prevalência de PIG alcançou 44% dos recém-nascidos entre 32 e 35 semanas em um outro estudo (105). Infelizmente não há dados nacionais compilados sobre a adequação do peso ao nascer; segundo dados do Ministério da

Saúde, o baixo peso ao nascer (<2500g) correspondeu a 8,3% recém-nascidos (106) e 65,8% dos natimortos em 2014 (107).

Repercussões da restrição de crescimento fetal

A restrição de crescimento fetal relaciona-se a desfechos adversos no período perinatal, na infância e na idade adulta. Considerando-se a longa latência de alguns eventos, como os atrasos cognitivos e doenças cardiovasculares, considera-se que a RCF é uma condição impactante do ponto de vista de saúde pública (102).

Não é surpreendente o fato de que os países que lideram o *ranking* em números absolutos de óbitos fetais e neonatais (108) e de RCF/PIG (102) sejam os mesmos: Índia, Paquistão e Nigéria. A morte intrauterina é o principal componente das taxas de mortes perinatais (109). A RCF pode responder por até metade dos óbitos fetais por causas originalmente desconhecidas (110), sendo cerca de seis vezes maior a chance de óbito fetal a termo (risco relativo, RR, 6.0; IC 95% 3.1-11.5) (75) ou quando o peso está abaixo do percentil 5 (comparado aos percentis 10-90) (111). A investigação e manejo adequado da RCF é uma das principais estratégias para redução das perdas fetais anteparto, e é mundialmente encorajada (40,112,113).

Além da morte neonatal (19,75,76,100,114), outros eventos adversos têm sido descritos para os recém-nascidos PIG: escore Apgar <7 (75,96) ou <5 (76,96,114) no 5º minuto de vida, admissão em UTI neonatal (75,96), síndrome da angústia respiratória do recém-nascido (19,29,76), suporte ventilatório (75,76), enterocolite necrotizante (76), sepse neonatal (19,29,76), convulsão (19,76,114), hemorragia periventricular (19,76,114), hipoglicemia (29), e icterícia (29).

Apesar da diversidade dos estudos e das definições utilizadas para a RCF/PIG, a restrição de crescimento esteve associada a menor escore na escala de Bailey na primeira infância, nas habilidades de comunicação (22) e na frequência de distúrbios do sono (115). O atraso das habilidades motoras e do desenvolvimento cognitivo foi mais marcante nos fetos PIG que sofreram redistribuição do fluxo sanguíneo intrauterino (ex., fluxo umbilical ausente ou reverso, dilatação da artéria cerebral média ou onda a ausente no ducto venoso) (22,116–118). Há também repercussões metabólicas: já na infância pré-púbere, os recém-nascidos PIG apresentam níveis séricos superiores de insulina (e do índice de resistência à insulina, HOMA-IR), e os que nasceram abaixo do percentil 3 também apresentaram níveis superiores de leptina (28).

A epigenética tem ajudado a explicar o papel dos estímulos ambientais no ambiente intrauterino sobre os desfechos a médio e longo prazos observados com os recém-nascidos PIG. A metilação do DNA ou das histonas, ou a acetilação das histonas, são os principais mecanismos epigenéticos descritos (119); é possível que a hipóxia, distúrbios na transferência de nutrientes (macro e micromoléculas), ou redistribuição do fluxo sanguíneo, por exemplo, influenciem nesses processos. Diferentes taxas de metilação do DNA de genes relacionados ao mecanismo de apresentação de antígenos, sinalização intracelular da insulina, e biossíntese de esteroides já foram identificadas em crianças nascidas PIG (120). Na idade adulta, neonatos PIG apresentam maior gordura visceral (121) e livre (122), especialmente na região abdominal (123). Estudos com adultos que nasceram durante o período da Fome Holandesa (124,125) ou Chinesa (126) mostram maior prevalência de síndrome metabólica, obesidade e intolerância aos carboidratos, mas a real

participação dos recém-nascidos PIG na prevalência populacional de diabetes mellitus é questionada (127).

2.4. Avaliação pré-natal da restrição de crescimento fetal

Avaliação de risco clínico

A história clínica e o exame físico são as primeiras abordagens durante a consulta pré-natal, e vários fatores de risco para distúrbios do crescimento fetal podem ser identificados.

A história de a mãe ter nascido com peso abaixo de 2500g (5) ou 3000g (27) tem aparecido como um importante item da anamnese: esteve associado a um risco duas vezes maior de a gestação atual ser de um recém-nascido PIG. Por outro lado, tanto a nuliparidade (75,128,129) como a história obstétrica pregressa de feto com restrição de crescimento são fatores de risco ainda mais fortes. Um recém-nascido PIG anterior, na presença ou não de pré-eclâmpsia, está associado a um novo PIG na gestação seguinte (129). Caso o PIG anterior tenha sido pré-termo, há uma chance 4 a 5 vezes maior de óbito intrauterino na gestação subsequente (26). Não se sabe quais mecanismos estão envolvidos neste processo: se algum fator já é inerente à mãe, ou se o feto anterior disparou algum processo metabólico que permanece na gestação seguinte.

O tabagismo é, possivelmente, o mais importante fator comportamental relacionado ao crescimento fetal. Estima-se que a diferença de peso chegue a ser cerca de 250g menor em fetos de mães fumantes (130). Em meados do século XX, quando o tabagismo era socialmente estimulado, o menor peso fetal surgia como vantagem adicional para as parturientes fumantes. Neste primeiro quarto do século

XXI, a prevalência do tabagismo diminuiu consideravelmente, mas seus efeitos sobre o crescimento fetal ainda são perceptíveis (6,27,75,129–131). O tabagismo induz a formação de espécies reativas de oxigênio e estresse oxidativo, com diminuição do volume dos capilares fetais placentários (132). A nicotina é uma substância vasoconstritora, e talvez atue como mediadora da hipóxia placentária (132). A hipóxia, por sua vez, teria repercussões fetais diferentes a depender da idade gestacional em que houve exposição ao tabaco. Uma outra hipótese é que o tabaco interfira com a atividade da 11-beta-hidroxisteróide desidrogenase, ou seja, com os níveis de glicocorticoides na circulação fetal (133). No cordão umbilical, os recém-nascidos de mães fumantes apresentam maiores níveis de interleucina-8, sugerindo alguma participação na mediação imunológica (134). Uma revisão sistemática recente não encontrou alterações das medidas fetais quando houve exposição ao tabagismo apenas no 1º trimestre, ao passo que as medidas do diâmetro biparietal, circunferência abdominal e fêmur mostraram-se significativamente inferiores quando o feto foi exposto no 2º ou 3º trimestre (130). Entretanto, a cessação do tabagismo ou diminuição de sua intensidade só se mostrou benéfica em relação ao peso ao nascer quando ocorreu até o 4º mês de gestação (135).

Estima-se, ainda, que 15-30% das gestações múltiplas apresentem RCF, principalmente as monocoriônicas (35). Entre as condições crônicas maternas, as síndromes hipertensivas e as trombofilias são as mais consistentemente relacionadas à RCF. A história de RCF em gestação anterior está associada a um risco relativo de 1,4 (IC 95% 0,6-3,0) para pré-eclâmpsia na gestação atual (136). A Sociedade Internacional para o Estudo das Síndromes Hipertensivas na Gestação (137) considera a identificação de RCF como parte do diagnóstico de pré-eclâmpsia.

O passado obstétrico de abortamentos ou mortes de fetos morfologicamente normais permanece sendo critério clínico para a síndrome do anticorpo antifosfolípide (138), e acredita-se que trombofilias hereditárias até então clinicamente não diagnosticadas podem estar envolvidas com a RCF (139).

No que tange ao exame físico, a altura e o peso maternos na gestação entram no cálculo dos percentis customizados de peso (74,99). A menor estatura e peso aparecem associados aos PIG em alguns estudos (97,100), mas demonstraram apenas 43% e 73% de sensibilidade, respectivamente, para a identificação destes (140). O índice de massa corpórea e o ganho de peso maternos ao longo da gestação também apresentaram baixa acurácia preditora, e a área sob a curva (AUC) r*eceiver operating characteristic (*ROC) foi de 0,56 e 0,60, respectivamente (140). A performance da medida da altura do fundo uterino em predizer o recém-nascido PIG aumenta com a idade gestacional (141). Quando comparada à palpação abdominal, uma revisão da Biblioteca Cochrane não identificou diferenças na detecção de PIG com o seu uso sistemático (RR, 1,32, IC 95% 0,92-1,90) (33). Entretanto, por ser de baixo custo e estar inserida na rotina do exame físico obstétrico, os autores aconselham seu uso, e os profissionais de saúde devem associá-la a alguma outra técnica ou avaliação do crescimento fetal.

O RCOG orienta que todas as gestantes com um fator maior de risco clínico (razão de chances, OR, >2,0) ou três menores devem ser avaliadas com o uso da Dopplervelocimetria no 2º trimestre (69). No Quadro 3 estão sumarizados os fatores clínicos maiores para PIG (peso ao nascer abaixo do percentil 10). Nuliparidade, baixa ingesta de frutas, intervalo entre as gestações inferior a 6 meses ou superior a 60 meses, entre outros, foram considerados fatores de risco menores.

Quadro 3. Fatores de risco clínico que indicam investigação adicional com Doppler de artérias uterinas ou umbilicais no 2º trimestre. (Adaptado de RCOG, 2013) (69).

Fatores de risco	Razão de chances	IC 95%
Características avaliadas na 1ª consulta pré-natal		
Idade materna ≥40 anos	3.2	1.9–5.4
Tabagismo (≥11 cigarros/dia)	2.21	2.03–2.4
Abuso de substâncias – cocaína	3.23	2.43–4.3
Exercício vigoroso diário	3.3	1.5–7.2
Mãe ter nascido PIG	2.64	2.28–3.05
Pai ter nascido PIG	3.47	1.17–10.27
Recém-nascido PIG anterior	3.9	2.14–7.12
Morte fetal intrauterina anterior	6.4	0.78–52.56
Hipertensão crônica	2.5	2.1–2.9
Diabetes mellitus com doença vascular	6.0	1.5–2.3
Doença renal crônica	5.3	2.8–10
Intercorrências na gestação atual		
Sangramento na 1ª metade da gestação	2.6	1.2–5.6
Pré-eclâmpsia	2.26*	1.22–4.18
Baixo ganho de peso materno	4.9	1.9–12.6

*Risco relativo.

Avaliação de risco com a ultrassonografia

A ultrassonografia é o mais estudado exame complementar no rastreio de complicações obstétricas. Para predição dos recém-nascidos PIG, há estudos com o uso de medidas biométricas ou de estudo Doppler de vasos maternos.

A medida biométrica isolada mais investigada como preditora do peso ao nascer é a circunferência abdominal, que também figura como um dos critérios

diagnósticos no recente consenso para definição de RCF (Quadro 2) (92). A estimativa de peso fetal também é estudada como preditora do peso ao nascer, com acurácia crescente quanto mais próxima do parto. Porém, estudos observacionais e ensaios clínicos não comprovam os benefícios do rastreio universal (20,142,143), e apenas a Sociedade Francesa de Ginecologia e Obstetrícia recomenda ultrassonografia universal no 3º trimestre, a partir de 32 semanas (16).

O estudo Doppler das artérias uterinas reflete a invasão trofoblástica das artérias espiraladas, vasos da junção miométrio-decidual. Em gestações normais, o número de artérias espiraladas remodeladas tem relação direta com o tamanho do leito placentário (144). Considerando-se que uma vascularização do órgão pressupõe seu adequado funcionamento, a presença de incisuras protodiastólicas ou de aumento da impedância ao fluxo sanguíneo são marcas da placentação deficiente (144). No primeiro trimestre, a sensibilidade e a especificidade do Doppler de uterinas atingem 39% e 93%, respectivamente, para a suspeição de RCF precoce (145), e a sensibilidade permanece em cerca de 40% quando o exame é feito no segundo trimestre (146). Há, portanto, quem desencoraje o rastreio universal nesses modos, pois o número necessário para tratar seria alto o suficiente para colocar muitas mulheres sob o risco teórico de RCF. Além disso, haveria poucas estratégias possíveis de serem implementadas quando já houve a implantação placentária inadequada.

Avaliação de risco com o uso de biomarcadores

A terceira estratégia para avaliação de risco é a dosagem de biomarcadores. Um biomarcador é qualquer fator que tenha a capacidade de predizer, diagnosticar ou identificar o prognóstico de uma condição, mas que não

necessariamente está envolvido com a fisiopatologia da doença (147). De acordo com a OMS, um biomarcador reflete a interação de um sistema biológico com um potencial evento de risco à saúde (148). Neste contexto, a mensuração laboratorial de substâncias relacionadas ao funcionamento placentário e a RCF tem tido crescimento expressivo nas últimas três décadas.

O traço comum nesses estudos é a dosagem de compostos, especialmente proteínas, muitas delas ligadas ao funcionamento placentário. No início do 2º trimestre (15 semanas), os níveis séricos da proteína placentária A (PAPP-A), do fator de crescimento placentário (PlGF) e da insulina são significativamente menores nas gestações que culminarão com o recém-nascido PIG (6). E maiores níveis plasmáticos de fator de crescimento vascular (VEGF), entre 34 e 37 semanas, se relacionaram a menor chance de fetos PIG (OR 0,8; IC 95% 0,71-0,92) (149).

A razão sFlt-1/PlGF≤38 é promissora para excluir casos suspeitos de pré-eclâmpsia pré-termo (150,151), mas a morbidade neonatal não é menor quando o manejo clínico da gestante é baseado nos valores de PlGF (152). Similarmente, os níveis de PlGF nas gestações com RCF são marcadamente reduzidos no 2º e 3º trimestres (153–155). Porém, ainda demonstra acurácia modesta para ser implementado isoladamente na prática clínica: área sob a curva ROC foi de 0,66 (IC 95% 0,44-0,87) para a predição de RCF (34). Talvez esta performance seja devida à definição de RCF utilizada pelos estudos incluídos na revisão sistemática, que consideraram tanto a medida estimada de peso fetal, o peso ao nascer ou a presença de achados adicionais de gravidade da condição patológica (ex., oligoâmnio).

Os estudos com melhor acurácia elaboraram modelos preditores com a combinação de múltiplos fatores clínicos maternos, ultrassonográficos e bioquímicos. Em uma coorte internacional de nulíparas (6), o PlGF mostrou área sob a curva ROC de 0,84 (IC 95% 0,78-0,89) para os casos de fetos PIG com doença hipertensiva materna quando combinado com tabagismo, proteinúria, índice de resistência da a. uterina, PAPP-A e triglicerídeos. No segundo trimestre (19-24 semanas), o PlGF e a alfa-feto proteína, também combinados com fatores maternos e da biometria fetal, compuseram um modelo cuja área sob a curva ROC foi superior a 0,96 para a predição de parto antes de 32 semanas em recém-nascidos PIG (97).

2.5. O papel das novas tecnologias em saúde reprodutiva

Atualmente, a suspeição antenatal dos recém-nascidos PIG acontece em menos da metade dos casos, mesmo quando se indica um parto prematuro terapêutico (29). Portanto, faz-se mister a procura de novas tecnologias e métodos de predição. A era pós-genômica tem sido marcada por avanços rápidos nas chamadas ciências ômicas, que também incluem a transcriptômica, proteômica e a metabolômica. A transcriptômica se refere à avaliação das moléculas do RNA transcritas pelos genes. Em seguida, a proteômica se preocupa em estudar quais as proteínas são traduzidas a partir das moléculas de RNA. Por último, a metabolômica se dedica ao estudo de moléculas pequenas, entre 50 e 2000 Daltons, e que representam, em última análise, a complexa interação entre o indivíduo e o meio ambiente (147,156).

A primeira menção ao termo ‘metaboloma’ ocorreu em 1998 (157), mas só surgiu como vocabulário controlado na base de dados PubMed em 2009. A metabolômica foi uma ciência que partiu dos estudos de plantas, e bases de dados

já foram enormemente alimentadas. Em relação ao metaboloma humano, avanços importantes foram descritos nos estudos sobre câncer, no sentido de oferecer uma medicina individualizada para o tratamento destas condições. Nesta última década, a quantidade de artigos publicados a respeito do tema é crescente. Porém, considerando-se as especificidades dos perfis clínicos de pacientes, a pesquisa de biomarcadores é específica para cada grupo. Por isso é importante que pesquisas com gestantes sejam bem delineadas, para que seus resultados possam ser adequadamente usados.

Na gestação, o perfil metabolômico para o sangue, urina e cabelos já foi descrito. Tais investigações evidenciam uma mudança clara do metabolismo materno para atender às demandas do crescimento fetal em diferentes idades gestacionais. No primeiro trimestre, encontram-se maiores níveis de amino ácidos e derivados, como valina, isoleucina e lisina (‘*branched chain aminioacids*’) (158,159). No segundo trimestre, ocorre maior demanda fetal por amino ácidos, e estes compostos têm sua concentração diminuída no cabelo materno. Por fim, quando o feto necessita principalmente acumular energia na forma de carboidratos e lipídios, os ácidos graxos são metabólitos que estão aumentados no cabelo materno.

O perfil metabolômico pode variar entre populações semelhantes (ex., recém-nascidos entre si) na dependência de fatores genéticos, sexo, comportamentais ou de microbiota intestinal, por exemplo. Robinson e colaboradores estudaram o perfil metabolômico de recém-nascidos em quatro coortes europeias e demonstraram que acilcarnitinas (decanoilcarnitina, tetradecanoilcarnitina e hexadecenoilcarnitina) estão mais fortemente associadas ao peso ao nascer entre meninas, e as fosfocolinas, em meninos (160). Ao mesmo tempo, as lisofosfocolinas mostraram resultados contraditórios, ora menos

associadas ao peso ao nascer (160), ora mais (161). Na China, Liu e colaboradores (162) encontraram menores níveis dos ácidos aspártico e glutâmico em recém-nascidos PIG masculinos, enquanto o ácido hexacosaenóico apresentou níveis elevados em recém-nascidos PIG do sexo feminino; neste estudo não foram observadas diferenças no metabolismo das carnitinas em relação ao sexo. Lindsay e colaboradores encontraram tendência a níveis crescentes de alguns aminoácidos não essenciais (asparagina, aspartato, citrulina, glicina, glutamina) com o avançar da idade gestacional entre não-hispânicas comparadas a hispânicas (159). Tais achados reforçam a interpretação de que o crescimento fetal é heterogêneo, e pontuam a relevância dos fatores ambientais.

3. OBJETIVOS

Objetiva-se, nesta tese, analisar a restrição de crescimento fetal e os recém-nascidos pequenos para a idade gestacional de uma forma ampla, abordando a predição e o diagnóstico da condição.

São objetivos específicos desta tese:

3.1 Compreender o rastreio dos recém-nascidos PIG na prática obstétrica atual;

3.2 Analisar os fatores clínicos preditores para PIG na coorte do Preterm-SAMBA;

3.3 Descrever a utilização da metabolômica no estudo da RCF;

3.4 Estabelecer a acurácia da tecnologia da metabolômica na predição dos recém-nascidos PIG;

3.5 Identificar biomarcadores metabolômicos relacionados à predição de recém-nascidos PIG.

4. MÉTODOS

Esta tese compõe-se de dois componentes: revisão de literatura e análise dos recém-nascidos PIG do estudo Preterm-SAMBA. Para o primeiro componente, discussões levantadas no Capítulo de Introdução foram adaptadas em dois artigos de revisão narrativa de literatura. Adicionalmente, uma revisão sistemática (RS) da literatura sobre PIG e metabolômica foi realizada. Neste Capítulo, descrevemos os métodos científicos envolvidos na coorte do Preterm-SAMBA e na elaboração da RS.

4.1. Estudo Preterm-SAMBA

O *Preterm Screening and Metabolomics Brazil and Auckland* (Preterm-SAMBA) (7) foi uma coorte longitudinal brasileira desenvolvida entre Julho/2015 e Julho/2018, e coordenada pela Universidade Estadual de Campinas (UNICAMP). O desfecho primário do estudo foi o parto prematuro espontâneo, enquanto que os desfechos secundários foram a RCF, pré-eclâmpsia e diabetes mellitus gestacional. Nesta tese, serão apresentadas análises relativas à adequação do peso ao nascer dos recém-nascidos.

Cenário da pesquisa

Os cinco centros terciários que participaram foram: Centro de Atenção Integral à Saúde da Mulher (CAISM) da Universidade Estadual de Campinas (UNICAMP, Campinas, São Paulo); Maternidade da Faculdade de Medicina de Botucatu da Universidade Estadual Paulista (UNESP; Botucatu, São Paulo); Maternidade do Hospital de Clínicas de Porto Alegre da Universidade Federal do Rio

Grande do Sul (HCPA/UFRGS; Porto Alegre, Rio Grande do Sul); Maternidade do Hospital das Clínicas da Universidade Federal de Pernambuco (HC-UFPE; Recife, Pernambuco); e Maternidade Escola Assis Chateaubriand da Universidade Federal do Ceará (MEAC-UFC; Fortaleza, Ceará). Todos os centros seguiram o mesmo protocolo de pesquisa (ANEXO A) (7). Treinamento específico foi oferecido pelo centro coordenador antes do início das atividades, e monitorado durante a execução das mesmas.

População do estudo

O universo da pesquisa Preterm-SAMBA foi constituído por todas as gestantes atendidas nos serviços de pré-natal dos cinco centros participantes do estudo. A amostra foi obtida por conveniência, de acordo com critérios de inclusão e exclusão descritos a seguir.

Critérios de inclusão e exclusão

Foram incluídas mulheres com gestação única, nulíparas, entre 19 e 21 semanas de idade gestacional. Os critérios de exclusão foram amplos e corresponderam aos fatores de risco para os desfechos estudados pelo Preterm-SAMBA. Portanto, as participantes elegíveis foram excluídas caso apresentassem uma ou mais das seguintes condições:

- Idade gestacional não confirmada (data da última menstruação incerta ou ausência de exame ultrassonográfico);
- Três ou mais abortamentos prévios;
- Malformação fetal maior congênita suspeita ou anomalia confirmada por testes genéticos na gestação em curso no momento do recrutamento;

- Anomalias anatômicas uterinas, como as malformações congênitas Müllerianas (ex., útero bicorno ou septado);
- Passado de conização a frio do colo uterino;
- Hipertensão arterial sistêmica crônica em tratamento prévio à gravidez ou com níveis iguais ou superiores a 160mmHg, para a pressão sistólica, ou a 100mmHg para a diastólica;
- Outras condições crônicas: nefropatias, diabetes mellitus pré-gestacional (quaisquer tipos), síndrome do anticorpo antifosfolípide, lúpus eritematoso sistêmico, doença falciforme, infecção pelos vírus da imunodeficiência humana (HIV) ou das hepatites (B ou C);
- Intercorrências na gestação atual: cerclagem do colo uterino ou ruptura da bolsa amniótica antes do recrutamento para o estudo;
- Uso diário de medicações, como corticoides, heparina ou ácido acetilsalicílico (doses superiores a 60mg/dia);
- Ingestão de suplementos nutricionais compatíveis com consumo diário superior a 1g de vitamina C, 400UI de vitamina E, 1g de cálcio, ou 2,7g de ácido eicosapentaenoico.

Cálculo da amostra

O desfecho principal do Preterm-SAMBA foi a ocorrência de prematuridade espontânea. Para o cálculo da amostra, foi considerada a prevalência de parto pré-termo (7%) para fins matemáticos, pois é o evento gestacional de menor prevalência quando comparado a RCF, pré-eclâmpsia, ou diabetes mellitus gestacional. Assim, o tamanho amostral da coorte foi avaliado em 1150 mulheres, ao

se considerar a área mínima sob a curva ROC de 0,68; os erros amostral de 3,5%, o tipo I de 5% (*alfa),* e o tipo II de 20% (*beta)*; e a perda de seguimento de 20%.

Procedimentos operacionais

O estudo só foi iniciado em cada um dos cinco centros participantes após anuência das chefias locais, aprovação ética e treinamento inicial da equipe que coletou os dados.

As gestantes elegíveis foram identificadas a partir das mulheres atendidas nos serviços de pré-natal dos hospitais envolvidos, das Unidades Básicas de Saúde, ou do uso de quaisquer outras estratégias locais para recrutamento. A equipe pesquisadora de cada centro convidou as gestantes elegíveis. Caso concordassem, assinaram o Termo de Consentimento Livre e Esclarecido (TCLE) (APÊNDICE C).

A inclusão da participante no estudo se deu com idade gestacional entre 19^{+0} e 20^{+6} semanas de gestação. Nesta ocasião, cada equipe de pesquisadores fez uma entrevista detalhada com a participante, abrangendo informações sociodemográficas, reprodutivas, clínicas e obstétricas da gestação atual. Em seguida, foi realizado um exame físico geral, incluindo massa corpórea, altura e três aferições da pressão arterial. Exames ultrassonográficos não foram incluídos de forma sistemática no protocolo do estudo.

Dados relativos ao parto e nascimento foram obtidos a partir de registros médicos, como prontuários (i.e., quando a assistência ao parto ocorreu nos próprios centros participantes), ou resumos de alta, e cartões da criança, além de contatos telefônicos ou por correio eletrônico quando as formas iniciais falharam.

Os dados coletados foram inseridos em tempo real numa base de dados *online*, *MedSciNet*® (AB, Suécia; www.medscinet.com/samba). Para atendimento

em locais onde o sistema não esteve disponível em tempo real, formulários impressos foram utilizados e armazenados, para alimentação do banco de dados em tempo oportuno.

Variáveis dependentes

Para que os objetivos da tese fossem alcançados, variáveis dependentes distintas foram elencadas:

- Recém-nascido pequeno para a idade gestacional P10 (PIG-P10; SGA<10th): recém-nascido com peso ao nascer abaixo do percentil 10 na curva customizada de peso proposta por Gardosi e colaboradores (163). Este cálculo leva em consideração características maternas (etnia, peso na 1ª visita do estudo, altura, paridade) e neonatais (sexo, nascido vivo), além da idade gestacional no parto. Variável dicotômica, tipo sim/não. Este foi considerado o 'padrão ouro' para o diagnóstico de restrição de crescimento fetal.
- Recém-nascido adequado para a idade gestacional (AIG; AGA): recém-nascido com peso ao nascer entre os percentis 10 e 90 na curva customizada de peso (163). Variável dicotômica, tipo sim/não.

Variáveis independentes

As variáveis independentes são descritas a seguir, de acordo com o momento em que foram coletadas.

- Variáveis independentes maternas obtidas por entrevista ou exame físico na 1ª visita:

- Idade gestacional quando da inclusão na pesquisa: Idade gestacional, calculada em dias. Variável numérica discreta.
- Assistência inteiramente pública do pré-natal: local de assistência pré-natal da participante, dentro do espectro do Sistema Único de Saúde. Variável categórica tipo sim/ não.
- Idade materna: idade da participante, em anos completos, quando da 1ª visita do estudo. Calculada a partir da data de nascimento obtida em documento oficial (ex., Registro Geral). Variável numérica contínua, categorizada em ≤19 anos, 20-34 anos, e ≥35 anos.
- Escolaridade: total de anos de estudo da participante até a 1ª visita do estudo, estabelecido em função da série e do grau mais elevado alcançado pela mulher (164). Calculada considerando a última série concluída com aprovação. Variável numérica discreta, categorizada em ≤12 anos e >12 anos
- Etnia: cor da pele auto referida pela participante (164). Variável nominal categorizada em branca ou não branca.
- Remuneração: fato de a mulher receber algum tipo de pagamento, em dinheiro, por suas atividades laborativas. Variável categórica tipo sim/ não.
- Situação conjugal: condição de conviver ou não regularmente com um companheiro, do mesmo sexo ou de sexos biológicos diferentes. Variável categórica, classificada em com companheiro (casada, união estável) ou sem companheiro (solteira, viúva, divorciada).
- Baixo peso materno ao nascer: história de a participante ter nascido com peso abaixo de 2500g (23). Considerado o relato da participante ou da sua genitora. Variável categórica tipo sim/ não.

- Gestação da participante com síndrome hipertensiva: história de a genitora ter apresentado qualquer síndrome hipertensiva durante a gestação da participante. Considerado o relato da participante ou da sua genitora. Variável categórica tipo sim/ não.
- Infertilidade: *status* do casal quando não há gravidez após 12 meses de atividades sexuais regulares e desprotegidas. Variável categórica tipo sim/ não.
- Reprodução assistida: gestação atual não espontânea, resultado de procedimentos como fertilização *in vitro*, inseminação artificial ou injeção intracitoplasmática de espermatozoides. Variável considerada para as participantes consideradas como “sim” para a variável Infertilidade; tipo categórica, sim/ não.
- Primiparidade: *status* da participante quando está gestante pela primeira vez, e sem passado de abortamentos. Variável dicotômica, tipo sim/não.
- Tabagismo: ato de fumar cigarros, industrializados ou não. Variável nominal categorizada em sim (tabagista atual ou cessou na gestação) ou não (não fumou até três meses antes da concepção).
- Alcoolismo: ato de ingerir bebidas alcóolicas. Variável nominal categorizada em sim (ingere álcool até a entrevista de inclusão ou cessou durante a gestação) ou não (não bebeu bebidas alcóolicas até três meses antes da concepção).
- Uso de drogas ilícitas: uso de drogas ilícitas, por qualquer via (ex., inalatória, venosa). Variável nominal categorizada em sim (usuária atual ou durante a primeira metade da gestação) ou não (não usou até três meses antes da concepção).

- Sangramento genital: relato de qualquer sangramento genital até a 1ª visita do estudo, independente da causa, duração ou aspecto. Variável categórica, tipo sim/ não.
- Admissão hospitalar: relato de qualquer internação hospitalar, seja para tratamento clínico ou cirúrgico. Variável categórica, tipo sim/ não
- Pressão arterial sistólica (PAS): aferida através de esfigmomanômetro digital, aneroide ou de mercúrio, quando da identificação do 1º som de Korotkoff. Mensurada preferencialmente no braço direito, na altura do coração, na ausência de qualquer ingesta de cafeína ou de uso de cigarro nos 30 minutos anteriores (165). Foram obtidas três medidas, e utilizada a média aritmética, para fins estatísticos. Variável numérica discreta, expressa em mmHg.
- PAS >130mmHg: variável obtida a partir da média aritmética da pressão arterial sistólica na 1ª visita do estudo. Variável categórica, tipo sim/ não.
- Pressão arterial diastólica (PAD): aferida na mesma ocasião da pressão sistólica, quando da identificação do 5º som de Korotkoff (165). Utilizada a média aritmética das três medidas obtidas. Variável numérica discreta, expressa em mmHg.
- PAD >75mmHg: variável obtida a partir da média aritmética da pressão arterial diastólica na 1ª visita do estudo. Variável categórica, tipo sim/não.
- Pressão arterial média na 1ª visita do estudo: Variável obtida a partir das pressões arteriais sistólica e diastólica. Calculada pela fórmula: (2 x PAD +PAS)/ 3. Variável numérica discreta, expressa em mmHg.
- Índice de massa corpórea (IMC): razão entre a massa corpórea (aferida em quilogramas) e o quadrado da altura (em metros) da participante. Variável

numérica contínua, expressa em Kg/m^2, sendo considerada uma casa decimal.

- Estado nutricional: adequação do IMC em relação à idade gestacional da 1ª visita do estudo, de acordo com os valores propostos por Morais e colaboradores (166) para a população brasileira. Variável ordinal, categorizada em baixo peso, peso adequado, sobrepeso e obesidade.

- Variáveis independentes maternas coletadas em registros médicos (p.ex., resultados de exames complementares, cartão pré-natal, resumo de alta ou prontuário):

- Proteinúria: presença de qualquer proteinúria até a 1ª visita do estudo, identificada por fita urinária (≥1+ ou 30mg/dL). Variável categórica, tipo sim/ não.
- Infecção urinária: diagnóstico de bacteriúria assintomática, infecção do trato urinário baixo ou pielonefrite até a 1ª visita do estudo. Variável categórica, tipo sim/não.
- Qualquer infecção até a 1ª visita do estudo: diagnóstico clínico, laboratorial ou de imagem de qualquer infecção até 19/20 semanas de idade gestacional, incluídas as do trato genital (vulvovaginites e vaginoses), urinário, respiratório ou gastrointestinal, por exemplo. Variável categórica, tipo sim/não.

- São variáveis independentes relacionadas ao recém-nascido, obtidas a partir de registros médicos:

- Peso ao nascer: massa corpórea do recém-nascido, aferida durante a internação hospitalar. Variável numérica discreta, expressa em gramas.

- Percentil do peso ao nascimento: cálculo do percentil customizado de peso do recém-nascido, e que varia de 0 a 100 (163). Variável numérica discreta.

Financiamento

O estudo Preterm-SAMBA foi financiado através da parceria entre o Governo Federal (Ministério da Ciência, Tecnologia e Inovação; Ministério da Saúde), através do CNPq (processo CNPq 401636/2013-5), e a Fundação Bill e Melinda Gates (OPP1107597), na chamada No 05/2013.

Aspectos éticos

O estudo Preterm-SAMBA seguiu a Declaração de Helsinki e as recomendações éticas emanadas pela Resolução 466/2012 do Conselho Nacional de Saúde. Obteve aprovação dos Comitês de Ética em Pesquisa (CEP) de todos os centros participantes, e o Comitê Nacional de Ética em Pesquisa (CONEP) referendou a aprovação do CEP da UNICAMP (*Campus* Campinas) (ANEXO B).

Todas as participantes concordaram que informações sobre suas gestações e referentes ao recém-nascido fossem utilizadas para fins acadêmicos e científicos. Todas assinaram o TCLE em duas vias, e mantiveram uma cópia consigo. A abordagem da participante para coleta de dados da pesquisa ocorreu em ambiente privativo e na presença de um acompanhante de escolha da própria mulher, caso desejasse.

Aspectos estatísticos

As informações armazenadas na base de dados *online MedSciNet*® foram condensados em planilhas Excel® e transferidos para o *Social Package for*

Social Sciences 21.0 (SPSS), para devida análise estatística. O preparo do banco de dados final incluiu a renomeação, categorização, cálculo ou transformação de variáveis, quando adequado ou necessário.

Variáveis quantitativas foram submetidas ao teste de Kolmogorov-Smirnov, para avaliação de normalidade, e de Levene, para homogeneidade de variância. Em seguida, foram apresentadas a média ou mediana, desvio padrão, ou valores mínimo e máximo. As diferenças entre os grupos foram analisadas pelo teste *t* de Student ou Mann-Whitney, a depender de a distribuição ter aproximação com a curva de Gauss. Variáveis qualitativas foram expressas como frequências ou porcentagens. Comparações entre os grupos foram realizadas através do teste qui quadrado de Pearson ou Exato de Fisher. O *p*-valor <0,05 foi considerado para significância estatística.

Para a análise, foram excluídos os óbitos intrauterinos e os recém-nascidos grandes para a idade gestacional (GIG) - cujo peso ao nascer é superior ao percentil 90 – por apresentarem fatores de risco e preditivos próprios (167). Para a identificação dos fatores clínicos preditores de recém-nascidos PIG, foi calculado o risco relativo de cada variável independente materna. Em seguida, foi aplicado o modelo *backward* para análise múltipla dos fatores de risco clínico para PIG. Por se considerar a heterogeneidade dos cinco centros participantes, as análises foram ajustadas por unidade de amostragem (PSU, *primary sampling unit*).

4.2. Revisão sistemática da literatura

A revisão sistemática seguiu a metodologia proposta pelo *Cochrane Handbook for Diagnostic Test Accuracy Reviews* (168,169). Foi cadastrada na plataforma *International Prospective Register of Systematic Reviews* (PROSPERO,

CRD 42018089985), e sua descrição respeitou o *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA *Statement*) (170). O protocolo foi publicado na revista *British Medical Journal Open* (8), e consta no capítulo de Resultados desta tese.

Buscou-se responder à seguinte questão: “Qual a acurácia da metabolômica na predição da restrição de crescimento fetal?”. Duas revisoras, de forma independente, realizaram a busca na literatura, a seleção dos estudos, a extração dos dados dos artigos incluídos e a avaliação do risco de viés. Um terceiro revisor dirimiu quaisquer discordâncias, e decisões a respeito da inclusão dos estudos ou interpretação dos dados foram obtidas através da maioria.

Para a elaboração e execução desta revisão sistemática, não houve participação de pacientes e não foi necessária aprovação por Comitê de Ética em Pesquisa.

Desfechos e análises de subgrupos

O desfecho primário para a RS foi o peso ao nascer abaixo do percentil 10, a partir de qualquer curva de referência. Como desfechos secundários, considerou-se o peso ao nascer menor ou igual aos percentis 3 e 5, a partir de qualquer curva de referência.

As análises de subgrupo propostas foram:

- Tipo de técnica metabolômica aplicada: ressonância nuclear magnética ou cromatografia (líquida ou gasosa) acoplada a espectrometria de massa;
- Estado de saúde materno pré-gestacional: gestantes saudáveis *versus* mulheres com qualquer condição crônica de saúde;
- Tipo de gestação: única *versus* múltipla;

- Suspeita de restrição de crescimento fetal durante a gestação: precoce *versus* tardia.

Busca na literatura

A busca na literatura ocorreu em fevereiro de 2018, e novamente em novembro de 2018, para a seleção dos mais recentes artigos publicados sobre o tema. Os conjuntos de palavras chave *'fetal growth restriction', 'metabolomics', 'pregnancy',* e *'screening'*, foram combinados com o operador Booleano 'AND'. A busca compreendeu artigos publicados entre 1998 e 2018, sem restrições de idioma.

As fontes da literatura incluíram: onze bases eletrônicas de dados (PubMed; EMBASE; *Latin American and Caribbean Health Sciences Literature* – LILACS; *Health Technology Assessment* – HTA; *Database of Abstracts of Reviews of Effects* – DARE; *Aggressive Research Intelligence Facility* – ARIF; *Cumulative Index of Nursing and Allied Health Literature* – CINAHL; *Maternity and Infant Care* - MIDIRS; *Scopus; Web of Science; Scientific Electronic Library Online* – Scielo), *Google Scholar*, resumos de congressos e busca manual nas listas de referência dos artigos incluídos. Os títulos encontrados foram importados para um gerenciador de referências (EndNote®).

Estudos originais com desenho de coorte ou caso-controle aninhado foram incluídos, desde que a coleta de material biológico ocorresse durante a gravidez e que fosse avaliado o peso ao nascer de recém-nascidos morfologicamente normais. Os critérios de exclusão dos artigos foram:

- Estudos transversais ou de intervenção (ensaios clínicos), ou quaisquer artigos não originais (ex., revisões narrativas ou sistemáticas, comentários, carta ao editor);

- Estudos experimentais com animais; ou
- Estudos duplicados. Neste caso, o artigo mais recente ou mais completo foi incluído.

Inicialmente, os artigos foram selecionados com base no título ou resumo. Os textos completos foram lidos quando não foi possível decidir sobre a inclusão. Um terceiro revisor mediou a decisão sobre a inclusão quando não houve consenso entre as revisoras.

Extração e síntese dos dados

As duas revisoras principais, de maneira independente, extraíram e sintetizaram as informações necessárias de cada estudo incluído. Tais dados incluíram:

- Nomes dos autores dos artigos e ano de publicação;
- Local e período de recrutamento das participantes;
- Desenho do estudo epidemiológico (coorte ou caso-controle) e laboratorial (prévia determinação dos metabólitos, '*targeted*', ou não, '*untargeted*');
- Número de mulheres em cada grupo (gestantes com recém-nascidos PIG e as com recém-nascidos de peso adequado - AIG);
- Idade gestacional em que houve a coleta de material biológico;
- Tipo de gestação (única ou múltipla);
- Paridade (nulíparas ou multíparas);
- Tipo de curva de adequação do peso (populacional ou customizada);
- Tipo de técnica metabolômica empregada (ressonância nuclear magnética, cromatografia acoplada a espectrometria de massa);
- Tipo de material biológico coletado e em que temperatura foi armazenado;

- Metabólitos que foram dosados (nos casos de estudos do tipo '*targeted*') ou que foram encontrados (nos casos de '*untargeted*'), e os que foram preditivos da RCF;
- Coeficiente de variação e limites mínimos de detecção dos compostos pelas técnicas descritas;
- Medidas de acurácia diagnóstica: sensibilidade, especificidade, área sob a curva ROC (AUC).

A síntese narrativa dos dados coletados foi realizada através de tabelas e discussão teórica. As classes e subclasses bioquímicas dos metabólitos foi checada na *Human Metabolome Database* (HMDB) (171), e as vias metabólicas, no *Kyoto Encyclopedia of Genes and Genomes* (172). O protocolo do estudo previa o cálculo das razões de verossimilhança e da curva hierarquizada (HSROC, *hierarchical summary receiver characteristic operating curve*) (173), além da avaliação de heterogeneidade (teste I^2) e viés de publicação (174).

Avaliação dos vieses

A análise de viés e de aplicabilidade do estudo à pergunta da revisão sistemática foi avaliada de acordo com a segunda versão do instrumento *'Quality Assessment of Diagnostic Accuracy Studies*' (QUADAS-2) (175). Cada estudo individual foi considerado como de baixo, alto ou indefinido risco de viés em quatro domínios: Recrutamento dos Participantes, Teste Índice (i.e., técnicas metabolômicas), Teste Padrão Ouro (i.e., peso ao nascer), e o Seguimento dos Participantes no estudo. Em seguida, os estudos foram considerados como de baixa, alta ou indefinida a preocupação a respeito da aplicabilidade dos resultados à

revisão sistemática nos três primeiros domínios. Os dados foram sintetizados e apresentados em figuras.

5. RESULTADOS

Os resultados desta tese são apresentados na forma de cinco artigos, referentes aos objetivos específicos descritos previamente.

Artigo 1: Leite DFB, Cecatti JG. Fetal growth restriction prediction: how to move beyond? (Submmitted to The Scientific World Journal).

Artigo 2: Leite DFB, Rocha Filho EAP, Melo Jr EF, Souza RT, Mayrink J, Calderon IM, Feitosa FE, Vettorazzi J, Sousa MH, Kenny LC, Baker PN, Cecatti JG, for the Preterm SAMBA study group. Assessing clinical risk factors for small for gestational age infants in a cohort of low-risk nulliparous pregnant women. (To be submitted to the Obstetrics and Gynecology.)

Artigo 3: Leite DFB, Cecatti JG. New approaches for fetal growth restriction: it is time for metabolomics. (Submmitted to the Revista Brasileira de Ginecologia e Obstetrícia.)

Artigo 4: Leite DFB, Morillon AC, Melo Jr EF, Souza RT, Khashan AS, Baker PN, Kenny LC, Cecatti JG. Metabolomics for predicting fetal growth restriction: protocol for a systematic review and meta-analysis. *BMJ Open* 2018; 8 (12): e022743.

Artigo 5: Leite DFB, Morillon AC, Melo Jr EF, Souza RT, McCarthy FP, Khashan AS, Baker PN, Kenny LC, Cecatti JG. Examining the predictive accuracy of metabolomics

for small for gestational age babies: a systematic review. (To be submitted for publication on the American Journal of Obstetrics and Gynecology)

Artigo 1: Fetal growth restriction prediction: how to move beyond?

Comprovante de submissão do artigo ao *The Scientific World Journal*.

UNICAMP

José Guilherme Cecatti <cecatti@unicamp.br>

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Dear Dr. Cecatti,

The Review Article titled "Fetal growth restriction prediction: how to move beyond?," by Debora F Leite and Jose Guilherme Cecatti has been received and assigned the number 1519048.

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

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Fetal growth restriction prediction: how to move beyond?

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Keywords

Fetal growth retardation, small for gestational age, Thrifty Phenotype Hypothesis, screening, prediction.

ABSTRACT

The actual and future burden of the small for gestational age (SGA) babies turn its screening in pregnancy a question of major concern for clinicians and policy makers. Half of stillbirths are due to growth restriction *in utero*, and possibly a quarter of livebirths of low- and middle-income countries are SGA. Growing body of evidence shows their higher risk of adverse outcomes at any period of life, including increased rates of neurologic delay, noncommunicable chronic diseases (central obesity, metabolic syndrome) and mortality. Although there is no strong consensus regarding its definition, birthweight centile threshold, or follow up, we believe birthweight<10th centile is the most suitable cutoff for clinical and epidemiological purposes. Maternal clinical factors have modest predictive accuracy; being born SGA appears to be of transgenerational heredity. Addition of ultrasound parameters improve prediction models, especially using estimated fetal weight and abdominal circumference in 3rd trimester of pregnancy. Placental growth factor levels are decreased in SGA pregnancies, and it is the most promising biomarker in differentiating angiogenesis-related SGA from other causes. Unfortunately, however, only few societies recommend universal screening. SGA evaluation is the first step of a multidimensional approach which includes adequate management and long-term follow up of these newborns. Apart from only meliorating perinatal outcomes, we hypothesize SGA screening is a key for socioeconomic progress.

INTRODUCTION

The intrauterine environment influence on fetus development is a well-known determinant of individual's long-term health and quality of life. From the initial description of 23 infants being born at term weighting less than 2000g, Warkany et al [1], introduced the idea of ‘intrauterine growth retardation’ (IUGR). Soon they were followed by others [2–4]. They considered IUGR ‘all conditions leading to marked reduction in size during intrauterine life’ [1], mainly represented by reduced birthweight. Although all of them have described pregnancies and infants with a wide variation of phenotype - with and without hypertensive syndromes or morphologic anomalies, for instance – the turning points were to consider the environment in which the fetus is developing, and the placenta role in this process.

In fact, human development goes far beyond genetic inheritance. Lessons learned from pregnancies subjected to smoking [5,6] or intermittent fasting [7], for instance, show how intrauterine growth is adjustable. Post-translational changes in small-for-gestational-age (SGA) infants [8] reinforce the Thrifty Phenotype Hypothesis [9]. According to Hales & Barker, the nutritional deficiency, especially regarding amino acids supply, would decrease the pancreatic beta cells function and induce changes of the muscular, hepatic and adipose tissue systems functioning, for example [9]. These newborns are at higher risk of neonatal morbidity and mortality [6,10–18]; in adolescence and adulthood, they present worse neurodevelopment [19,20], metabolic [21,22], and cardiovascular [23] adverse outcomes. On the other hand, the placenta – a shared organ by both the mother and the fetus - is responsible for adjusting maternal supply to the fetus demands. Since it is difficult to realize which are the normal placental functioning patterns and the optimal fetal growth, it is

reasonable to use the birthweight as a measure of the intrauterine environment [24], and SGA newborns as surrogates for fetal growth restriction (RCF) [10–13].

Therefore, considering the long latency of some events, such as cognitive delays and cardiovascular diseases, SGA has impacts of public health magnitude, especially in low and middle income countries (LMIC) [13,25]. In this review, we will discuss the importance of SGA screening in pregnancy, and which are the best approaches and moment to perform it.

WHY WE SHOULD SCREEN FOR FETAL GROWTH RESTRICTION

The identification of RCF as a distinct pathophysiological entity is merged with preterm birth history. In the first half of the 20th century, gestational age at birth and birthweight concepts overlapped; the World Health Organization recommended a birthweight of 2500g or less to characterize prematurity [26]. However, several authors and clinicians were intrigued by 'pseudopremature' newborns - who would be in chronic suffering due to placental insufficiency – and would benefit from earlier delivery [2–4]. Only in 1961, the terminology IUGR was first cited [1]. Apart from only birthweight (<2000g), Warkani et al suggested that preterm infants whose birthweight were 40% below the expected for a given gestational age should be considered IUGR. Two years later, Lubchenco et al proposed to use the birthweight as a proxy for intrauterine development [27] – and this is still a common practice in the 2000's [10–13], due to difficulties in defining and measuring fetal growth [28–30].

Currently, the birthweight <10th centile – either by population-based or customized charts – is the most accepted definition for SGA infants [28]. This mathematical

threshold was initially chosen due to (i) the increased neonatal mortality observed in this group when compared to those born between the 10th and the 90th centiles, and (ii) the agreement on the 10th centile among studies up to the 1960's [27]. There are concerns that some of these infants are 'constitutionally small' - not at higher risk of (neonatal) adverse outcomes, and lower limits for SGA, such as ≤5th [31], ≤3rd or even ≤2,3rd centile [32], are considered by some researchers. However, little is still known about the long-term health endpoints of the 'constitutionally small' newborns. Therefore, the 10th centile seems the most suitable cutoff for epidemiological and clinical purposes, and it is the adopted threshold in this review.

The SGA prevalence varies according to the reference standards applied; it tends to be higher with customized curves [11,12,14]. Using population-based charts, between 19,3% [13] and 27% [25] of livebirths in LMIC could have been classified as SGA in 2000's. Majority of them were term-SGA (98% and 95,6%, respectively). This turns SGA the most important pregnancy-related syndrome, since other pathological conditions, such as pregnancy hypertension and preterm birth, have markedly lower prevalence [11,12,14]. It is interesting to note, however, that these 'great obstetrical syndromes' may share pathophysiological pathways [33], and it is possible that SGA may represent an underlying condition for the other ones.

As a matter of fact, some clinical risk factors are similar between them. Multiple pregnancy and maternal chronic conditions, such as previous hypertension, systemic lupus erythematosus, and diabetes mellitus are all associated to the 'great obstetrical syndromes' [34–36]. Nulliparity [11,14,37], shorter height [11,14,37], lower pre-pregnancy weight [11,14,37] or body mass index [11,14], previous history of SGA [6,11,37], smoking [5,6,11,32,37], and being born SGA [38] are frequently related to

SGA pregnancies. Maternal age shows conflicting results, as well as ethnicity [14,39], socioeconomic and marital status [34], which may explain how maternal culture background and environment influence SGA patterns in a given population.

Regarding the outcomes of SGA newborns, extensive investigation has been performed on immediate [10–14,16,18,32,40] and long term endpoints [19–23,41,42], demonstrating worse health performance at any period of life. Not surprisingly, the leading countries in absolute numbers of fetal and neonatal deaths [43] are the same of SGA [25]: India, Paquistan and Nigeria. Indeed, growth restriction can account for up to half of fetal deaths of unknown causes [44], being about 6-fold higher the chance of stillbirth at term (relative risk, RR, 6.0; 95%CI, 3.1-11.5) [11], or when the birthweight is <5th percentile (compared to the 10-90th centiles) [17]. Besides perinatal death [6,11,12,15–18], preterm birth [6,11,14] and other short-term adverse events are described for SGA infants (Box 1); the adjusted odds ratio (aOR) for composite neonatal morbidity can be as high as 3.22 (95%CI, 3.07–3.39) [12]. Interestingly, SGA suspicion in pregnancy is associated with better neonatal outcomes [18,45], which turns SGA screening a cornerstone strategy for reducing antepartum fetal loss [13,46] and meliorating neonatal morbidity ratios.

Unfortunately, the higher risk of mortality goes beyond neonatal period. Data from Sweden shows a hazard ratio (HR) of 1.37 (95% CI 1.28–1.47) of death up to 18 years-old, which increased to 2.61 (95% CI 2.19-3.10) for those neonates born <3rd centile [47]. Additionally, growth restriction is associated with a lower Bailey score, especially in communication skills domain [19], sleep disorders [42], and hyperactivity [48]. If SGA fetuses experience any degree of brain-sparing effect, the delayed motor skills and cognitive development are even more pronounced [19,41]. Regarding

metabolic repercussions, insulin and insulin resistance index (HOMA IR) are higher in SGA children at 6-8 years old, and those born <3rd centile also have higher levels of leptin [22].

Evidence from adults exposed to famine *in utero* shows increased odds for metabolic syndrome [21] and obesity [49] in SGA newborns, perhaps in a sex-specific manner, depending on childhood nutritional parameters (especially weight gain velocity). Proportionate biometric measurements at birth were the initial observations of Barker et al, who related the ponderal index, head circumference and birthweight <2495g to cardiovascular mortality [23]. Although maternal undernourishment is not synonymous of SGA infant, and considering that birthweight approach has changed overtime, these findings mean that the adequate fetal development is the standpoint for a long-term health. There is greater visceral fat thickness (in women) [50], higher fat free soft tissue mass [51] and increased trunk and abdominal fat mass proportion (of both sexes) [52] in adults born SGA. These epidemiological data ground current theories of epigenetic modifications in SGA infants, leading to enriched (i.e., with increased DNA methylation) pathways involved with fat, sugar and protein metabolism [8].

Therefore, timely recognition of SGA – still in pregnancy – is a real concern for obstetricians, perinatologists, health workers and policy makers. Unfortunately, only small proportion of SGA babies are suspected before birth [18,45], leading to lack of appropriate short- and long-term follow-up of these newborns. SGA suspicion will provide adequate management of the mother and fetus/newborn, including referencing to specialized facility for antenatal care and delivery, and individualized follow-up in childhood, adolescence and adulthood.

WHEN AND HOW WE SHOULD SCREEN FOR FETAL GROWTH RESTRICTION

Clinical factors

Clinical risk assessment is the first approach in antenatal care. A detailed maternal history at booking can identify several risk factors, and guide referencing to tertiary care facilities.

Single maternal clinical factors demonstrate poor prediction accuracy (Table 1), and, as a result, are generally considered in a multidimensional model. Smoking, although less prevalent in the early years of the 21st century, still demonstrates effects on fetal growth [5,6,37], and is the most common maternal variable to compose a prediction model. Lower maternal stature and weight appear associated with SGA in some studies [11,14,37], but showed only 43% and 73% of sensitivity, respectively [53]. Body mass index (BMI) and maternal weight gain throughout pregnancy demonstrate an area under the (AUC) receiver operating characteristic (ROC) curve of 0,56 and 0,60, respectively [53]. The performance of symphysial-fundal height (SFH) measurement in predicting SGA newborns increases with gestational age [54], but it is not different to the Leopold maneuvers (RR1.32, 95%CI 0.92-1.90) [55]. However, since it is inexpensive and already part of routine obstetrical examination, Cochrane reviewers advise its use, and health professionals should associate it with some other technique or evaluation of fetal growth.

Other maternal factors have been combined differently, evidencing how SGA syndrome can be heterogeneous in distinct settings. In a multicenter international nulliparous cohort, family history of coronary heart disease, maternal birthweight <3000g, infertility, being college student, smoking at the 2nd trimester, proteinuria,

daily vigorous exercise, diastolic blood pressure ≥80mmHg, combined with the protective factors rising random glucose, recreational walking (≥4x/week), *Rhesus* negative blood group, provided an AUC of 0.63 [6]. This same AUC (0.66, 95%CI 0.61-0.70) was achieved by combining maternal age and height, smoking, previous SGA infant, and chronic hypertension in Spain [56]. In the United Kingdom, a logistic regression model included maternal height, weight, parity, ethnic background, smoking, and previous history of preeclampsia or SGA [57]. In this model, maternal factors evaluation between 35 and 37w have had similar AUC for delivery within two weeks (0.744; 95%CI 0.731–0.756) and term delivery (0.712; 95% CI 0.700–0.725) for SGA without preeclampsia.

Ultrasound scans

Adding ultrasound scan (US) parameters to maternal clinical factors improves performance of prediction models, although not consistently [6,57]. Crown-rump length (CRL) [58]; nuchal translucency (NT) [58]; head circumference (HC) [6]; abdominal circumference (AC) [6,59]; AC growth velocity (ACGV) [59,60]; femur length (FL) [61]; estimated fetal weight (EFW) [32,57,60,62]; uterine arteries pulsatility (UtA-PI) or resistance (UtA-RI) index, or notches [6,32,57]; umbilical artery PI (UA-PI); middle cerebral artery PI (MCA-PI); cerebral-placental ratio (CPR: MCA-PI/UA-PI) [32,63]; and umbilical vein blood flow (UVBF) [32] were studied for SGA prediction. Except for NT, the lower the fetal biometry, the higher the odds for SGA; in general, there is a trend towards better US predictive accuracy for lower birthweight centiles (especially <3rd) [64]. Unfortunately, participants selection criteria, study protocol of follow up, and outcome measures differ between studies, precluding

interpretation and evaluation of US in clinical practice [64]. In Table 1, predictive accuracy measures of EFW and AC are shown.

In 1st trimester, decreased values of NT were associated to lesser odds for SGA (OR 0.79; 95%CI 0.70-0.89), but the CRL has shown no relationship (OR 0.99, 95%CI 0.99-1.00) [58]. In 2nd trimester, McCowan et al [6] demonstrated only a limited increase in AUC (from 0.66 to 0.73) was observed with addition of 20w US data to maternal data: HC z-score <10th centile, AC z-score <10th centile, and UtA-RI≥0.05. The higher the UtA-RI, the higher the OR for SGA, reaching 4.56 (95%CI 2.45 to 8.48) when 0.8-1.0. At 35-37w, Fadigas et al [57], have combined maternal variables with EFW z-score, which improved AUC from 0.81 (95%CI 0.802–0.824) to 0.98 (95%CI 0.98–0.98) for delivering an SGA infant <3rd centile in less than two weeks. In this cohort, adding mean arterial pressure and UtA-PI have not improved the prediction performance (AUC 0.98; 95%CI 0.98–0.99).

Interestingly, a single measurement is better than longitudinal follow up [32,59,60,62,64]. In 2nd trimester, the femur length <5th centile is associated with increased odds for IUGR or SGA (3.24, 95%CI 2.34-4.48) [61]. In another example, Triunfo et al have demonstrated better prediction performance of EFW at 37w for birthweight below the 3rd centile (0.85; 95%CI 0.82–0.89), when compared to the 4-10th centiles (0.93; 95%CI 0.89–0.97), but reached a disappointing AUC of 0.54 (95%CI 0.48-0.61) for predicting adverse perinatal outcomes [32]. This is also true for AC cross-sectional evaluation at 32w when compared to ACGV (difference from 32w results and 2nd trimester) [59]. The detection rate (DR) of SGA<10th centile was 49.1 (95%CI 44.2-52.8; false positive rate, FPR, 10%), and 81.2 (95%CI 75.3-88.1) for SGA<3rd centile or suspected before birth by abnormal Doppler results. This finding

partially contradicts the Pregnancy Outcome Prediction (POP) Study, which found relative risk of 17.6 (95%CI 9.2-34.0) for delivering a SGA infant when both EFW and ACGV (between 28 and 36w) were <10th centile [60]. In this research, sensitivity of EFW<10th centile was higher with universal screening for SGA<10th (57%) or <3rd centile (77%) than with clinically-oriented US evaluation (20% and 32%, respectively) [60].

More recently, magnetic resonance imaging (MRI) has been explored in maternal-fetal surveillance. Carlin et al [65] have demonstrated no difference in EFW ≤3rd or ≤5th centile by US or MRI before delivery (48h). However, DR of SGA ≤10th centile was superior with MRI (100.0; 95%CI 81.5–100.0, FPR, of 10%) than US (77.8; 95%CI 52.4-93.6, FPR 10%).

Biomarkers

Biomarker measurements of placental functioning-related substances have had significant development in the last three decades. Many of these compounds are also involved with antenatal detection of chromosomal anomalies, or preeclampsia, such as placental protein A (PAPP-A), alpha-fetoprotein (AFP), placental growth factor (PlGF), or sFLt-1 [66]. (Table 1). In early 2nd trimester (15w), serum levels of PAPP-A, PlGF, and insulin are significantly lower in SGA pregnancies [67], while increased plasma levels of vascular growth factor (VEGF) between 34-37 weeks were related to a lower chance of restricted fetuses (OR 0,8; 95%IC 0,71-0,92) [68]. Conversely, a model built by EFW, UtA-PI, and PlGF at 35-37w has provided an AUC 0.883 (95%CI 0.867-0.899) [69].

PlGF has consistently lower levels in SGA pregnancies, in 2nd and 3rd trimesters [70–72], especially for BW<5th or <10th centiles. For higher sFlt-1/PlGF ratios, there is batter AUC for preeclampsia-associated SGA [66,73]. These findings point in the direction of an angiogenesis-mediated pathophysiology of SGA. Unfortunately, PlGF shows poor accuracy to be implemented in clinical practice: the combined AUC was 0,66 (95%IC 0,44-0,87) for RCF prediction [74]. Perhaps this finding is due to the diverse PlGF measurements and FGR definitions used by the studies included in the systematic review, which considered either the estimated fetal weight, birth weight or the presence of additional findings of severity (e.g., oligohydramnios).

After all, better accuracy was achieved by combining multiple maternal, ultrasonographic and biochemical clinical factors. In an international cohort of nulliparous women [67], PlGF has had an AUC of 0.84 (95% CI 0.78-0.89) for hypertensive-SGA when combined with smoking, proteinuria, uterine artery Doppler, PAPP-A and triglycerides. In the 2nd trimester (19-24w), PlGF, AFP, combined with maternal factors and fetal biometry, made up an AUC of 0,96 for birth below 32 weeks in SGA newborns [31].

CONCLUSIONS

Fetal growth restriction is related to adverse outcomes in the perinatal period, childhood, and adulthood; the estimated actual burden of SGA [13,25] might be even higher in the next few years. Starting antenatal care at early pregnancy leads to adequate risk management and additional evaluation assessment, with US or biomarkers. The ‘inverted pyramid’ of prenatal care, claims attention to the early

pregnancy risk evaluation [75], and we strongly believe screening is the first step towards a better disease diagnosis and management. Screening for FGR is a major cornerstone for a coordinating care from pregnancy to postpartum period, which affects both maternal and fetal/ neonate outcomes [76]. The low velocity in which stillbirth and neonatal death rates has decreased in the past 30 years is an ‘unfinished agenda’ [76].

Although the cost-effectiveness of short-term pregnancy-related adverse outcomes is still a matter of debate [77], little is known about the future consequences of a health policy devoted to primary prevention of pregnancy-associated illness in a long-term [38,47,78]. On the other hand, the lack of definition of a high-risk group of women that could benefit from a more directed approach delay scientific and clinical evaluation of SGA. As maternal factors have different magnitude between settings, and placental biomarkers are not a reality in most LMIC countries, currently, the 3rd trimester US seems the best approach for SGA prediction. In near future, we envision an integrated approach of pregnant women at booking [75], aiming a transgenerational [38] effect of long-term health, both at individual and populational levels.

AUTHORS' CONTRIBUTIONS

DFBL has proposed the review and drafted the first manuscript. JGC has supervised and checked the drafting. Both authors have read and agree with this submission.

CONFLICTS OF INTEREST

Both authors are involved with original research about SGA prediction with metabolomics and have performed a predictive accuracy systematic review on this topic. JGC has presented talks in conferences about FGR.

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Box 1. Neonatal adverse events associated with being born SGA
Perinatal asphyxia
5th minute Apgar score <7 [10,11,14]
5th minute Apgar score <5 [10,12,16,18]
Admission to neonatal intensive care unit [6,10,11]
Hypoglycemia requiring treatment [40]
Phototherapy [40]
Respiratory distress syndrome [12,14,16,40]
Ventilatory support [11,12,16,48]
Necrotizing enterocolitis [12,48]
Neonatal sepsis [12,16,40]
Seizures [12,16,18]
Intraventricular hemorrhage [12,16,18]
Neonatal death [11,12,15,16]

Table 1. Accuracy for clinical factors, ultrasound parameters and placental biomarkers for SGA prediction (birthweight <10th centile).

Predictive factors	**AUC**	**S (95%CI)**	**Sp (95%CI)**	**When**
Maternal height [53]	0.59	0.43 (0.27-0.60)	0.70 (0.53-0.83)	At booking
Maternal weight [53]	0.57	0.73 (0.60–0.83)	0.35 (0.23–0.51)	At booking
Maternal weight gain [53]	0.60	0.50 (0.42–0.59)	0.66 (0.57–0.73)	At booking
PAPP-A [58]		0.16 (0.14–0.19)	0.90 (0.89–0.90)	1st trimester
PlGF [74]	0.66	0.49 (0.44–0.53)	0.64 (0.63-0.66)	2nd trimester
Cerebroplacental ratio[a] [63]		0.43 (0.39-0.47)	0.94 (0.84-0.98)	3rd trimester
Estimated fetal weight [64]	0.79	0.38 (0.31-0.46)	0.95 (0.93-0.97)	>32w
Abdominal circumference [64]	0.92	0.35 (0.20-0.52)	0.97 (0.95-0.98)	>32w

[a]MCA-PI/UA-PI <10th centile or ≤1.08. [b]Estimated fetal weight<10th centile for gestational age.
AUC: area under the receiver operating curve. S: sensitivity. Sp: specificity. PAPP-A: pregnancy-associated plasma protein-A. PlGF: placental growth factor.

Artigo 2: Assessing clinical risk factors for small for gestational age infants in a cohort of low-risk nulliparous pregnant women. *(To be submitted for publication on the Obstetrics and Gynecology.)*

Assessing clinical risk factors for small for gestational age infants in a cohort of low-risk nulliparous pregnant women

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Short title: risk factors for small-for-gestational-age

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Précis

Clinical risk must be assessed at every antenatal visit. Fetal growth restriction may not present an angiogenic pathogenesis in this population.

Abstract

Objective: To estimate incidence and clinical risk factors for small for gestational age (SGA) infants in nulliparous low-risk women at mid pregnancy.

Methods: This is a secondary analysis of PRETERM-SAMBA study, designed to evaluate early predictors of adverse outcomes in healthy nulliparous women. It was a longitudinal cohort conducted in five tertiary Brazilian facilities, from July, 2015 to July, 2018. Standardized research protocol was followed, based on ethical statements. Demographic and obstetric data was obtained at recruitment (19-20w); medical charts were reviewed for perinatal outcomes. SGA was characterized as birthweight<10th customized centile, and these newborns were compared to the adequate for gestational age (AGA; 10-90th centile) ones. Stillbirths and infants with birthweight>90th centile were excluded from analysis. A backward logistic regression model was applied for estimating risk factors, providing risk ratios and their 95%CI.

Results: In this cohort, the incidence of SGA was 12,8%; 1,032 women were enrolled in this analysis. Entirely public antenatal care (adjusted Risk Ratio, aRR, 2,02; 95%CI 1,23-3,33) and any infection in 1st half of pregnancy (aRR 1,36; 95%CI 1,10-1,68) increased the risk of SGA. There was no association with smoking (aRR 1,05; 95%CI 0,52-2,11), systolic blood pressure >130mmHg (aRR 1,31; 95%CI 0,85-2,03) or diastolic blood pressure >75mmHg (aRR 1,38; 95%CI 0,68-2,82) at booking with SGA.

Conclusion: Clinical risk must be assessed at every antenatal visit. Fetal growth restriction may not present an angiogenic pathogenesis in this population.

Keywords: small for gestational age infant, fetal growth restriction, risk factors,

Background

There is growing evidence that poor perinatal outcomes and adult non-transmissible chronic diseases (NCD) are related to birth weight and fetal growth during pregnancy.[1–3] Newborns with birth weight <10th centile, i.e. small for gestational age (SGA), show greater risks of perinatal mortality and composite neonatal morbidity,[4–7] and impaired glucose tolerance, hypertension and metabolic syndrome in adulthood.[8,9] This turns the restriction of growth *in utero* the main condition to be assessed in pregnancy with potential long-term consequences.

As a matter of fact, SGA has been used as proxy for fetal growth restriction[4–6,10] (FGR) in epidemiological studies due to its better relationship with adverse outcomes than fetal parameters[7]. Although they might represent distinct pathological conditions, identification of factors associated with SGA at booking might guide further investigation with ultrasound scans (US) or biomarkers, where available. SGA pathogenesis is controversial. Its hemodynamic[11] abnormalities and lower levels of angiogenic biomarkers[12] suggest placental insufficiency, comparable to hypertensive syndromes. Then, clinical risk assessment for SGA is recommended;[13] nulliparity[5], lower pre-pregnancy body mass index (BMI)[5], smoking,[5,10] hypertensive syndromes[5] and maternal lower birth weight[10] have been associated with increased risk for SGA. In this context, evaluation of nulliparous pregnant women is cornerstone, since SGA, preterm birth and stillbirth are strong risk factors for reccurrence.[14]

In Brazil, there is lacking evidence about early risk assessment in nulliparous low-risk women, mainly followed at primary care facilities. Therefore, the main purpose of this study was to stablish the incidence and the clinical risk factors in first half of pregnancy for SGA.

Methods

The Preterm-SAMBA (Preterm Screening And Metabolomics in Brazil and Auckland) was a longitudinal, multicenter cohort study conducted in five Brazilian centers, from July, 2015 through July, 2018. They are all public tertiary facilities and local references for specialized obstetric care. A standard research protocol was followed by all research team, as outlined previously.[15] This report follows the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) Statement.[16]

Participants: Healthy nulliparous women were assessed by convenience and enrolled between 19^{+0} and 20^{+6} weeks of a single pregnancy. Conditions already known to be related to preterm births were exclusion criteria, and maternal chronic illness as well.[15] On first visit, a full clinical assessment was carried, including evaluation of: demographic data (e.g., age, marital status, schooling); reproductive history (maximum of two previous abortions); previous or current diseases (e.g., mild chronic hypertension without anti-hypertensive treatment[17]); and recreational habits (e.g., any smoking, alcohol or illegal drugs use up to 1st visit). Any infections in pregnancy were considered, such as urinary, gastrointestinal or respiratory tract infections. Weight, height and blood pressure were measured. BMI was classified according to reference ranges for Brazilian pregnant women.[18] The antenatal care of included women could have been at public or private institutions. Data from delivery and perinatal period were retrieved from medical records plus direct contact with women though telephone or electronic messages.

A safe online database was used (MedSciNet® AB, Sweden), allowing real time data registry during participant's assessment and an initial data consistency

checking. When not available, printed forms were used, stored and data was inserted later.

Definitions: We have included only liveborn children. In this study, a SGA baby was defined as a newborn <10th customized birth weight centile.[19] Adequate for gestational age (AGA) babies were those whose birth weight was between the 10th and the 90th centile. The large for gestational age (LGA) babies, i.e. >90th of birthweight centile, were excluded from this analysis.

Statistical analysis: This is a secondary analysis of Preterm-SAMBA, which was primarily designed to validate a prediction model for preterm birth.[15] Assuming a type I error of 0.05 and a type II error of 0.2 (power of 80%), it would be necessary 1150 women for the preterm birth outcome[15]. Then, estimating a prevalence of 10% of SGA, we had to had at least 115 cases of SGA.

Difference in means or medians were evaluated by the Student *t* test (if Gaussian distribution was achieved), and in proportions, by the Pearson Chi-squared test. Groups for comparison were: (1) all SGA babies; and (2) AGA babies. Crude and adjusted Risk Ratios (RR) were estimated for variables in bivariate and multivariate analyses. Backward multivariate analysis was performed including all variables. The Stata v. 7.0 (StataCorp) and SPSS v. 20.0 (IBM) packages were used for statistical analysis. All analyses were adjusted for the primary sampling unit (PSU) considering the heterogeneity of the five participating centres.

Ethical issues: Preterm-SAMBA Study has respected the Helsinki Declaration (1975, revised in 2013) and followed standards of ethics in research. It has obtained ethical

approval by the Institutional Review Boards of all participating centers (letter of approval 1.048.565 issued on 28th April 2015 by the coordinating center) and was endorsed by the National Ethics Committee for Research (CONEP). All enrolled women have signed a two-way informed consent and were free to quit at any time. Participants agreed that data would be published with scientific purposes.

Results

In total, 1,373 women were eligible for Preterm-SAMBA Study, and 1,181 were enrolled (Figure 1). Outcome data was available for 1,165 (1.3% of follow-up loss). The incidence of SGA babies was 12,8% (149/1165). After exclusions, the final sample included 1,032 women.

Tables 1 and 2 demonstrate maternal characteristics evaluated at booking. Participants who delivered SGA or AGA newborn had similar age, schooling, blood pressure and BMI. Rates of ethnicity; marital status; paid employment; being primigravid; being born <2500g or subjected to hypertension *in utero*; having chronic hypertension; smoking, alcohol or illegal drugs abuse; having proteinuria, urinary tract infection, or bleeding; and hospital stay up to 21w of pregnancy were also equivalent between groups. The overall prevalence of smoking was 7,1% (7,4% for SGA and 7% for AGA), and of overweight/obesity, 42,8% (44,3% and 42,6%, respectively). However, women who delivered SGA newborns were more frequently assisted at public antenatal care and have presented infections in first halt of pregnancy.

The univariable relative risks for delivering an SGA neonate are shown in Tables 3 and 4. Having an entirely public prenatal care and presenting any infection before 19-20w increased the risk for delivering an SGA baby in 2.02 (95%CI 1.24-

3.29; *p* 0,016) and 1.36 (95%CI 1.10-1.68; *p* 0,016). There was no difference in SGA risk regarding other demographic or clinical characteristics, such as maternal age, BMI, ethnicity, or smoking status. Presenting raised systolic or diastolic blood pressure at booking, or having mild chronic hypertension, have had no relationship with SGA risk.

Discussion

We have presented the largest assessment of clinical risk factors for SGA infants in a Brazilian population, to the best of our knowledge. We have found that antenatal care at public facilities or presenting infections in the first half of pregnancy were associated with higher risk of SGA.

The incidence of SGA in our study (12,8%) is comparable to those reported for North America (11.7%[6] – 15.2%)[4], Oceania (11.6%)[5] and Europe (10.7%).[10] Indeed, classification of birth weight with customized charts is worldwide endorsed;[13] they are more strongly associated with adverse perinatal outcomes.[4–6,10] Although there is no consensus if birth weight is the best measure of intrauterine development, it shows better performance in identifying at-risk children than ultrasound parameters for FGR.[7]

However, ‘SGA syndrome’ seems to have different phenotypes. Assessment of hypertensive syndromes-SGA is different from normotensive ones,[20] and this raises the question of which the main pathogenesis of SGA in our population is. First, although placental insufficiency is suspected to be involved with SGA pregnancies, it does not explain its recurrence.[14] Microchimerism[21] evaluations might deeper, in future, paternal role in SGA. Second, in low- and middle-income countries, there are widespread chronic micronutrient deficiencies, known to have impact on

birthweight.[22,23] Therefore, the lack of association with blood pressure in mid-pregnancy might indicate other fetal adaptations to endogenous or environmental[24] factors in our population that need further assessment.

In Brazil, the national public health system has a widespread distribution and is responsible for the care of mostly low socioeconomic level population. All facilities involved with this study were public and were referral units for obstetric high-risk care. Then, we believe that public antenatal care, in our sample, is as a proxy for low socioeconomic level. This group of women may benefit from predictive, diagnostic or even preventive strategies for SGA. We hypothesize that women being attended at a tertiary care facility are more prone to engage in healthy habits.

Regarding recreational habits, smoking is associated with impaired growth, both measured by ultrasound[25] or at birth.[5,10,26] Women who quit smoking before or at the first trimester of pregnancy have infants with similar birth weight to nonsmokers'.[26] Smoking rates have decreased over years, but SGA prevalence has levelled off,[27] which points in the direction of other factors increasingly affecting birth weight.[24] Our numbers have not allowed control for gestational age of ceasing smoking, and we have not assessed tobacco use till the end of pregnancy. Although we could not demonstrate an increased risk in our sample, we cannot rule out tobacco effect on fetal development. There is some evidence to support impairment on glucocorticoid-related genes functioning in infants exposed to inflammation-related disorders, such as asthma;[28] with birthweight <3rd or in the presence of Doppler abnormalities;[29] and exposed to smoking.[30]

Although innovative, our study had limitations. Firstly, we have used SGA as proxy for FGR. We acknowledge they are different concepts, but FGR criteria[31] lacks validation. Secondly, participants were enrolled in the second trimester. However,

majority of women will be enrolled in prenatal care at this stage, when it is still possible to manage modifiable risk factors for adverse outcomes, like smoking or excess weight gain. Finally, we have not included fetal biometry in this analysis. We have prioritized clinical risk factors, which can be evaluated by specialized consultants or general practitioners, nurses or midwives. The two major strengths of this report were the use of customized charts, and the exclusion of LGA infants for final analysis. These latter ones present distinct risk factors and would impair proper data evaluation.

Being born SGA has been associated with increased risk of morbidity and mortality in any period of life, and some authors suggest a transgenerational transmission.[32] Possibly epigenetic modifications mediate these outcomes, and studies with protein activity[29] in placentas of SGA<10th or <3rd are promising in differentiating the truly growth restricted fetuses from the constitutional small ones. Understanding clinical profile of pregnancies at risk of SGA is the first step to promote other appropriate investigations, like the role of ultrasound scans and biomarkers, such as angiogenic or metabolomic. Future research should focus on customized fetal weight. Adequate identification of growth restricted fetuses could possibly stratify perinatal morbidity and mortality according to gestational age of diagnosis and its relationship with adulthood health. Therefore, identifying SGA pregnancies have long-lasting effects. This underestimated potential to improve health indices at populational level is even more important in low- and middle-income countries.

Declarations

Disclosure of Interests: Authors declare no competing interest for the current analysis.

Author's contributions: JGC, DFBL and RTS have performed the study design. DFBL, RTS and JM have prospectively collected data. DFBL, EPRF, and EFMJ and have written the first draft. RTS and JM have participated in research management and have helped in writing the document. JGC, IMC, FEL, JV, PNB, and LCK have revised the document. All authors have read and endorsed final manuscript.

Ethical Approval: The current study was approved by each local Institutional Review Board (IRB). The Brazilian National Committee for Ethics in Research (CONEP) has amended the ethical statements - 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.

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Table 1. Maternal characteristics 19^{+0} and 20^{+6} weeks of pregnancy according to adequacy of birth weight to gestational age.

Maternal history	SGA (N=149)		AGA (N=883)		*p*
	n	%	n	%	
Maternal age, years [mean (±SD)]	24.77 (±6.1)		24.41; (±6.2)		0.56
Schooling, years [mean (±SD)]	11.62 (±3.3)		11.78 (±3.4)		0.60
Schooling (>12 years)	47	31,5	276	27,8	0.96
White ethnicity	47	31,5	355	40,2	0.18
Single	41	27,5	244	27,6	0.96
Paid employment	78	52,3	445	50,4	0.67
Primigravid	134	89,9	770	87,2	0.46
Being born with <2500g	5	3,3	31	3,5	0.95
Being born with any hypertension in pregnancy	16	10,7	81	9,1	0.48
Chronic hypertension	1	0,6	9	1	0.78
Entirely public prenatal care	139	93,3	762	86,3	**0.012**
Infertility	7	4,7	72	8,1	0.38
Assisted reproduction[a]	1	0,6	3	0,3	0.58
Smoking in pregnancy	11	7,4	62	7	0.86
Alcohol intake in pregnancy[b]	18	12,1	131	14,8	0.59
Illicit drugs in pregnancy[c]	8	5,4	42	4,7	0.70

SGA, small for gestational age; birthweight<10^{th} centile. AGA, adequate for gestational age; birthweight 10-90^{th} centile. BMI: body mass index[18]. [a] Information suitable only for infertile women. Missing data for [b]125 and [c]155 participants.

Table 2. Maternal risk factors at 19^{+0} and 20^{+6} weeks of pregnancy according to adequacy of birth weight to gestational age.

Maternal characteristics at booking	**SGA (N=149)**		**AGA (N=883)**		*p*
	n	%	n	%	
Gestational age at enrollment [days; (±SD)]	139; (±4.6)		139,3; (±4.3)		0.52
Systolic blood pressure, mmHg [mean, (±SD)]	107,4; (±10.7)		108,2; (±10.7)		0.63
Diastolic blood pressure, mmHg [mean, (±SD)]	65.9; (±9.2)		65,4; (±8.2)		0.62
Mean blood pressure mmHg [mean, (±SD)]	79.7; (±9.0)		79,7; (±8.4)		0.98
BMI at booking (kg/m^2)[a]	26.35; (±5.5)		26,3; (±5.34)		0.90
Underweight	28	18,8	153	17,3	
Normal weight	54	36,2	354	40,1	0.58
Overweight/ obesity	66	44,3	376	42,6	
Proteinuria (>1+ or 30mg/dL)[b]	2	1,3	7	0,8	0.34
Any infection <19/20w	66	44,3	315	35,7	**0.016**
Urinary tract infection	33	22,1	146	16,5	0.20
Bleeding <19/20w	33	22,1	173	19,6	0.22
Hospital admission <19/20w	4	2,7	24	2,7	0.98

SGA, small for gestational age; birthweight<10^{th} centile. AGA, adequate for gestational age; birthweight 10-90^{th} centile. Missing data for [a]1 and [b]255 participants.

Table 3. Clinical risk factors for SGA babies at 19^{+0} and 20^{+6} weeks of pregnancy (adjusted for primary sampling unit).

Maternal history	SGA (N=149)	AGA (N=883)	*RR*	*95% CI*
	n	n		
Maternal age (years)				
≤19y	36	217	0.99	0.53-1.85
20-34y	102	606	Ref.	
≥35y	11	60	1.08	0.50-2.31
Schooling (years)				
<9y	102	607	0.99	0.94-1.04
>12y	47	276	Ref.	
Ethnicity				
White	47	355	Ref.	
Non-white	102	528	1.38	0.80-2.39
Marital status				
With partner	41	244	Ref.	
Single	108	634	0.99	0.53-1.84
Paid employment				
No	78	445	Ref.	
Yes	71	438	1.07	0.71-1.60
Primigravid				
Yes	134	770	Ref.	
No	15	113	0.79	0.35-1.78
Being born with <2500g				
No	5	31	Ref.	
Yes	144	852	0.96	0.21-4.40
Being born with any hypertension in pregnancy				
No	133	802	Ref.	
Yes	16	81	0.86	0.51-1.45
Chronic hypertension				
No	148	874	Ref.	

Yes		1	9	0.69	0.02-23.99
Entirely public prenatal care					
No		10	121	Ref.	
Yes		139	762	**2.02**	**1.24-3.29**
Infertility					
No		142	811	Ref.	
Yes		7	72	0.59	0.15-2.41
Assisted reproduction [a]					
No		2	13	Ref.	
Yes		1	3	1.88	0.11-33.48
Smoking	No smoker	138	821	Ref.	
	Smoking in pregnancy	11	62	1.05	0.52-2.11
Alcohol intake [b]	No intake	112	646	Ref.	
	Any intake in pregnancy	18	131	0.82	0,30-2,19
Illicit drugs [c]	Non-user	117	710	Ref.	
	Any use in pregnancy	8	42	1.13	0.51-2.52

SGA, small for gestational age; birthweight<10th centile. AGA, adequate for gestational age; birthweight 10-90th centile. BMI: body mass index[18]. [a] Information suitable only for infertile women. Missing data for [b]125, [c]155, [d]1 and [e]255 participants.

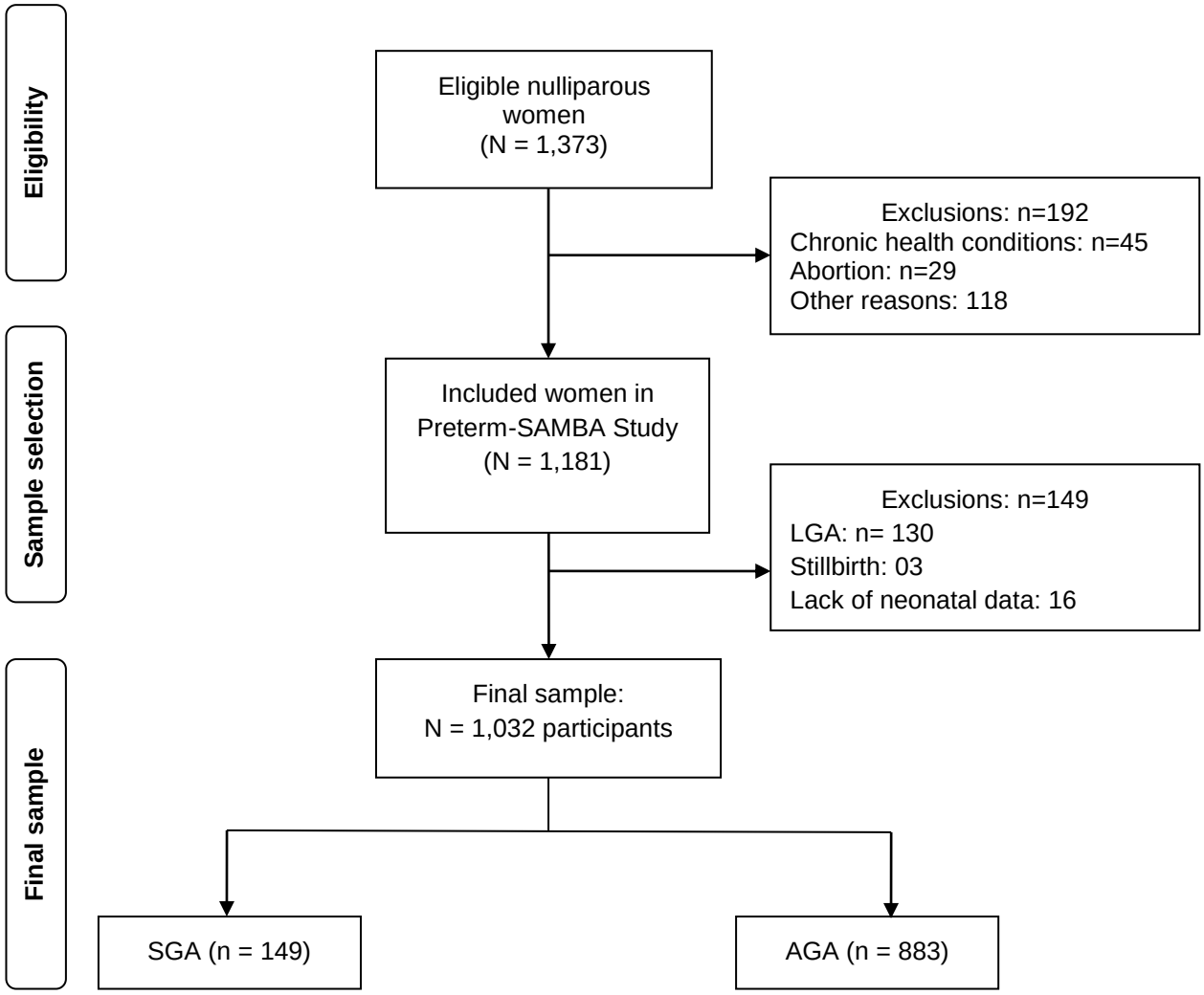
Table 4. Clinical predictors for SGA babies at 19^{+0} and 20^{+6} weeks of pregnancy (adjusted for primary sampling unit).

Maternal characteristics at booking	SGA (N=149)	AGA (N=883)	*RR*	*95% CI*
	n	n		
Systolic blood pressure >130mmHg				
No	143	857	Ref.	
Yes	6	26	1.31	0.85-2.03
Diastolic blood pressure >75mmHg				
No	119	754	Ref.	
Yes	30	129	1.38	0.68-2.82
BMI at booking (kg/m^2) [a]				
Underweight	28	153	1.17	0.88-1.56
Normal weight	54	354	Ref.	
Overweight/ Obesity	66	376	1.13	0.71-1.78
Proteinuria (>1+ or 30mg/dL) [b]				
No	114	654	Ref.	
Yes	2	7	1.50	0.54-4.18
Any infection < 19/20w				
No	83	568	Ref.	
Yes	66	315	**1.36**	**1.10-1.68**
Urinary tract infection				
No	116	737	Ref.	
Yes	33	146	1.36	0.78-2.35
Bleeding < 19/20w				
No	116	710	Ref.	
Yes	33	173	1.14	0.89-1.47
Hospital admission < 19/20w				
No	145	859	Ref.	
Yes	5	24	0.99	0.31-3.18

SGA, small for gestational age; birthweight<10^{th} centile. AGA, adequate for gestational age; birthweight 10-90^{th} centile. Missing data for [a]1 and [b]255 participants.

Figure captions

Figure 1. Flow chart of eligible participants, excluded infants from analysis and final sample.



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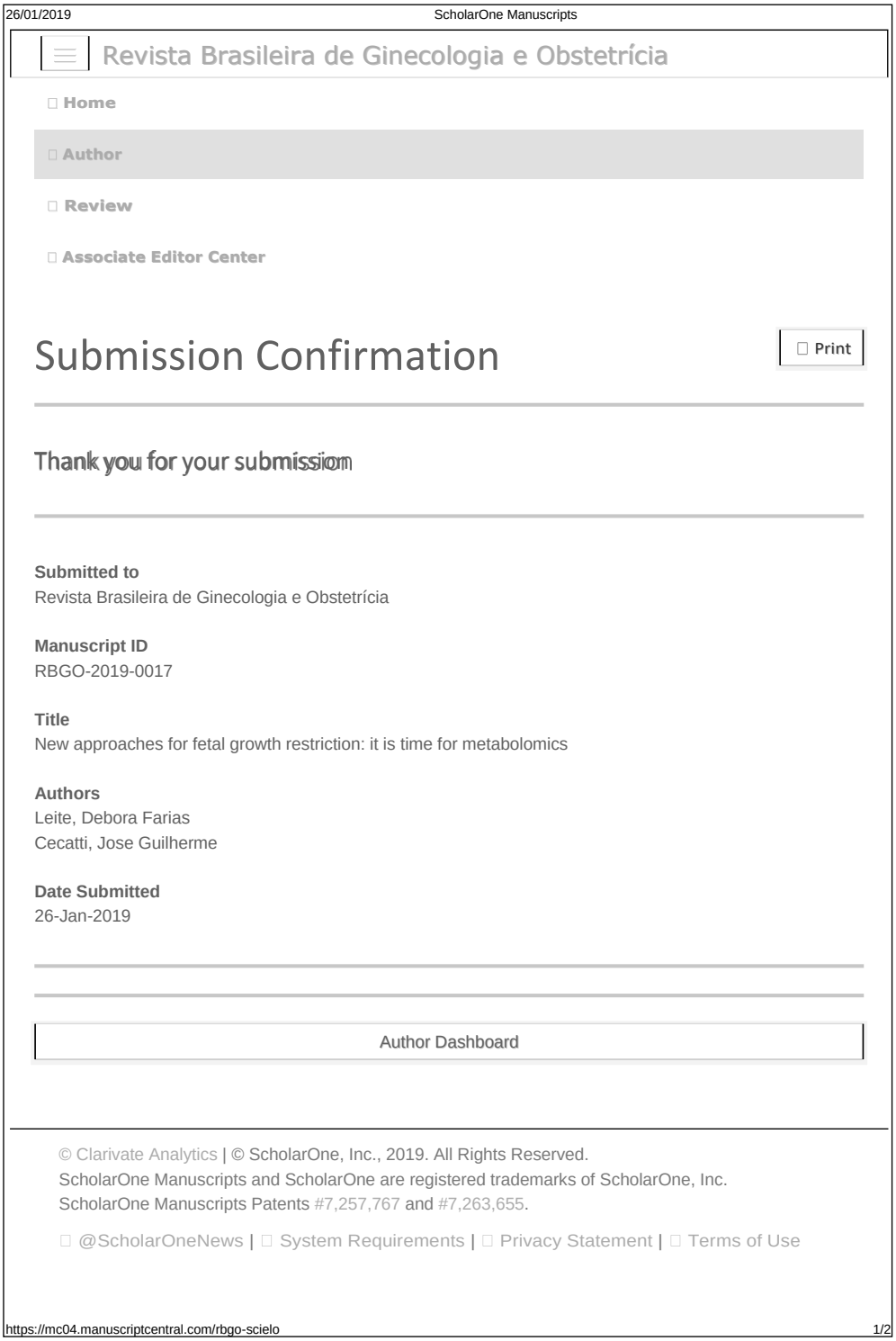

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REVIEW ARTICLE

New approaches for fetal growth restriction: it is time for metabolomics

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ABSTRACT

Fetal growth restriction (FGR) diagnosis is often made by fetal biometric ultrasound measurements or Doppler evaluation, but most babies are only diagnosed after birth, using the birthweight as proxy for intrauterine development. The higher risks of neurodevelopment delay, metabolic syndrome, and cardiovascular illness associated with FGR impose a shift on the focus during pregnancy. New methodological approaches, like metabolomics, can display novel insights about intrauterine fetal development. Information already available on metabolites involved with fetal growth and weight show a consistent role played by lipids (especially fatty acids), amino acids, vitamin D and folic acid. In near future, the establishment of a core set of outcomes for FGR studies may improve the identification of each metabolite's role for its development. Then, we will concretely progress with the perspective of a translational capacity of metabolomics for this condition.

Keywords: fetal growth restriction, small for gestational age, prediction, metabolomics

Resumo: O diagnóstico da restrição do crescimento fetal (RCF) frequentemente é feito por medidas biométricas ultrassonográficas ou avaliação pela Dopplervelocimetria, mas na maioria dos casos o diagnóstico é apenas pós-natal, usando o peso ao nascimento como um marcador para o desenvolvimento intrauterino. Os maiores riscos de atraso do neurodesenvolvimento, síndrome metabólica e doenças cardiovasculares associadas com a RCF impõem uma mudança no foco durante a gestação. Novas abordagens metodológicas, como a metabolômica, podem prover novos biomarcadores para o desenvolvimento fetal intrauterino. As informações já disponíveis sobre os metabolitos envolvidos com o

crescimento e peso fetal mostram um papel consistente desempenhado pelos lipídios, (especialmente ácidos graxos), aminoácidos, vitamina D e ácido fólico. Em um futuro próximo, o estabelecimento de um conjunto de desfechos a serem descritos para os estudos de RCF pode melhorar a identificação do papel de cada metabolito para seu desenvolvimento. Assim, iremos progredir no entendimento da RCF numa perspectiva translacional.

Palavras chave: restrição do crescimento fetal, pequeno para a idade gestacional, predição, metabolômica.

Introduction

The impairment of fetal growth has gained major importance in the last years. There is an increasing body of evidence suggesting that long-term health outcomes could be managed still during pregnancy. Findings from children[1] and adults[2–4] being born below birthweight average or exposed to maternal undernutrition *in utero*[5,6] support the hypothesis of the Developmental Origins of Health and Disease (DOHaD). Fetal growth restriction (FGR), i.e. when the fetus does not reach its 'optimal' growth potential, is possibly the underlying condition of future epidemiological burden of noncommunicable chronic diseases (NCD).

FGR has been recognized as a distinct condition in Perinatology only in the 1960's.[7] Unfortunately, there is still little consensus, both from Obstetric and Neonatology standpoints, of how clinicians should screen, diagnose and manage these fetuses and newborns. In fact, FGR is responsible for half of stillbirths,[8] and suspicion of fetal growth impairment in pregnancy clearly improves perinatal outcomes.[9] Clinical factors, ultrasound scan (US) parameters or placental biomarkers have shown modest clues about FGR pathophysiology. Small for gestational age (SGA) neonates are frequently used as a surrogate for FGR, and there are concerns that some of these babies are 'constitutionally small', i.e. not at higher risk of immediate worse outcomes.

Therefore, the development of new strategies for FGR and SGA evaluation is necessary. The post-genomic era is marked by rapid advances in the so-called *omics* sciences, including transcriptomics, proteomics, lipidomics, and metabolomics. This latter one is dedicated to studying small molecules, between 50 and 2000 Daltons, which represent the complex interaction between each individual and the

environment.[10,11] With metabolomics platforms, it is possible to evaluate endogenous compounds or exposure to contaminants, for instance, and to offer personalized care based on disease phenotype. In pregnancy, it is still an open field for appraising maternal and fetal adaptive responses to the intrauterine environment.

Metabolomics studies have shown maternal metabolic changes during normal pregnancies and have emerged as a reliable predictive and diagnostic tool for preeclampsia.[12] We hypothesize that recent advances in FGR evaluation have a similar potential, at least. Therefore, the aims of this review are to summarize the investigations of FGR with metabolomics approach, and the future perspectives of translating this knowledge to bedside practice.

What is metabolomics and its application on Obstetrics?

The first mention of the term 'metabolome' occurred in 1998,[13] and much has been done since then. The metabolome is dynamic by nature and represents a meaningful simultaneous evaluation of genetic and environmental influences.[14] As FGR is a heterogeneous syndrome and appears to be a metabolic disorder, both for mother and fetus, metabolomics is thought to be the best approach to investigate it.

Nuclear magnetic resonance (NMR) and mass spectrometry (MS) are the most common analytical platforms applied; MS can be coupled with liquid or gas chromatography, for example (Dunn et al[14] provide a comprehensive review on this issue). Two main types of investigations can be drawn, with different objectives: untargeted or targeted. In the first place, untargeted or ‘metabolic profiling’, evaluate thousands of metabolites in a given sample, simultaneously.[10] Peaks must be

matched by retention time and accurate mass; the Human Metabolome Database is an example of repository.[15] With untargeted analysis, it is not possible to determine absolute quantities of compounds, but a relative change between groups. Then, they are generally applied for hypothesis-generating purposes, attempting to comprehend biological processes.[11,14] In sequence, they should be validated in large-scale studies.[14] On the other hand, a targeted analysis is hypothesis-driven, i.e. devoted to measuring prespecified biomarkers,[10,14] with acceptable accuracy measures (e.g., sensitivity, specificity, area under the receiver operating curve, AUC) for differentiating health conditions.[11] Sample preparation will ultimately depend on study design and type of biological sample chosen.[14] Important to note, however, that biomarkers developed for a given population, are only suitable for that population.[11]

It is known that uneventful pregnancies show metabolic disruption when subjected to any pathological condition, such as chromosomal abnormalities, hypertensive disorders, or any composition of them.[16–20] Multiple pregnancies[21] and gestation following assisted reproduction also demonstrate distinct metabolic pathways when compared to single or spontaneous pregnancies, respectively. Changes can be detected in any biological sample, even breastmilk.[22] However, the main difficulty for conducting and interpreting metabolomics studies in reproductive medicine is the significant variety of definitions. For FGR, estimated fetal weight (EFW) by US,[23,24] reduced growth velocity of abdominal circumference, uterine or umbilical arteries blood flow abnormalities, or birth weight (BW)[16] are all criteria applied to identify these fetuses and newborns. Then, in order to offer a deeper evaluation of the available knowledge, we have kept the definitions applied by each study.

Fetal metabolism depends on its interaction with the maternal organism, and it is mediated at the placental level. There is probably a trend towards higher levels of nonessential amino acids with increasing gestational age in maternal blood,[25] while they show decreasing levels in maternal hair.[18] Some metabolic pathways are suspected to influence birthweight, such as the carnitine shuttle, de novo fatty acids biosynthesis, C-21 steroid biosynthesis and metabolism, prostaglandin formation, and glycerophospholipid, glycosphingolipid and tryptophan pathways.[26,27] Environmental exposure to organochlorine compounds, such as phthalate metabolites and perfluorooctanoic acid, are associated to decrease birthweight,[28] in a sex-specific manner.[29]

What metabolomics has found in growth-restricted fetuses and newborns

Maternal blood, urine, and hair have been explored for FGR evaluation with metabolomics, as well as amniotic fluid, venous cord blood, and newborn urine. In pregnancy, some studies have evaluated maternal levels of certain metabolites to birthweight. Our group has recently suggested a disruption of lipids metabolism in the 2nd trimester of SGA pregnancies (BW<10th centile).[30] Untargeted analysis of maternal blood[16] and hair[17,18] have provided reliable predictive accuracy, that should be validated in different settings. In the third trimester, there is major deposition of fat in fetal tissues and in the brain, which has led some investigations on maternal fatty acids metabolism. Between 26-28w, linoleic acid levels are positively associated with birthweight and abdominal adipose tissue volume, while docosahexaenoic acid is related to the proportionality of growth (length/height).[31] Near delivery, mother/newborn ratio of medium chain fatty acids is downregulated in pregnancies

affected by IUGR with Doppler abnormalities,[32] suggesting the increased need of energetic and structural metabolites by these newborns.

Most metabolomics studies with newborns have been concentrated in samples collected near delivery, to get the closest snapshot of fetal metabolism (Table 1). Favretto et all have found 22 metabolites that could differentiate adequate for gestational age (AGA) babies from FGR (suspected in pregnancy and confirmed after birth, both EFW and BW<10th centile). Seven were alpha-aminoacids, and all compounds were upregulated in FGR newborns. Tryptophan, phenylalanine, glutamate individually have had the best accuracy, reaching 100% of sensitivity (the former two compounds) and at least 85% of specificity (the latter one).[23] However, in the newborns sampled by Sánz-Cortez et al, amino acids were only significant in late-onset IUGR (BW<10th centile with delivery >35w and normal Doppler evaluation).[33]

In neonatal urine, Dessí et al[34,35] and Barberini et al[24] have found increased levels of myo-inositol in FGR cases (both EFW and BW<10th centile), although Barberini et al have grouped SGA and large for gestational age (LGA) babies for a final comparison. In fact, both SGA[36] and LGA[37] newborns show increased risk of metabolic events later in life, and more research is needed to elucidate which pathways are affected in each condition. Liu et al have searched for amino acids and acylcarnitines in neonatal blood. Homocysteine, methionine, tyrosine, alanine, ornithine, and serine have shown decreased levels in IUGR<3rd centile of BW.[38] Interestingly, these latter two amino acids were upregulated in SGA children without catch-up growth.[39]

Vitamin D has been involved in a multiplicity of biological pathways. Liquid chromatography coupled to mass spectrometry is the best approach for measuring

vitamin D. Evidence from trials suggest a protective effect of vitamin D maternal supplementation on birthweight,[40] but less is known if it directly impacts birthweight or if it is implicated with SGA pathogenesis. Indeed, vitamin D concentration varies according to ethnicity and smoking patterns,[41] variables already associated with impaired fetal growth. In the 1st trimester, vitamin D<50nmol/L was statistically associated with SGA (BW<5th centile) infants.[42] In the 2nd trimester of high-risk women for preeclampsia, vitamin D levels ≥75nmol/L were associated to decreased risk for BW<10th centile (adjusted risk ratio, aRR, 0.46; 95%CI 0,24-0,87).[43] However, in low-risk women, levels <30nmol/L at 15w were not associated with SGA (BW<10th centile),[44] even when there were increased parathyroid hormone levels.[45] These findings suggest that the thresholds of vitamin D that confer either a risk or a protective effect are not the same as those used in clinical practice for defining normal levels in pregnancy. Indeed, apart from the high prevalence of vitamin D deficiency in pregnancy and in cord blood, it appears to have no impact on infant musculoskeletal development at 2y.[41]

This raises the question of whether there is constitutional or truly impaired fetal growth and birthweight. Some researchers have investigated differences between newborns with BW<10th with or without Doppler abnormalities.[32] As a matter of fact, metabolic differences are understandable, and perhaps expected, due to fetal blood flow redistribution. Unfortunately, discriminating the ‘truly restricted fetuses’ from the ‘constitutionally small’ ones may need more than BW evaluation, but include adverse perinatal outcomes, at least.

What should be explored

The World Health Organization now recommends iron and folic acid (at least 400mcg) supplementation during all pregnancy.[46] Apart from its role in preventing neural tube defects, epidemiological data indicate folate participation on birthweight. For instance, its depletion is suspected to justify the repeated SGA in case of interpregnancy intervals lower than 23 months.[47] In fact, a recent systematic review has pointed that folic acid supplementation before conception decreases the risk of SGA <10th BW centile (adjusted odds ratio, aOR, 0.80, 95%CI 0.71-0.90) or <5th (aOR 0.78, 95%CI 0.66-0.91).[48] Additionally, at *nuclei* level, folate acts as a methyl donor, and little is known if its involved with methylated enriched pathways observed in SGA pregnancies.[49] Therefore, whether folic acid or homocysteine mediate FGR pathogenesis or are only biomarkers of disease merit consideration in further metabolomics researches.

Amino acid supplementation to improve fetal weight is another intriguing relationship. L-arginine is a precursor of nitric oxide, which regulates placental perfusion. Arginine in amniotic fluid is directly correlated with BW, length and head circumference.[50] Evidence from small trials show a marked increase in BW (mean difference 0.41; 95%CI 0.24-0.58), although participants characteristics and follow up, and route and duration of arginine supplementation, were heterogeneous.[51] FGR placental explants in hypoxic (O_2 1%) conditions have half of the metabolites in common with AGA pregnancies under normal oxygen tension (O_2 6%), suggesting that hypoxia should play a role in FGR pathogenesis.

Conclusion

Metabolomics is a novel and promising area of research in reproductive medicine. Although some results may contradict each other, maternal and fetal metabolism are

highly dynamic and may adapt according to several influences. Levels of metabolites in cord blood might represent increased fetal demands or catabolism, for instance. Future validations of metabolomics studies[11] in different populations will set the ideal thresholds for clinical practice, and we envision possible distinction of fetuses that reach ‘optimal growth’ from others that do not.

As metabolomics is a very sensitive and holistic approach, extra care must be taken in participants selection. Evaluating pregnant women or newborns different from those found in clinical practice will limit the translational potential of this technology. Although guidelines for reporting observational epidemiologic[52] or metabolomics[53] studies are available, they do not fulfill the needed details for translational investigations. Meaningful transfer of the bench side advancements to clinical practice is a real concern and will be achieved only if researchers and clinicians speak the same language [11]. In the near future, the establishment of a core set of outcomes for FGR studies may organize a description of clinical data and avoid duplicate effort. Then, we believe that concrete progress with metabolomics will be faster.

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Table 1. Metabolomics studies with newborns.

Authors	Country	Type of sample	Time of sampling	Technique	Targeted metabolites	Definition for FGR/ SGA	Participants	Increased metabolites	Decreased metabolites
Untargeted evaluations									
Dessi et al, 2011 [34]	Italy	Urine	<24h after birth (before any nutrition)/ again in 96h after birth	H-NMR		IUGR: <10th of BW by populational curves suspected in pregnancy	26 IUGR[b] 30 AGA, preterm births	Creatinine and myo-inositol	
Horgan et al, 2011 [16]	Australia	Blood	20' after birth	UPLC-MS		<10th customized birthweight	8 SGA 6 AGA	DG(32:0)	Phenylacetylglutamin, Leucyl-leucyl-norleucine, Cervonyl-carnitine, (15Z)-Tetra-cosenoic acid, Hexacosanedioic acid, Pentacosenoic acid, Cycloheptanecarboxylic acid, Hydroxybutyrate, LysoPC (18:2), PC-more than 20 hits, PC, Lyso-PC, Acetylleucyl-leucyl-norleucinal, LysoPC(16:1),Pregnaned iol-3-glucuronide, Sphinganine 1-phosphate, Sphingosine 1-phosphate, Pregnanediol-3-glucuronide, 6-hydroxysphingosine
Favretto et al, 2012 [23]	Italy	Venous cord blood	Immediately after birth	Metabolic profiling/ LC-HRMS		IUGR[a]: EFW <10th AGA: EFW 10-90th – both confirmed after birth by populational curves	22 IUGR 21 AGA; antepartum C-section.	Tryptophan, phenylalanine, glutamate, valine, isoleucine, hystidine, proline, methionine, dopamine, uric acid,	

								5-methylundecenoic acid, L-thyronine, hexadecanedioic acid, (OH)VitD3-3-D-glucopyranoside	
Sanz-Cortez et al, 2013 [33]		Venous cord blood	At delivery	H-NMR		IUGR: <10th of BW and Doppler abnormalities	76 IUGR[a] 55 AGA[d]	Unsaturated lipids, creatine, glutamine	Choline
Dessi et al, 2014 [35]	Italy	Urine	1st urine after birth	H-NMR		IUGR: <10th of BW by populational curves suspected in pregnancy	12 IUGR[b] 17 AGA	Citrate, creatinine, creatine, myo-inositol, bataine/TMAO, glycine	Urea, aromatic, compounds, branched-chain amino acids
Miranda et al, 2018 [54]	Spain	Blood	After birth	H-NRM		FGR: EFW<3rd centile or <10th + Doppler abnormalities SGA: EFW and BW<10th without Doppler abnormalities		Fatty acids, formate (SGAxAGA/ FGRxAGA) Acetate (FGRxAGA)	
Targeted evaluations									
Liu et al, 2016 [38]	China	Blood	3-7 days of birth	HPLC-MS	21 amino acids and 55 acylcarnitines	IUGR: <10th of BW by populational curves	60 IUGR[a] 60 AGA		Alanine, homocysteine, methionine, ornithine, serine, tyrosine[c]
Visentin et al, 2017 [32]	Italy	Venous cord blood	At birth	GC-MS	Medium chain fatty acids	IUGR: EFW<3rd centile or <10th + Doppler abnormalities SGA: EFW and BW<10th without Doppler abnormalities	11IUGR 12 AGA 10 SGA	decanoic and dodecanoic acid (SGAxIUGR) octanoic, decanoic and dodecanoic acid (SGAxAGA)	

IUGR: [a]intrauterine growth restriction or [b]retardiation; LC-HRMS: Liquid chromatography coupled with high-resolution mass spectrometry. [c]IUGR<3rd centile x AGA; 20 early IUGR, and 56 late IUGR; highlighted metabolites for both late and early IUGR cases.

Table 2. Targeted metabolomics studies with mothers near delivery.

Authors	Country	Type of sample	Time of sampling	Approach	Targeted metabolites	Definition for FGR/ SGA	Participants	Increased metabolites	Decreased metabolites
Visentin et al, 2017 [32]	Italy	Blood	After birth	GC-MS	Medium chain fatty acids	IUGR: EFW<3rd centile or <10th + Doppler abnormalities. SGA: EFW and BW<10th without Doppler abnormalities	11IUGR 12 AGA 10 SGA	Hexanoic, octanoic, decanoic and dodecanoic acid (SGAxIUGR) octanoic, decanoic and dodecanoic acid (SGAxAGA)	
Miranda et al, 2018 [54]	Spain	Blood	After birth	H-NRM		FGR: EFW<3rd centile or <10th + Doppler abnormalities SGA: EFW and BW<10th without Doppler abnormalities	27 FGR 25 SGA 28 AGA		Fatty acids, 2-oxoisovaleric acid, citrate (SGAxAGA/ FGRxAGA) Alanine (FGRxAGA)

Artigo 4: Metabolomics for predicting fetal growth restriction: protocol for a systematic review and meta-analysis.

Open access Protocol

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Metabolomics for predicting fetal growth restriction: protocol for a systematic review and meta-analysis

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ABSTRACT

Introduction Fetal growth restriction (FGR) is a relevant research and clinical concern since it is related to higher risks of adverse outcomes at any period of life. Current predictive tools in pregnancy (clinical factors, ultrasound scan, placenta-related biomarkers) fail to identify the true growth-restricted fetus. However, technologies based on metabolomics have generated interesting findings and seem promising. In this systematic review, we will address diagnostic accuracy of metabolomics analyses in predicting FGR.

Methods and analysis Our primary outcome is small for gestational age infant, as a surrogate for FGR, defined as birth weight below the 10th centile by customised or population-based curves for gestational age. A detailed systematic literature search will be carried in electronic databases and conference abstracts, using the keywords 'fetal growth retardation', 'metabolomics', 'pregnancy' and 'screening' (and their variations). We will include original peer-reviewed articles published from 1998 to 2018, involving pregnancies of fetuses without congenital malformations; sample collection must have been performed before clinical recognition of growth impairment. If additional information is required, authors will be contacted. Reviews, case reports, cross-sectional studies, non-human research and commentaries papers will be excluded. Sample characteristics and the diagnostic accuracy data will be retrieved and analysed. If data allows, we will perform a meta-analysis.

Ethics and dissemination As this is a systematic review, no ethical approval is necessary. This protocol will be publicised in our institutional websites and results will be submitted for publication in a peer-reviewed journal.

PROSPERO registration number CRD42018089985.

Strengths and limitations of this study

- ► This systematic review covers a great range of electronic databases and will also search for grey literature.
- ► Two researchers will perform literature search, data extraction and study quality assessment independently, and any disagreement will be resolved by a third reviewer.
- ► Careful statistics procedures will be performed to identify accuracy of metabolomics in predicting fetal growth restriction.

InTROduCTIOn

Fetal growth restriction (FGR) is usually defined as a fetus that has not reached its intrauterine growth potential,[1 2] with no major congenital abnormalities[1] and has also been named as fetal growth retardation, intrauterinegrowthrestrictionorretardation.[3] This heterogeneous condition is associated with increased risks of stillbirth,[4 5] neonatal intensive care unit admission,[6] neonatal mortality,[5] cognitive and behavioural impairment in infancy[7] and chronic non-transmissible disease in adulthood.[8] FGR is mainly diag- nosed according to the estimated fetal weight in ultrasound scans below the 10th centile,[2 9] although it is anticipated that misdiagnosis can occur: fetuses below the 10th centile, but with normal outcomes ('constitutionally' small), or fetuses above the 10th centile, but who did not follow personal growth poten- tial.[2] In this context, antenatal recognition of truly restricted fetuses, that is, those at higher risk of morbidity and mortality in any period of life, followed by adequate obstetrical care, can improve neonatal outcomes.[10]

Unfortunately, in current practice, there is no gold standard for FGR diagnosis. Recent consensus has added ultrasound criteria (eg, abdominal circumference, umbilical and uterine artery Doppler measurements) and lowered estimated fetal weight cut-offs (<3rd centile),[1] to improve specificity. In these terms, the concept of FGR can overlap with that of small for gestational age (SGA), which includes infants with birth weight below the 10th (or fifth, or third) centile for gender and gestational age.[11] In fact, it is common to use SGA as a surrogate for FGR,[6 12 13] as an indication of real intrauterine growth impairment. Besides that, neonatal parameters seem more

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adequate as 'patient important outcomes', but regrettably, ultrasound have still low accuracy to determine them.[6]

Since 1990s, when the thrifty phenotype theory was introduced,[14] a huge effort has been undertaken to investigate the pathologically growth restricted fetuses and newborns and to enhance antenatal screening.[6] Clinical data has been intensively studied, with conflicting risk factors[15 16] and, in general, poor accuracy is achieved for identifying impaired birthweight[17] orneonatal morbidity,[6] even when first,[18] second[13 17] or third trimester[19] ultrasound parameters are added to prediction model. Using only clinical or ultrasound variables, the great majority of SGA babies will only be recognised after birth, by population-based[5] or customised curves.[17] Biomarkers, such as placental growth factor (PlGF), soluble fms-like tyrosine kynase 1 (s-Flt-1) and alpha-fetoprotein,[20] have each been found to show promise as aids to understanding FGR. However, the performance of these angiogenic factors as predictors of FGR has been limited (positive likelihood ratio, LR+, of 1.3 for PlGF and 1.4 for s-Flt-1).[21] Similarly, placental proteins are not robust enough biomarkers for FGR (eg, LR+ of 3.7 for placental protein-13 in first trimester).[22] Therefore, there is a real need for better methods of FGR prediction.

The disappointing evidence may be due to the multifactorial nature of FGR; the aetiology of the condition is complex and poorly defined. Moreover, placental structure and functioning, maternal and fetal metabolism vary during pregnancy.[23] In this context, contemporary metabolomics approaches have identified several pathways and metabolic processes that may contribute to FGR, such as disruptions in DNA methylation,[24] cellular signalling,[25 26] neurotransmitter precursors[26 27] and energy generation.[25 26]

Despite excellent performance of some metabolites in predicting FGR (area under the curve, above 0.9),[25 26] these studies have shown an overall modest accuracy.[21] However, only two 'omics' studies were included in Conde-Agudelo *et al*[21] review, and issues related to gestational age of sampling and delivery, or analysis of composite outcomes, could have introduced bias and confounders to metabolomics findings. In recent years, many authors have applied diverse metabolomics techniques to predict FGR, suggesting that metabolite biomarkers may have a role to play in disease screening. Thus, the main objective of this systematic review is to define the accuracy of metabolomics techniques for predicting FGR. As secondary aims, we will try to deter- mine which metabolites are robust candidates for a prediction model of FGR and which chemical class they belong to.

METhOdS And AnAlySIS

Review question

What is the accuracy of metabolomics for predicting FGR?

Condition or domain studied

SGA infant and FGR.

Participants/population

Inclusion criteria: Original studies including pregnant women.
Exclusion: Congenital malformation.

Interventions/exposure

Screening for SGA/FGR with metabolomics approach. Biomarker analysis should have been performed on samples taken before clinical recognition of neonatal outcome.

Inclusion and exclusion criteria

Original studies (cohort or case control studies) involving pregnant women, as the studied population and SGA infant (and variations of terminology), as the outcome of interest, will be included in this systematic review.

The reasons for excluding studies are: (1) if they are Cross-sectional studies, Case Reports, Editorials, Letter to Editors, Commentaries, Expert Opinions, or any type of Reviews; (2) if they describe only experimental studies with animals; (3) if they show duplicate publication of the same data; in these cases, we will use the most recent publication.

Outcomes

Primary outcome

SGA infant, defined as a birth weight below the 10th centile according to population-based or to customised charts.

Secondary outcomes

Birth weight below the fifth or the third centile by population-based or customised parameters.

literature search

The primary source of information will be these electronic databases: PubMed, EMBASE, Latin American and Caribbean Health Sciences Literature, Scientific Electronic Library Online, Health Technology Assessment, Database of Abstracts of Reviews of Effects, Aggressive Research Intelligence Facility, Cumulative Index of Nursing and Allied Health Literature, Maternity and Infant Care, Scopus and Web of Science. Secondary sources include Google Scholar, hand-held searching of the reference list of eligible studies, conference proceedings and contact with authors when necessary.

The keywords linked to the outcomes of interest will be combined with terms related to 'metabolomics' technique, 'pregnancy' and 'screening', using Boolean connectors. The same search strategy will be applied for each database, adapting for individual filters, main language, their own syntax and mechanisms of search; the complete search strategy is provided as online supple- mentary material.

Considering that the term metabolome was first used in 1998,[28] we will take into account studies published in

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the last 20 years (1998–2018). The preliminary searches for this systematic review have started in February 2018. The search strategy will be re-run before final analysis, to check for recently published eligible studies. There are no language restrictions.

data extraction and management

All searches will be exported to a reference manager (EndNote). Individually, two researchers (DFBL and ACM) will select papers according to (1) title or abstract and (2) full text, that will be read only when abstracts are not sufficient to decide about inclusion criteria. Any disagreement about selected studies will be dealt by a third researcher (EFMJ or RTS); in these cases, only after majority decision (2:1 ratio) the next step will be performed. A fifth investigator (JGC) will revise all procedures before approving the data extraction. DFBL, ACM and ASK will deal with the statistic procedures. JGC, PNB and LCK will re-examine all steps and supervise data interpretation.

A standardised form will be applied to extract the variables of interest—by two independent researchers—which will include: authors and year of publication, country of participants' enrolment, study design, definition used for FGR/SGA (customised or population-based charts) and outcome measured, number of affected (who later delivered a FGR/SGA baby) and non-affected pregnant women, gestational age of assessment (throughout pregnancy), laboratory methods and biological sample analysed (eg, blood, amniotic fluid). In addition, data regarding growth impairment suspicion in pregnancy—such as gestational age, criteria applied for diagnosis and follow-up—will be retrieved once available. Researchers will contact authors (by electronic address) if any clarification of data is needed. The metabolites described will be matched with the Human Metabolome database to check their characteristics.[29]

Strategy for data synthesis

Details about data search and selection will be presented as a flow diagram, according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement recommendations.[30] An aggregate partici- pant data synthesis will be performed with all included studies; narrative data will be analysed and structured according to birth weight centile (10th, 5th and 3rd) and curve type (population-based or customised curves). Additionally, the metabolites will be grouped and synthesised according to their biological function and chemical subclass. Studies' characteristics and risk of bias assessment will be demonstrated in tables. Once possible, we will perform subgroup analysis according to:

- Which metabolomic methods were applied (gas or liquid chromatography coupled with mass spectrometry; or proton nuclear magnetic resonance).
- Maternal health status before pregnancy (healthy ones vs women with any chronic health condition).
- Gestational age of first fetal growth impairment suspicion (early vs late FGR).[1]
- Type of pregnancy (single vs multiple).

Depending on data availability, accuracy measures will be calculated and a meta-analysis will be drawn. Considering the quantitative nature of the metabolo- mics approach and the expected different thresholds for metabolites in each study, we will try to perform the hierarchical summary receiver characteristic operating curve.[31] Heterogeneity will also be assessed, through I^2 test.

Risk of bias assessment

Both investigators initially involved with literature search (DFBL and ACM) will assess methodological quality and applicability of all included studies, and they must check their judgements. A third researcher (EFMJ or RTS) will resolve any disagreement if necessary and the final decisions will be made by majority. We will use the 'Quality Assessment of Diagnostic Accuracy Studies'[32] tool, which comprised four domains: patient selection, characteristics of index test (metabolomics technique), the reference standard test (measurement of birth weight) and flow and timing of patient inclusion and follow-up. Every study will be labelled as 'low', 'high' or 'unclear' risk of bias for each domain. For example, there is 'low risk of bias' if the study clearly states how the metabolomics techniques were performed, or which birth weight curve was applied to identify the SGA babies.

Regarding publication bias, we will assess the symmetry of funnel plots if more than ten studies are included in the meta-analysis.[33]

Potential limitations to this review

Concerning the publication bias, we expect to encounter more published positive results and data interpreta- tion must take this issue in consideration. The metabo- lomics approach is highly detailed and meticulous, has shown great technological advancements in recent years, and results from mass spectrometry and from nuclear magnetic resonance complement each other. Therefore, we acknowledge that we may find distinct metabolites in each study and generalisation may be challenging.

In this systematic review, we have considered SGA as a proxy for FGR, as other authors.[6 12 13] The consensus for FGR diagnosis was published recently[1] and past investigations may have used distinct terminology or conflicting criteria for this condition in pregnancy. Additional confounders to interpret the selected studies will include clinical factors potentially associated to FGR/SGA, like parity, smoking habits and history of previous fetal growth impairment. These characteristics will be appraised during data extraction and synthesis, and detailed evidence will be retrieved.

Ethics and dissemination

This protocol follows the PRISMA Protocols statements.[34] A report of this systematic review will be sent to our

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sponsors. This protocol will be electronically available on UNICAMP-CNPq-Gates Foundation project website (www.medscinet.com/samba) and Infant Centre website (infantcentre.ie). Our results will be submitted to publication in peer-reviewed journal.

Patient and public involvement

Patients and or public were not involved at all in elaborating this systematic review protocol.

COnCluSIOn

This systematic review will synthesise data about metabolomics and FGR/SGA, a promising field for understanding disease pathophysiology and natural history. By highlighting the metabolites and chemical classes that they belong to, this review might present solid data to future research protocols, that can target the most promising compounds, or assess the participants in a more reliable gestational age, for example. A robust FGR/SGA prediction assumes great importance in reproductive health and epidemiology, since this condition is associated with short and long-term adverse outcomes for the offspring.

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Contributors DFBL (the guarantor of the review) and A-CM developed the systematic review protocol and will perform the literature search, study selection, data extraction and risk of bias assessment. ASK, PNB, RTS, EFMJ and JGC supervised protocol elaboration and the latter three will resolve any discrepancy about methodology. ASK, DFBL and A-CM will deal with statistics procedures. PNB and LCK performed the last amendments of protocol and will revise the final systematic review draft. All authors have read this manuscript and have agreed with this submission.

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Competing interests DFBL and A-CM are studying this technology in predicting FGR. JGC, LCK and PNB have presented conference talks about this field. LCK and PNB are principal investigators of Metabolomic Diagnostics.

Patient consent Not required.

Ethics approval As this is a systematic review protocol, no ethics committee approval is necessary.

Provenance and peer review Not commissioned; externally peer reviewed.

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Supplementary material. Complete strategy for literature search.

	# Date
1	fetal growth retardation
2	fetal growth restriction
3	intrauterine growth restriction
4	intrauterine growth retardation
5	small for gestational age
6	#1 OR #2 OR #3 OR #4 OR #5
7	metabolomic*
8	metabonomic*
9	metabolit*
10	H NMR
11	proton NMR
12	proton nuclear magnetic resonance
13	liquid chromatogra*
14	gas chromatogra*
15	UPLC
16	ultra-performance liquid chromatograph*
17	ultra performance liquid chromatograph*
18	#7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17
19	pregnan*
20	antenat*
21	ante nat*
22	prenat*
23	pre nat*
24	#19 OR #20 OR #21 or #22 OR #23
25	screen*
26	predict*
27	metabolic profil*
28	#25 OR #26 OR #27
29	#6 AND #18 AND #24 AND 28

Artigo 5: Examining the predictive accuracy of metabolomics for small for gestational age babies: a systematic review. (*To be submitted for publication on the American Journal of Obstetrics and Gynecology*)

SYSTEMATIC REVIEW

Examining the predictive accuracy of metabolomics for small for gestational age babies: a systematic review

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CONFLICTS OF INTEREST

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CONDENSATION

LC-MS was the most common metabolomics technique for predicting SGA. Compounds related to lipid metabolism pathway are involved in the pathophysiology of SGA in the 2nd trimester.

SHORT TITLE

Metabolomics for SGA prediction: a systematic review.

AJOG AT A GLANCE

- *A. Why was this study conducted?* There are limited tools for predicting small for gestational age (SGA) infants. Metabolomics is a promising approach to clinical prediction in reproductive health.
- *B. What are the key findings?* Liquid-chromatography coupled to mass spectrometry (MS) was the most commonly used technique for the evaluation of SGA. Untargeted studies in the 2nd trimester of pregnancy provided more compounds of interest. Although vitamin D and homocysteine were the most commonly targeted metabolites, the inclusion of fatty acids and other lipid-like compounds in many predictive models demonstrates that lipid metabolism may play a critical role in the pathophysiology of SGA.
- *C. What does this study add to what is already known?* There is an impairment of lipid and energy pathways in SGA pregnancies. Efforts must be focused on sampling women up to the 2nd trimester of pregnancy.

ABSTRACT

Objective: To determine the accuracy of metabolomics in predicting small for gestational age babies and elucidate which metabolites were found to be predictive of this condition.

Data sources: Following a published protocol, two independent researchers explored 11 electronic databases in February 2018 and November 2018, covering published articles from 1998 to 2018. Both researchers performed data extraction and quality assessment independently. Database search was supplemented by a hand-held search of bibliographies of eligible studies. Discrepancies were resolved by a third researcher.

Study eligibility criteria: Cohort or nested case-control studies were included, which investigated pregnant women and performed metabolomics analysis to evaluate small for gestational age infants. The primary outcome was birthweight <10th centile - as a surrogate for fetal growth restriction - by population-based or customized charts.

Study appraisal and synthesis methods: Data on study design, obstetric variables and sampling, metabolomics technique, chemical class of metabolites, and prediction accuracy measures were extracted by two independent researchers. Authors were contacted to provide additional data when necessary.

Results: A total of 9,181 references were retrieved. Of these, 273 were duplicate data, 8,620 were removed by title or abstract, and 273 were excluded by full text content. Thus, 15 studies were included in this systematic review. Only two studies used the 5th centile as a cutoff, and the majority of reports sampled 2nd trimester pregnant women. Liquid-chromatography coupled to mass spectrometry was the most common metabolomics approach. Untargeted studies in the 2nd trimester

provided the largest number of predictive metabolites, using maternal blood or hair. Although vitamin D (i.e., steroid) was the most frequently studied metabolite, fatty acids, phosphosphingolipids, and amino acids were the most prevalent predictive chemical subclasses.

Conclusions: There was a significant heterogeneity of participant characteristics and methods employed among studies, precluding a meta-analysis. Compounds related to lipid metabolism should be validated up to the 2nd trimester in different settings.

Keywords: small for gestational age, fetal growth restriction, metabolomics, prediction, gas-chromatography, mass spectrometry, vitamin D, homocysteine, lipids, fatty acids.

INTRODUCTION

Fetal growth restriction (FGR) and small for gestational age (SGA) infants are major concerns in modern obstetrics[1–3]. SGA is commonly used as a proxy for FGR[4], despite the subtle differences between these two pathological conditions. The prevalence of both varies according to criteria applied and on the population and setting, although it reaches as much as 25% in low and middle-income countries[5]. SGA newborns may have adverse health effects, such as stillbirth,[4] perinatal asphyxia,[6] impaired neurodevelopment,[7] and increased cardiovascular risk[8,9]. To date, there are no robust prediction tools for SGA using clinical factors,[10,11] ultrasound data,[12,13] or placental biomarkers.[14]

For hypothesis generating or validation purposes, metabolomics is a novel area of biomarker, discovery, development and clinical diagnostics in translational medicine.[15,16] Metabolomics is the study of all metabolites[15-17] in a given sample, i.e. low molecular weight compounds (50-2000 Da) that are intermediates of biochemical reactions and metabolic pathways, considered to directly reflect cellular activity and phenotype.[15,16] Recent studies have evaluated the pathophysiology[17–20] of SGA with metabolomics. However, little is known about the potential of metabolomics to identify predictive compounds of SGA.

Since metabolomics can identify multiple metabolites from low volume samples, and create a model from a collection of these samples,[15] it is a promising technology for hypothesis generation in a heterogeneous condition such as SGA. The prediction of SGA in pregnancy would help refer women to specialized care facilities, improving maternal and neonatal outcomes.[21,22]

OBJECTIVE

In this context, the main objective of this systematic review was to assess the accuracy of metabolomics techniques in predicting SGA. As a secondary aim, we intended to determine which metabolites are predictive of this condition.

METHODS

The protocol for this systematic review was published previously.[23] This study follows international guidelines for transparency (PROSPERO, CRD 42018089985) and respects the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement.[24] This systematic review was conducted without any public involvement, and ethical approval was not necessary.

Literature Search Strategy

Two independent researchers (DFBL and ACM) assessed 11 electronic databases (PubMed, EMBASE, Latin American and Caribbean Health Sciences Literature (LILACS), Scientific Electronic Library Online (Scielo), Health Technology Assessment (HTA), Database of Abstracts of Reviews of Effects (DARE), Aggressive Research Intelligence Facility (ARIF), Cumulative Index of Nursing and Allied Health Literature (CINAHL), Maternity and Infant Care (MIDIRS), Scopus, and Web of Science) and grey literature. There were no limits or language constraints; the search strategy covered published documents between 1998 and 2018. Keywords ‘small for gestational age’, ‘metabolomics’, ‘prediction’, ‘antenatal’, and variations of each, were combined with Boolean operators depending on each database requirements. The full EMBASE literature search was, as follows: (‘fetal growth retardation’ OR ‘fetal growth restriction’ OR ‘intrauterine growth restriction’ OR ‘intrauterine growth retardation’ OR ‘small for gestational age’) AND (‘metabolomic*’ OR ‘metabonomic*’

OR ‘metabolit* ‘H NMR’ OR ‘proton NMR’ OR ‘proton nuclear magnetic resonance’ OR ‘liquid chromatogra*’ OR ‘gas chromatogra*’ OR ‘UPLC’ OR ‘ultra-performance’ OR ‘ultra performance liquid chromatograph*’) AND (‘pregnan*’ OR ‘antenat*’ OR ‘ante nat*’ OR ‘prenat*’ OR ‘pre nat*’) AND (‘screen*’ OR ‘predict*’ OR ‘metabolic profil*’).

Outcomes and subgroup analysis

The primary outcome was SGA, as a surrogate for FGR and defined as birthweight <10th centile, by population-based or customized charts. Secondary outcomes were birthweight ≤5th or ≤3rd centile.

The intended subgroup analysis comprised: type of metabolomics technique applied (nuclear magnetic resonance, NMR; gas or liquid chromatography coupled with mass spectrometry, GC-MS or LC-MS respectively); maternal health status before pregnancy (women with *versus* without any chronic health condition); type of SGA suspected during pregnancy (early *versus* late SGA); and type of pregnancy (singleton *versus* multiple pregnancy).

Selection Criteria of Studies, Data Collection and Analysis

Cohort or nested case-control studies were included if maternal samples were collected before the clinical diagnosis of SGA, if any metabolomics technique was applied, and if the results of SGA were presented. Articles presenting data from the same research project but analyzing distinct metabolites or showing data from different countries were included. Studies were excluded (i) according to study design; (ii) if they had not applied any metabolomics technique; (iii) if they were only experimental studies; (iv) if it was not possible to extract data on SGA; (v) or if they

presented duplicate data, in which case the most complete publication was included for final analysis.

Two researchers (DFBL and ACM) independently selected studies, extracted data and discussed discrepancies. One additional reviewer (EFMJ or RTS) helped to decide, by majority, when no consensus was reached.

Piloted standardized forms were applied for data extraction, including pregnancy characteristics and experimental details. The Human Metabolome Database (HMDB)[25] and the Kyoto Encyclopedia of Genes and Genomes[26] were used for matching chemical class and metabolic pathways of each metabolite, respectively.

Risk of bias and Assessment of concerns regarding applicability

Two researchers (DFBL and ACM) independently evaluated individual studies using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool.[27] One of the third reviewers (EFMJ, or RTS) helped in decision-making when no consensus was achieved.

Each study was classified as high, low, or unclear risk of bias in four Domains (Patient Selection, Index Test, Reference Standard, and Flow and Timing), and as high, low, or unclear concerns regarding applicability in the first three Domains. We did not consider two signaling questions (“Was a case-control design avoided?”, “Was there an appropriate interval between the index test and reference standard?”). The nested case-control design was an inclusion criterion and maternal samples should have been collected during pregnancy, i.e. before the SGA diagnosis. Studies were considered ‘low risk’, for example, (i) if pregnancy or neonatal complications were not excluded in just one group of participants or data on

participant selection had been provided; (ii) if methods for sample preparation and interpretation were standardized or metabolite threshold was defined before the experiments (for targeted analysis); (iii) if the adequacy and reasons for choosing the reference birthweight chart had been explained; or, (iv) if large for gestational age babies had been excluded from the final comparative analysis.

Data synthesis

A quantitative summary of data was performed when any predictive accuracy measures could be extracted. Authors were contacted to provide additional information, when necessary. However, only Delplancke et al[28] replied. The estimation of likelihood ratios and hierarchical summary receiver operator characteristic curve[29] were planned, as well as assessment of heterogeneity and publication bias.[30] However, due to lack of data, a meta-analysis could not be performed.

RESULTS

Literature search characteristics

The literature search for this systematic review was performed in February 2018, and re-run in November 2018. A total of 9,181 references were retrieved (Figure 1). After the removal of duplicate records (n=273), title and abstract screening, and analysis of the remaining 148 full-text articles, 15 articles were included.[17,18,28,31–42] See Appendix A for excluded studies.

Characteristics of the included studies

The characteristics of the included studies are shown in Table 1. The prevalence of SGA ranged from 7.3%[38] to 21.5% in cohort studies.[28] There were no studies using a birthweight ≤3rd centile for a definition of SGA. The time interval between initial participant enrollment and publication varied from three[17] to 54 years,[33] although these data were unclear in 38% of the reports.[18,28,37,38,42] In nested case-control studies, participants were matched by maternal age,[17,18,31,35] ethnicity,[17,18,35] parity,[31] body mass index,[17,18,35] or infant gender.[18,31]

Participant characteristics varied between studies. Regarding gestational age at assessment, samples were collected in the 2nd trimester in one half of the studies.[17,18,32,35,38,40,42] In three reports, women were assessed at least twice.[31,34,39] In one study, maternal blood was drawn either in the 1st or 2nd trimester;[33] and in another three studies, only samples from the 3rd trimester were considered.[28,34,41] In the latter case, maternal hair was divided according to length, allowing evaluation of 2nd and 3rd trimester metabolites.[28] Studies considering the 5th centile as the cutoff, sampled women in the 1st trimester.[36,37] Twin pregnancy was a clear exclusion criterion in most studies.[17,18,33–36,38–40,42] Pregnancy aided by assisted reproduction[18,42] or women with pre-existing conditions[17,18,35,40,42] were also excluded, although these data were incompletely reported[28,31,32,34,37,41]. When both nulliparous and multiparous women were enrolled, there was no data analysis according to parity. Half of the studies considered term deliveries exclusively,[18,28,31–34,41] and the remaining studies did not differentiate results according to gestational age at birth.

Regarding clinical risk factors for SGA, only one paper mentioned a previous history of SGA, but findings were not adjusted for this variable.[37] All studies, except one,[28] cited participant smoking status. The rate of smoking habit ranged from 2.4%[18] to 47.5%.[33] It is important to note that Gernand et al[33] analyzed samples from women

recruited between 1959 and 1965, when smoking while pregnant was encouraged, which explains the high rate of smoking participants. The duration of smoking or any differences in birthweight (absolute measures or centiles) were not clearly stated. Although more prevalent in SGA pregnancies, results did not change with this variable control.[33,36,37,40,42] Only Gong et al[34] mentioned the suspicion of SGA in pregnancy, exhibiting decreasing abdominal circumference growth velocity between 20-36 wks. However, on final analysis, these babies were grouped with infants not suspected during pregnancy.

Subgroup analysis

Due to unavailable data, the only subgroup analysis performed was related to the metabolomics approach applied (Table 2). There was no mention of adherence to metabolomics reporting data guidelines. LC-MS was the leading technique used. Three studies have investigated metabolites related to environmental exposure, from contaminated water,[36] consumer products,[41] or pesticides,[35] while others have analyzed endogenous compounds.[31–33,37–40,42] Only Luthra et al conducted a biomarker validation study,[31] while Gong et al[34] chose to analyze the top ten statistically different metabolites according to infant sex.

Maternal blood was the most common biological sample analyzed by LC-MS in all studies,[17,32–34,37,39–42] except for one, which used GC-MS.[32] Maternal urine was analyzed by NMR,[31] GC-MS (36) or LC-MS.[35] There was only one report using amniotic fluid[38]; both two studies using maternal hair[18,28] have applied GC-MS. The period of laboratory analysis was rarely specified, which made it impossible to estimate total time of sample storage.

Untargeted studies reported diverse metabolic features. Authors matched the peaks with an in-house library[18,28] or HMDB-related database.[17,35] Horgan et al[17] found 785 compounds both in maternal and newborn samples; their predictive model included 19 metabolites (only five could be putatively identified, Table 2) and used 2nd trimester maternal blood. Sulek et al[18] and Delplancke et al[28] prepared and analyzed samples with GC-MS using similar protocols. Sulek et al[18] identified 32 statistically different chromatographic features from which they built a predictive model using five metabolites, including two fatty acids (2-methyloctadecanoate and margarate). In contrast, Delplancke et al,[28] identified 198 metabolites, including three fatty acids (margaric, pentadecanoic, and myristic acid) showing significantly higher levels in SGA cases, when 2nd trimester maternal hair segments were studied.

Analysis of identified metabolites

The identified compounds refer to eleven HMDB chemical classes. Fatty acids[18,28,32] comprised the most prevalent chemical class, followed by amino acids [18,38] and phosphosphingolipids[17] (Table 3).

A total of 5,974 women were assessed for vitamin D status. Results were presented as total vitamin D,[33,37,40,42] although vitamin D_2, D_3 or 3-epi-25(OH)D_3[40] metabolites were measured. Results were stratified according to season of maternal sampling or latitude. Either <15ng/mL (<37.5nmol/L)[33] or <20ng/mL (<50nmol/L)[37,40,42] levels characterized vitamin D deficiency, but were statistically different in SGA pregnancies only in the 1st trimester.[37] Horgan et al found a metabolite that could represent a vitamin D derivative, but it was only predictive in combination with 18 other compounds; this model had an area under the curve (AUC) of 0.90 (optimal odds ratio (OR), 44; 95%CI 9-214).[17]

The second most frequent targeted metabolite was homocysteine,[38,39] although levels were only differentiated between normal and SGA pregnancies when measured in 2nd trimester amniotic fluid, with a multiple linear regression model r^2=0.012 and p=0.029.[38] Comparatively, the only common metabolite in 2nd trimester maternal hair was margarate, with conflicting results since it was found to be either increased (AUC 0.72, 95%CI 0.58-0.86) [28] or decreased.[18] The N1,N12-diacetylspermine and the perfluorocarboxylic acids were associated to female SGA babies, not males. The former presented a 5-fold decreased risk of SGA across quintiles. The perfluorodecanoic and perfluoroundecanoic acids presented OR of 3.14 (95%CI 1.07-9.19) and 1.83 (95%CI 1.01-3.32). [41] Tyrosine, an essential amino acid for infants, was part of the predictive model of maternal hair, combining 5 metabolites with an AUC of 0.998 (95%CI 0.992-1.0)[18]. However, tyrosine did not predict SGA when urine samples were studied [31]. Methylmalonic acid [39], acetate, formate, or trimethylamine,[31] did not differentiate SGA when compared to uncomplicated pregnancies (p>0.05).

Risk of bias and Applicability Concerns

Figure 2 shows synthesized data for all included studies. See Appendix B for individual QUADAS-2 data.

Regarding the risk of bias, all cohort studies conducted a consecutive participant inclusion.[28,32,38–42] Nested case-controls matched cases and controls randomly (33–35,41) or according to maternal and infant characteristics.[17,18,31,35] One study[34] failed to mention matching procedures (‘Patient Selection’ domain). Researchers were not blinded to SGA status when interpreting metabolomics results,[17,18,28,31–34,37,40–42] and thresholds of targeted metabolites were not pre-

specified[31,32,36,38,41] ('Index Test' domain). Conversely, SGA identification was not influenced by the metabolomics test, although it was unclear when laboratory experiments were performed in some studies.[18,28,34,36,38,39] Birthweight charts were adequate, except for two studies. The first did not report which centile was chosen,[18] and the second used a centile designed for a different population[38] ('Reference Test' domain). Two studies were ranked as 'high risk' because not all participants were included in the analysis[36,42] ('Flow and Timing' domain).

The QUADAS-2 tool also highlights the importance of how the findings of the included studies are suitable to the review question. In the Patient Selecion domain, it was ranked as 'high applicability concerns' when infants born between the 4th and the 10th centile, but with normal abdominal circumference growth velocity, were not included in final analysis. [34] It was 'unclear' when the gestational age of maternal assessment was not standardized,[39] or was inferred by hair segment length;[28] or when few metabolites from untargeted studies were chosen for interpretation[34] ('Index Test' domain). Finally, it was 'high' when the birthweight charts applied did not correspond to the study population[18,38] ('Reference Standard' domain).

Meta-analysis

From the 15 included studies, only three were designed for prediction purposes[17,18,35] and provided the AUC. The remaining reports described statistical differences of metabolites between SGA pregnancies and controls. [28,31–34,36–42] Accuracy measures were extracted when available (Table 2). However, due to marked heterogeneity (Tables 1 and 2) of gestational age at sampling, type of samples used, type of birthweight chart chosen, thresholds for vitamin D deficiency, metabolomics approach, and identified compounds, a meta-analysis could not be performed.

COMMENTS

Main findings

In this first systematic review of metabolomics and adverse pregnancy endpoints, we presented techniques and metabolites, which were studied for the prediction of SGA. Any effect on birthweight has important implications for perinatal research, since it is related to short and long-term outcomes,[43–46] and in different generations.[47,48] Intrauterine environment influences fetal growth through epigenetic processes: altered gene expression potentially leads to distinct phenotypes.[49] Metabolomics is the most adequate approach to study this outcome, since it is most directly related to phenotype.[50]

Interpretation of metabolomics findings in pregnancy can be challenging. Firstly, maternal metabolites concentrations are influenced by placental transfer to and from the fetus. The ‘mirror effect’, seen for maternal plasma and venous cord blood metabolites at birth[51], cannot be ruled out when only maternal specimens are studied. Secondly, maternal exposure to distinct compounds may affect metabolite levels. Statistically significant differences between SGA infants and controls may not express the totality of underlying pathological pathways and have no clinical meaning. Finally, it is unclear when the processes leading to SGA are initiated. The disruption in maternal metabolism can theoretically occur at any time. In general the lower the gestational age at which the condition is suspected, the more severe the phenotype will be at birth.[52,53] Thus, the description of clinical data in translational studies must deal with all these confounding factors.

Gestational age at sampling is probably the most important parameter for prediction purposes. With timely prediction, women could be referred to specialized

care, have increased surveillance, and this in turn may lead to a reduction in perinatal mortality. There are temporal changes in the maternal metabolome during pregnancy;[28,54–57] therefore, it is reasonable to expect distinctive metabolites at different stages of pregnancy, as reported here. Unfortunately, a wide or unclear definition of gestational age of sampling[31,33,39,41] render a more precise interpretation impossible, and may limit the clinical application of these results.

In contrast, gestational age at birth and birthweight centile seem to be the hallmarks of severity and prognosis of growth restriction.[6,58] Indeed, term and preterm SGA babies show distinct clinical phenotypes, and there are concerns that some babies <10th centile of birthweight are constitutionally small infants.[59–61] If only term deliveries are evaluated, the most severe cases of growth restriction may be potentially missed. Moreover, when term and preterm births are analyzed together, or when lower cutoffs are not specified (e.g. ≤3rd or ≤5th centile), the lack of predictive metabolites might mean that they are distinct conditions. Thus, we hypothesize that the predictive performance of metabolomics may be improved if data is analyzed by gestational age at delivery, and by different cutoffs of birthweight centiles.

Evidence suggests that tobacco smoke has an impact on birthweight,[62–64] although it is uncertain how and when fetal growth is impaired. It is possibly related to oxidative stress,[65] and both maternal and fetal metabolism may be disturbed at delivery.[66,67] Studies that were included did not investigate cigarette-related chemicals or quantify exposure to tobacco smoke. Therefore, no relationship between SGA and tobacco was found. Hence, we suggest that tobacco interferes with ongoing metabolic pathological processes, or its disturbance is related to additional metabolic pathways other than the one examined by the included studies.

Subgroup and metabolite findings

No reports have explored data on any maternal chronic condition, suspicion of SGA in pregnancy, or number of fetuses. The lack of clear statements about participant selection have hindered data interpretation and precluded these analyses.

The majority of included studies performed a targeted approach, i.e. a hypothesis-testing evaluation,[16,50] driven by epidemiological or experimental data regarding SGA newborns. None of the targeted metabolites[31–33,36–42] were in common with those found by 'hypothesis-generating' metabolic profiling[17,18,28,34,35] investigations. This reinforces the suggestion that various maternal metabolic pathways may be triggered by the SGA condition, and be detected by different biological samples. However, since blood is a very complex sample and GC-MS only evaluates volatile molecules,[50] therefore our findings may be biased by study methodologies.

Untargeted studies, as expected, have characterized several metabolites that may be validated in future investigations. Nine lipids and fatty acid metabolites,[17,18,28,32] two amino acids,[18,38] and a steroid[17,37] have been identified as potential biomarkers of SGA.

All lipid-related metabolites identified are intermediates for energy storage and breakdown. Most metabolites were found in maternal blood[17] or hair of the SGA group.[18,28] Blood levels of saturated and monounsaturated non-esterified fatty acids apparently remain stable throughout pregnancy, while long chain polyunsaturated fatty acid (DHA and EPA, for example) measurements seem to show ethnicity-related changes.[57] Experimental data shows the importance of hypoxia and oxidative stress to placental function and ultimately, to birthweight.[68,69] Findings from included studies may represent a dysregulation of lipid pathways at the placental level, but an

association with maternal background is unclear. Therefore, we hypothesize that disorders of lipid metabolism may be the ‘metabolic snapshot’ of defective deep placentation,[70] and might reflect maternal efforts to respond to impaired fetal growth.

Recommendations on the assessment of vitamin D and cutoffs to define vitamin D deficiency in pregnancy are controversial.[71] However, vitamin D supplementation decreases SGA risk.[72] In early pregnancy, vitamin D status has been related to SGA,[73,74] which is in accordance with this review, despite the inconsistent findings.[75] There is evidence that trophoblasts actively produce and secrete vitamin D metabolites,[76] but it is not clear how they mediate fetal growth impairment. Altered hepatic gene expression and liver function in vitamin D deficient female rats,[77] and single nucleotide polymorphisms[78] in vitamin D receptor gene have been suggested as mechanisms to be explored by a multidimensional omics approach.

Finally, homocysteine is an intermediate metabolite of the folate cycle. It is indirectly involved with DNA methylation and is a marker of folate deficiency.[79] Maternal levels rarely reach hyperhomocysteinemia limits,[80] but folate depletion[81–83] and homocysteine itself[80] are thought to be associated with a higher SGA risk. In this review, homocysteine was only statistically different in SGA pregnancies when measured in amniotic fluid,[38] although within the normal ranges proposed for 17-21 weeks.[84] Since amniocentesis is generaly performed in women at higher obstetrical risk, future studies should investigate wheter homocysteine in amniotic fluid represents a confounding factor or a new biomarker.[85]

Methodological quality

The majority of studies were ranked as ‘low risk’ of bias or applicability to the review question. However, the lack of clear descriptions of laboratory experiments, including sample preparation and storage, and blinding of the researchers to the case/control status, are major pitfalls of the included studies.

Strengths and limitations

To our knowledge, this is the first systematic review of metabolomics and an adverse pregnancy outcome (SGA). We presented possible biomarkers of SGA pathophysiology, metabolites implicated in lipid transport and metabolic pathways, as well as gluconeogenesis.

However, this analysis has some limitations. First, included studies showed heterogeneity, which is fundamental in systematic reviews. Indeed, there was a wide variety of participant characteristics and methods used, and not all authors provided a detailed description of methods employed. Although the Metabolomics Standard Initiative was released in 2007,[86] there is still poor adherence to guidelines.[87,88] Clear reporting[15,87,88] and data sharing in repositories are crucial steps in identifying features of interest, specifically possible biomarkers to be validated in the clinical studies.[15] Secondly, we could not perform a meta-analysis of the extracted data, impacting the translational potential of metabolomics.

Thirdly, we considered that birthweight was a surrogate measure of intrauterine development. SGA and FGR are not interchangeable concepts. However, SGA has been used as a surrogate for FGR in many clinical studies due to difficulties in defining optimal intrauterine growth: (i) FGR diagnosis relies mostly on ultrasound measurements of fetal biometry,[3,89] which in turn is subject to systematic errors;[90] (ii) intrauterine development is adaptive, rather than uniform [91] or only

genetically driven;[49] (iii) growth impairment at birth better identifies adverse neonatal outcomes than during pregnancy.[58] It is recognized that changes in obstetric care occur when growth restriction is suspected, and neonatal outcomes are improved.[21,22] Thus, an accurate prediction of SGA during pregnancy will be a turning point in modern obstetrics.

CONCLUSIONS AND IMPLICATIONS FOR PRACTICE

Using the available clinical tools, efforts to predict SGA remain disappointing. Since SGA is a heterogeneous condition, it benefits from metabolomics. This novel area of research allows analysis of numerous types of biological fluids and detects thousands of metabolites in complex samples.[15,16,25] However, findings of this systematic review must be interpreted with caution. The type of samples used may have influenced LC-MS (2nd trimester maternal blood) and GC-MS (2nd trimester maternal hair) findings in individual studies. Furthermore, the prediction of SGA in the context of maternal disorders, suspected FGR and twin pregnancies is an open field for future metabolomics studies, and environmental exposure investigation as well.

Surprisingly, none of the studies used ≤3rd centile of birthweight as a cutoff or analyzed preterm deliveries and hypertensive syndromes. Considering our findings and the different phenotypic manifestations of SGA, we envision a better performance when (i) cutoffs other than the 10th centile are tested; (ii) data on gestational age at sampling and at birth are standardized; and (iii) other pregnancy-related syndromes are considered, especially hypertension. Thus, future metabolomics results should advance in these critical points.

Finally, all detected biomarkers were related to lipid pathways and energy metabolism. We consider that research efforts to predict SGA should focus on compounds involved in these pathways, up to the 2nd trimester of pregnancy.

AUTHORS CONTRIBUTIONS

DFBL and ACM have equally contributed to this report. They elaborated the protocol, searched the literature, selected studies, extracted data, assessed risk of bias, and drafted the initial manuscript. RTS and EFMJ have participated in judging inclusion of studies, interpreting data, and revising the manuscript. FM have supported data extraction and have critically examined the clinical interpretation of results. ASK has discussed the quantitative data synthesis, and supervised the report writing. PNB, LCK, and JGC have supervised and approved all steps. All authors have read and agree with this submission.

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Table 1. Main characteristics of included studies.

Authors, year	Country, year of participants enrolment	Study design	Affected/ non-affected	Gestational age at assessment	Type of pregnancy	Parity	Birthweight curve
Outcome: SGA <5th centile							
Costet N et al, 2011	France, 2002-2006 (PELAGIE Cohort)	Nested case-control	134/ 399	11w	Single pregnancy	Nulliparous and parous women, unclear proportions	Customized curve
Ertl R et al, 2012	United Kingdom [a]	Nested case-control	150/ 1,000	11^{+0}-13^{+6}w	Unclear	55,3% nulliparous in SGA group, 48.1% nulliparous in control group	Population-based charts
Outcome: SGA <10th centile							
Grandone E et al, 2006	Italy [a]	Cohort	31/ 393	17.1 ± 1.2w[b] (mean)	Single pregnancy; no maternal pre-existing conditions	Unclear	Population-based charts
van Eijsden M	Netherlands, 2003-	Cohort	429/ 3275	13.5 ± 3.3w	Term deliveries, no	57.6% nulliparous	Population-based

et al, 2008	2004 (ABCD Study)			(mean)	diabetes or hypertension		charts
Horgan RP et al, 2011	Australia, 2008-2011 (SCOPE Cohort)	Nested case-control	40/ 40	14-16w	Single pregnancy; no other pregnancy complications	Nulliparous	Customized curve
Gernand AD et al, 2013	United States, 1959-1965 (Collaborative Perinatal Project)	Nested case-control	395/ 1751	≤26w	Single pregnancy; term deliveries	Parous women	Population-based charts
Sulek K et al, 2014	Singapore [a] (GUSTO Study)	Nested case-control	41/ 42	26-28w	Single pregnancy; term deliveries; no maternal pre-existing conditions	Nulliparous and parous women, unclear proportions	Population-based charts
Choi R et al, 2016	South Korea, 2012-2013	Cohort	39/ 217	1st, 2nd or 3rd trimester	Single pregnancies	Nulliparous and parous women, unclear proportions	Population-based charts
Kiely ME et al, 2016	Ireland, 2008-2011 (SCOPE Cohort)	Cohort	190/ 1578	14-16w	Single pregnancy; no maternal pre-existing conditions	Nulliparous	Customized curve

Ong YL et al, 2016	Singapore [a] (GUSTO Study)	Cohort	83/ 827	26-28w	Single pregnancy; no maternal chronic illness	43,5% nulliparous	Population-based charts
Wang Y et al, 2016	Taiwan, 2000-2001 (Taiwan Maternal and Infant Cohort Study)	Cohort	35/ 188	3rd trimester	Unclear; term deliveries	48% nulliparous	Population-based charts
Delplancke TDJ et al, 2018	New Zealand [a]	Cohort	20/ 73	34-37w	Unclear; term deliveries	Unclear	Customized curve
Luthra G et al, 2018	United States, 2010-2012 (TIDES Study)	Nested case-control	53/ 106	1st and 2nd trimester	Single pregnancies; term deliveries	60% nulliparous	Customized curve
Gong S et al, 2018	United Kingdom, 2008-2012 (POP study)	Nested case-control	162/259	36w	Single pregnancies; term deliveries	Nulliparous	Customized curve
Morillon A-C et al, 2018	2008-2011 (SCOPE Study)	Nested case-control	40/40	20w	Single pregnancies	Nulliparous	Customized curve

[a] Unclear period of participant recruitment. [b] Mean for all study participants.

Table 2. Subgroup analysis of included studies according to which metabolomics technique was applied.

Authors/ year	**Metabolomics Technique**	**Maternal sample/ Storage temperature**	**Prediction model***	**Targeted compounds**	**Coefficient of variation/ Limits of quantitation**	**Predictive compounds**	**Sensitivity/ Specificity**	**AUC**
Nuclear magnetic resonance								
Luthra G et al, 2018	^{1}H-NMR 1D NOESY with pre-saturation and homonuclear 2D *J*-resolved at 300 K Bruker 600 MHz Advance III HD spectrometer	Urine/ -80^{o}C	Targeted	Tyrosine, acetate, formate, trimethylamine	NA	None		
Gas chromatography coupled to mass spectrometry								
Costet N et al, 2011	GC-MS Simple head space SPME-Capillary GC	Urine/ -20^{o}C	Targeted	Trichloroacetic acid	<5%/ 0.01mg/L	None	0.1/ 0.93	
Sulek K et al, 2014	GC-MS Thermo Trace GC Ultra	Hair/ -20^{o}C	Untargeted	NA	NA	↓ Lactate ↓ Levulinate		0.998

	system coupled to ISQ mass selective detector Capillary GC column: Phenomenex ZB-1701 (30 m x 250 µm id x 0.15 µm with 5 m guard column)					↑2-methyloctadecanate ↑Tyrosine ↓ Margarate	
Delplancke TDJ et al, 2018	GC-MS: Agilent 7890B gas chromatograph, capillary column ZB-1701 (30m x 250µm id x 0.15µm with 5m guard column) 5977 A mass spectrometer, electron impact ionisation	Hair/ -20°C	Untargeted	NA	NA	↑ Margaric acid ↑ Pentadecanoic acid ↑ Myristic acid[c]	0.72 0.73 0.73
Liquid chromatography coupled to mass spectrometry							
Grandone E et al, 2006	LC-MS/MS triple quadrupole Applera API 3000, TurboIonSpray	Amniotic fluid/ -80°C	Targeted	Homocysteine	Unclear	↑Homocysteine (1,29µM; 1,05-1,51µM)	

	ionisation						
Horgan RP et al, 2011	UPLC- MS/MS Thermo Fisher LTQ Orbitrap, ESI	Plasma/ -80°C	Untargeted	NA	NA	Hexacosanedioic acid, diglyceride, lyso-phosphocholine, sphinganine 1-phosphate; sphingosine 1-phosphate[d]	0.90
Ertl R et al, 2012	HPLC- MS/MS Shimadzu Prominence HPLC system with a column Phenomenex Luna C8 3 x 50 mm; AbSciex API-5000 triple quadrupole, ESI	Serum/ -80°C	Targeted	25(OH)D_2; 25(OH)D_3	6.3%[a], 6.6%[b] (D_2); 6.5%[a], 7.3%[b] (D_3)/ unclear	↓25,OH,Vitamin D (12.16ng/mL; 8.09-20.54ng/mL)	0.72/ 0.45
Gernand AD et al, 2013	LC-MS/MS	Serum/ -20°C	Targeted	25(OH)D_2; 25(OH)D_3	8.2%[a] (D_2) 5.9%[a] (D_3)/ <1ng/mL	None	0.39/ 0.66

Choi R et al, 2016	HPLC- MS/MS Waters HPLC system, Applied Biosystems API-4000 MS/MS mass spectrometer	Serum/ -20ºC	Targeted	Methylmalonic acid; homocysteine	<10%[a]; <10%[b]/ Unclear	None	
Kiely ME et al, 2016	UPLC- MS/MS Waters Acquity UPLS system, Waters Triple Quadrupole TQD mass spectrometer	Serum/ -80ºC	Targeted	25(OH)D_2; 25(OH)D_3; 3-epi-25(OH)D_3.	<6%[a]; <5%[b]/ 0.57ng/mL (D_2); 0.26ng/mL (D_3), 0,41ng/mL (epi-D_3)	None	
Ong YL et al, 2016	LC-MS/MS Applied Biosystems ThermoHypersil BDS C8 reverse-phase column	Plasma/ Unclear	Targeted	25(OH)D_2; 25(OH)D_3	≤10,3%[a,b]/ <1,6ng/mL	None	0.12/ 0.87
Wang Y et al, 2016	LC-MS Agilent HPLC system, Applied Biosystems Sciex	Serum/ Unclear	Targeted	PFOA; long-chain PFCA	0,83-7,94%[a]; 1,57-	PFDeA (OR 3,14; 95%CI 1,07-9,19), PFUnDA (OR 1,83;	

	API-4000 triple quadrupole mass spectrometer				24,7%[b]/ 0,07-0,45ng/mL[e]	95%CI 1,01-3,32)[f]
Gong S et al, 2018	LC-MS/MS Shimadzu UK Limited UPLC system, ACE Excel 2 C18-PFP LC-column; Thermo Fisher Scientific Exactive orbitrap mass spectrometer	Serum/ Unclear	Untargeted	NA		↑N1,N12-diacetylspermine[f]
Morillon A-C et al, 2018	UPLC- MS/MS Waters Acquity UPLS system, Waters Synapt G2-S mass spectrometer	Urine/-80oC	Untargeted	NA		None
Others						
van Eijsden M et al, 2008	GC-FID Solid phase extraction SPE, Capillary GC	Plasma/ -80^{o}C	Semi-targeted, Lipid	Elaidic, linoleic, alfa-linolenic, eicosatetraenoic,	≤2 - 22%[b]/ Unclear	↓ Eicosatetraenoic acid (OR 1,5; 95%CI 1,07-2,11),

	extraction	EPA, DPA, DHA DGLA, AA, Adrenic, and Osbond acids	↓DPA (OR 1,49; 95% CI 1,06-2,1)

[a]Intra-assay and [b]inter-assay coefficients of variation. [c]These metabolites were found in 2nd trimester hair segments. [d]And more 14 metabolites that could not be identified certain based on chromatographic peak and mass: Phenylacetylglutamine or formyl-N-acetyl-5-methroxykynurenamine; leucyl-leucyl-norleucine or sphingosine 1-phosphate; cervonyl carnitine and/or 1-alpha,25-dihydroxy-18-oxocholecalciferol; (15Z)-tetracosenoic acid or 10,13-dimethyl-11-docosyne-10,13-diol or trans-selacholeic acid; pencosenoic acid or cyclohexyl acetate or octanoic acid or methyl-heptenoic acid or 4-hydroxy-2-octenal or DL-2-aminooctanoic acid or 3-amino-octanoic acid; hydroxybutyrate or hydroxy-methylpropanoate or methyl methoxyacetate; lysophosphocoline and phosphocoline (more than 10 hits); phosphocoline (more than 20 hits); phosphocoline or ubiquinone-8; acetylleucil-leucil-norleucinal or oleoylglycerone phosphate or LPA(0:0/18:2(9Z,12Z)) or 1-16:1lysoPE or phosphocoline(O-11:1(10E)/2:0) or (3s)-3,4-Di-N-hexanoyloxybutyl-1-phosphocoline or N-(3-hydroxy-propyl) arachidonoyl amine or N-methyl N-(2-hydroxy-ethyl) arachidonoyl amine or similar; lysophosphocholine (16:1) or cervonyl carnitine; preganediol-3-glucuronide or 3-alpha,20-alpha-dihydroxy-5-beta-pregnane-3-glucuronide; 6-hydroxyshingosine or (4OH,8Z,t18:1) sphingosine or 15-methyl-15-prostaglandin D2 or 15-R-prostaglandin E2 methylester. [e]Values for all studied metabolites. [f]Predictive compounds only for female babies.

AUC: area under the receiver operating characteristic curve; ^{1}H-NMR: hydrogen nuclear magnetic resonance; NOESY: nuclear Overhauser effect spectroscopy; GC-MS: gas chromatography coupled to mass spectrometry; SPME: solid phase micro extraction; LC-MS: liquid chromatography coupled to mass spectrometry; UPLC: ultra-performance liquid chromatography; ESI: Electrospray ionisation; FID: flame ionisation detection; PFOA: perfluorooctanoic acid; PFCA: perfluorocarboxylic acid; PFDeA: perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; EPA: eicoisapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; DGLA: dihomo-gama-linolenic acid; AA: arachidonic acid; OR: odds ratio; CI: confidence interval; NA: not applicable.

Table 3. Predictive metabolites summarized according to their chemical class, subclass, and biological process.

Predictive metabolites	**Chemical class**	**Chemical subclass**	**Metabolic pathway**
Margarate	Fatty acyls	Fatty acids and conjugates	Lipid transport, metabolism, peroxidation
Pentadecanoic acid	Fatty acyls	Fatty acids and conjugates	Lipid transport, metabolism, peroxidation; fatty acid metabolism and biosynthesis
Myristic acid	Fatty acyls	Fatty acids and conjugates	Lipid transport, metabolism, peroxidation; fatty acid metabolism and biosynthesis
Eicosatetraenoic acid	Fatty acyls	Fatty acids and conjugates	Lipid transport, metabolism, peroxidation; lipid metabolism pathway
Docosapentaenoic acid	Fatty acyls	Fatty acids and conjugates	Lipid transport and metabolism, fatty acid metabolism, alpha linolenic acid and linoleic acid metabolisms
Tyrosine [a]	Carboxylic acids and derivatives	Amino acids, peptides, and analogues	Catecholamine biosynthesis; phenylalanine and tyrosine metabolism; thyroid hormone synthesis; transcription and translation
Homocysteine	Carboxylic acids and derivatives	Amino-acids, peptides, and analogues	Glycine and serine metabolism; methionine metabolism
Hexacosanedioic acid	Carboxylic acids and derivatives	Dicarboxylic acid and derivatives	Fatty acid biosynthesis
Sphinganine 1-phosphate	Sphingolipids	Phosphosphingolipids	Sphingolipid signalling pathway, nneuroactive ligand-receptor interaction
Sphingosine 1-phosphate	Sphingolipids	Phosphosphingolipids	Lipid metabolism pathway, sphingolipid metabolism
PFDeA	Alkyl halides	Alkyl fluorides	Not reported[b]

PFUnDA	Alkyl halides	Alkyl fluorides	Not reported[b]
25,OH,Vitamin D	Steroids and steroids derivatives	Vitamin D and derivatives	Lipid metabolism pathway
Diglyceride	Glycerolipids	Diradylglycerols	Adipocytokine signaling pathway
Lactate	Hydroxy acids and derivatives	Alpha hydroxy acids and derivatives	Gluconeogenesis, glycogenosis types IB and IC, pyruvate metabolism, triosephosphate isomerase
N1,N12-diacetylspermine	Carboximidic acids and derivatives	Carboximidic acids	Not reported[b]
Lyso-phosphocholine	Glycerophospholipids	Glycerophosphocholines	Not reported[b]
2-methyloctadecanate	Saturated hydrocarbons	Alkanes	Not reported[b]
Levulinate	Keto acids and derivatives	Gamma-keto acids and derivatives	Not reported[b]

[a] Essential amino acid for infants. [b] No human metabolic pathways reported at KEGG. PFDeA: perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid.

Figure captions

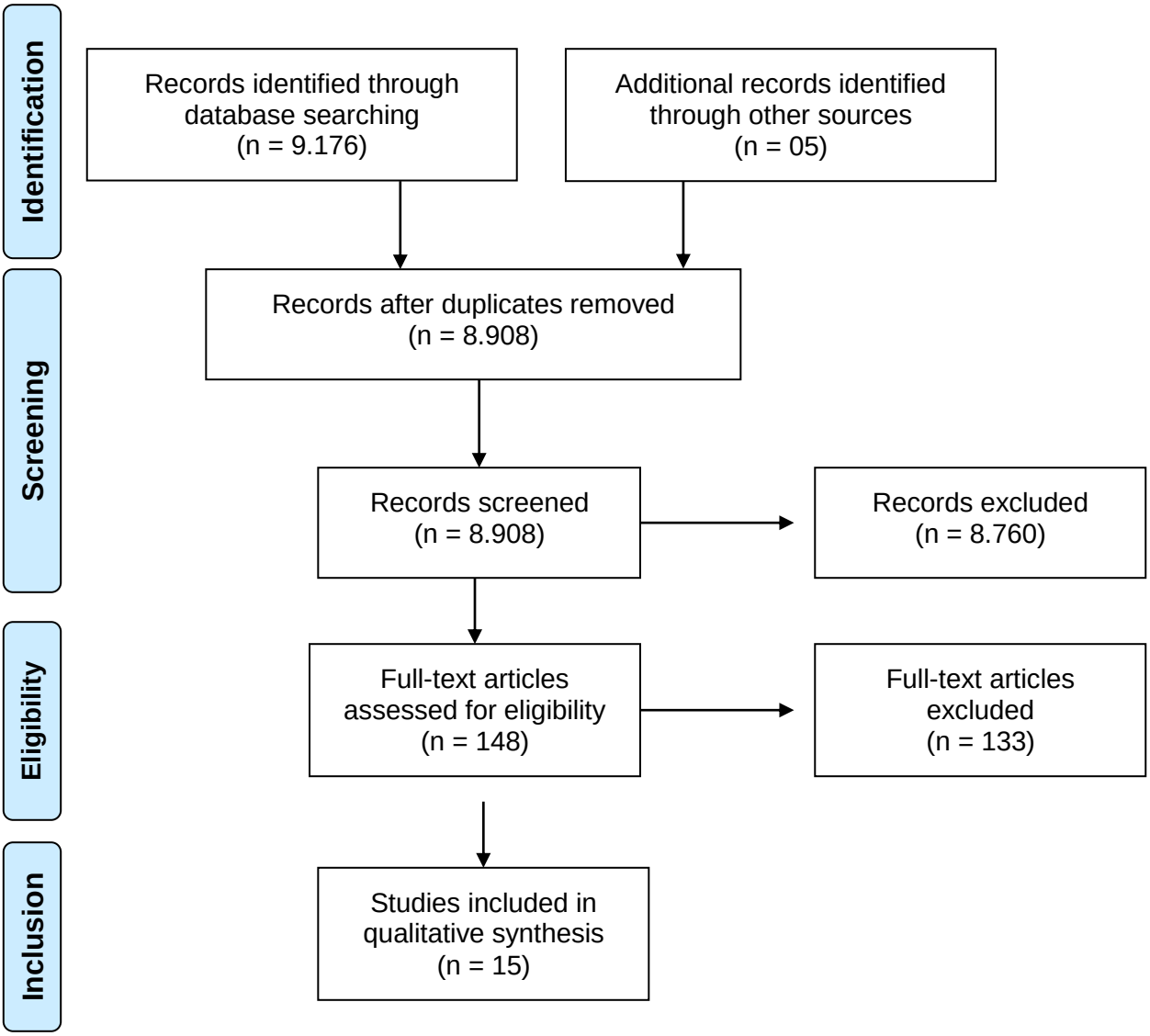
Figure 1. PRISMA flowchart of study identification, screening and selection.

Figure 2. Assessment of risk of bias (A) and applicability concerns (B) of individual studies.

Appendices description

Appendix A – List of excluded studies and reasons.

Appendix B - Individual QUADAS-2 data for all 15 included studies.



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit www.prisma-statement.org.

Figure 2A

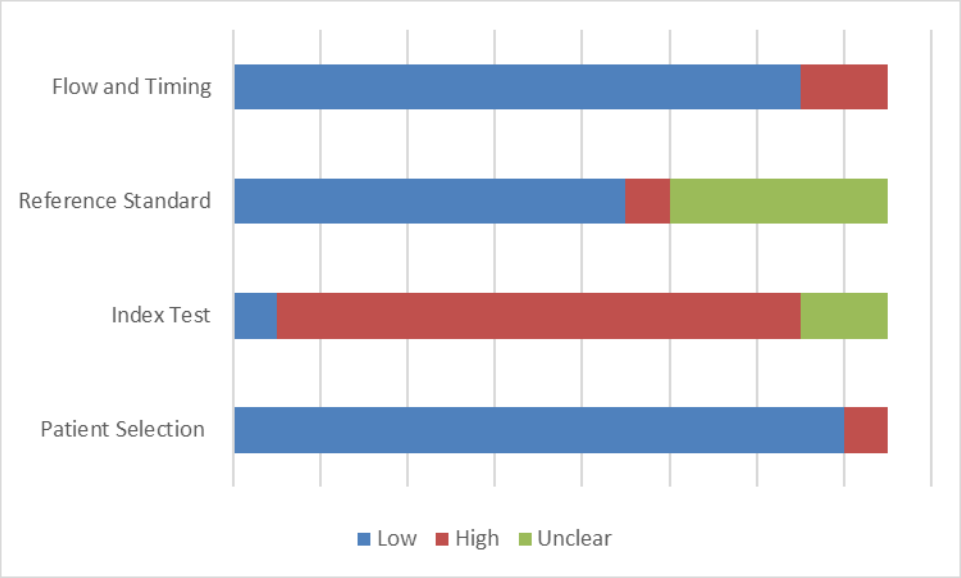
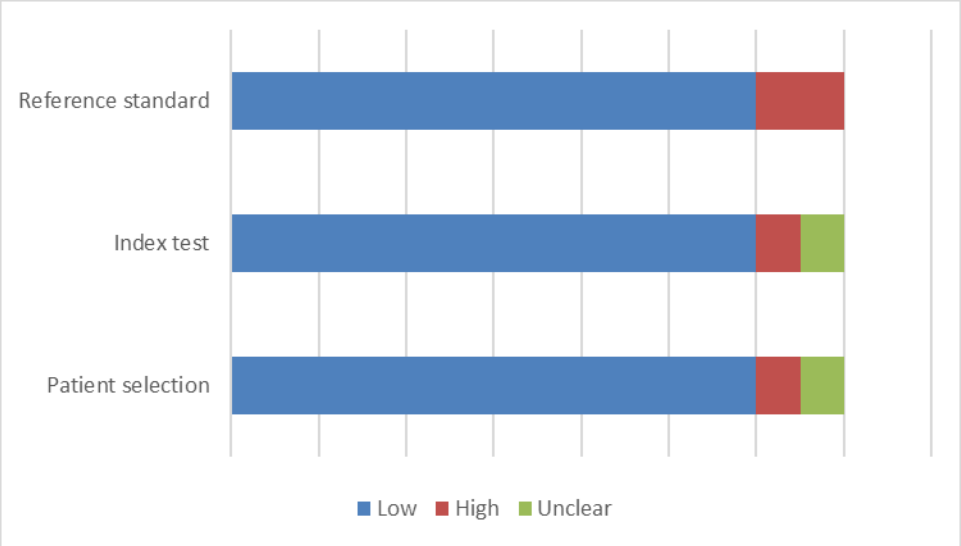


Figure 2B



Appendix A – List of excluded studies and reasons.

Authors/ year	**Country of enrollment**	**Additional comments**
Exclusions according to study design or statistical analysis		
Barnes CM et al, 2010	United States	Maternal samples collected at delivery.
Bobinski R. 2013	Poland	Cross-sectional study.
Bobinski R. 2014	Poland	Cross-sectional study.
Cao WC et al, 2016	China	Cross-sectional study. The metabolomics technique was not applied.
Chen TT et al, 2017	China	Cross-sectional study.
Cinelli et al, 2018	Italy	
D'Anna R et al, 2004	Italy	Cross-sectional study. The metabolomics technique was not applied.
Guo H et al, 2014	China	Cross-sectional study.
Guo J et al, 2016	China	Cross-sectional study.
Maekawa R et al, 2017	Japan	Cross-sectional study.
Mao D et al, 2010	China	Cross-sectional study.

Miranda J et al 2018	Spain	Cross-sectional study.
Powell et al, 2018	Australia	SGA babies not suspected before birth were considered healthy infants.
Spanou L. et al, 2017	Greece	Cross-sectional study.
Stein TP et al, 2008	United States	Newborns with birth defects were included in the analysis.
Tang R et al, 2013	China	Cross-sectional study.
Visentin S et al, 2017	Italy	Maternal samples collected after clinical recognition of FGR/SGA.
Zhu Y et al, 2018	China	Cross-sectional study.
Zota AR et al, 2009	United States	Cross-sectional study. The metabolomics technique was not applied.

Studies that have not applied metabolomics technique		
Baker PN, 2009	United Kingdom	
Berkowitz GS et al, 2004	United States	
Bodnar LM et al, 2012	United States	
Braun JM et al, 2011	United States	There is no data about FGR.
Cetin I et al, 2002	Italy	
Chong MFF et al, 2015	Singapore	There is no data about birth weight.

Colapinto CK et al, 2015	Canada	The metabolomics technique was not applied for pregnant women’s specimens.
Cupul-Uicab LA et al, 2013	United States	
Fruscalzo A et al, 2015	Italy	There is no data about birth weight.
Jusko TA et al, 2006	United States	
Koepke R et al, 2004	Mexico	
Lõpez-Alarcõn M et al, 2015	Mexico	There is no data about birth weight.
Maruta E et al, 2017	Japan	
Miranda ML et al, 2015	United States	
Morley R et al, 2006	Australia	
Muthayya S et al, 2006	India	
Paşaoğlu H et al, 2003	Turkey	
Rahman A et al, 2009	Bangladesh	
Rajasingam D et al, 2009	United Kingdom	
Savitz DA et al, 2002	United States	The metabolomics technique was not applied for pregnant women’s specimens.
Savvidou MD et al, 2003	United Kingdom	
Schneuer FJ et al, 2014	Australia	

Snijder CA et al, 2013	Netherlands
Sweeney AM & Symanski E, 2007	United States
Takimoto H et al, 2007	Japan
Terrell ML et al, 2015	United States
Wei Y et al, 2017	Bangladesh
Weisskopf MG et al, 2005	United States
Whyatt RM et al, 2009	United States
Xue F et al, 2007	United States

Studies that have not presented specific data about FGR/SGA	
Bach CC et al, 2016	Denmark
Bachkangi P et al.	United Kingdom
Bahado-Singh RO et al, 2012	United Kingdom
Bahado-Singh RO et al, 2015	United Kingdom
Bahado-Singh RO et al, 2017	United Kingdom
Bentley-Lewis R, 2015	United States

Braun JM et al, 2009	United States
Buckley JP et al, 2016	United States
Cantonwine D et al, 2010	Mexico
Cantonwine D et al, 2015	United States
Casas M et al, 2016	Spain
Castorina R et al, 2017 (a)	United States
Chou WC et al, 2014.	Taiwan
Cunha Figueiredo AC et al, 2017	Brazil
Dalsager L et al, 2018	Denmark
De Renzy-Martin KT. et al, 2014	Poland
Debost-Legrand A et al, 2016	France
Desert et al, 2015	France
Diaz SO et al, 2011	Portugal
Diaz SO et al, 2013	Portugal
Dobierzewska A et al, 2017	Chile

Dudzik D et al, 2015	Spain.
Engström KS et al, 2010	Bangladesh
Ettinger AS et al, 2017	Canada
Feng L et al, 2016	China
Ferguson KK et al, 2014	United States
Ferguson KK et al, 2015	United States
Ferguson KK et al, 2017	United States
Finkelstein JL et al, 2015	United States
Fischer ST et al, 2017	United States
Gao H et al, 2017	China
Gardner RM et al, 2011	Bangladesh
Ghartey J et al, 2017	United States
Graça G et al, 2010	Portugal
Graça G et al, 2012	Portugal
Graça G et al, 2012 (b)	Portugal
Hogeveen M et al, 2010	Netherlands

Huang J et al, 2017	China	
Kalhan SC et al, 2003	United States	
Khalil AA et al, 2013	United Kingdom	
Kuc S et al, 2014	Netherlands	
Lenters V et al, 2013	Greenland, Poland, Ukraine	
Lenters V et al, 2016	Greenland, Poland, Ukraine	
Liu K et al, 2017	China	
Lopez-Espinosa MJ et al, 2015	Spain	
Marchlewicz EH et al, 2016	United States	
Minatoya M et al, 2017	Japan	
Minatoya M et al, 2017 (b)	Japan	
Minatoya M et al, 2018	Japan	
Murphy MM et al, 2007	Spain	There is no data about any pregnancy outcomes.
Odibo AO et al, 2011	United States	
Pinney SE et al, 2017	United States	
Polanska K et al, 2014	Poland	

Polanska K et al, 2014 (b)	Poland	
Porter A et al, 2018	United States	
Rejc B et al, 2016	Slovenia	
Rijvers CAH et al, 2013	Netherlands	
Robledo C et al, 2013	United States	
Sachse D et al, 2012	Norway	
Scholtens DM et al, 2016	United Kingdom	
Shisler S et al, 2017	United States	Not all analysis were performed with metabolomics approach.
Tamblyn JA et al, 2018	Ireland	Duplicate data. Check Kiely ME et al, 2016.
Thomas MM et al, 2015	New Zealand	
Van Lee L et al, 2015	Singapore	
Virgiliou C et al, 2017	Greece	
Walsh J et al, 2012	Ireland	
Wang PW et al, 2015	Taiwan	
Watkins DJ et al, 2016	United States	
Wolff MS et al, 2008	United States	

Woods MM et al, 2017	United States	
Yang P et al, 2018	China	

Duplicate data		
Horgan R et al, 2009	Australia	Check Horgan R et al, 2011.
Horgan R et al, 2011	Australia	Check Horgan R et al, 2011.
Khashan AS et al, 2013	Ireland	Check Kiely ME et al, 2016.
Sulek et al, 2014	Singapore	Check Sulek et al, 2014.

Appendix B - Individual QUADAS-2 data for all 15 included studies

Studies	Risk of bias								Applicability concerns		
	Patient selection		Index test		Reference standard		Flow and timing		Patient selection	Index test	Reference standard
	Was a consecutive or random sample of patients enrolled?	Did the study avoid inappropriate exclusions?	Were the index test results interpreted without knowledge of the results of the reference standard?	If a threshold was used, was it pre-specified?	Is the reference standard likely to correctly classify the target condition?	Were the reference standard results interpreted without knowledge of the results of the index test?	Did all patients receive the same reference standard?	Were all patients included in the analysis?	Are there concerns that the included patients do not match the review question?	Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Are there concerns that the target condition as defined by the reference standard does not match the review question?
Grandone E et al, 2006	Yes	Yes	Unclear	No	No	Unclear	Yes	Yes	No	No	Yes
van Eijsden M et al, 2008	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	No
Horgan R et al, 2011	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	No	No
Costet N et al, 2012	Yes	Yes	Yes	No	Yes	Unclear	Yes	No	No	No	No
Ertl R et al, 2012	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	No	No
Gernand AD et al, 2013	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	No	No
Sulek K et al, 2014	Yes	Yes	No	Yes	Unclear	Unclear	Yes	Yes	No	No	Yes
Choi R et al, 2016	Yes	Yes	Unclear	Yes	Yes	Unclear	Yes	Yes	Unclear	No	No
Kiely ME et al, 2016	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	No	No
Ong YL et al, 2016	Yes	Yes	No	Yes	Yes	Yes	Yes	No	No	No	No
Wang Y et al, 2016	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	No
Delplancke TDJ et al, 2018	Yes	Yes	No	Yes	Yes	Unclear	Yes	Yes	No	Unclear	No
Luthra G et al, 2018	Yes	Yes	No	No	Yes	Yes	Yes	Yes	No	No	No
Gong S et al, 2018	No	Yes	No	No	Yes	Unclear	Yes	Yes	Yes	Yes	No
Morillon AC et al, 2018	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	No	No

6. DISCUSSÃO

A interpretação da restrição de crescimento como condição patológica presente em toda a vida do indivíduo levanta dois questionamentos principais. O primeiro diz respeito aos impactos imediatos e a longo prazo de sua crescente prevalência; e o segundo, ao papel que a assistência prestada durante a gestação desempenha neste cenário.

Literatura crescente nos últimos anos tem relacionado o inadequado peso ao nascer com desfechos negativos ao longo da vida, incluindo hipertensão arterial, diabetes mellitus e síndrome metabólica (124–126,176). As DCNT relacionam-se principalmente à morbidade cardiovascular de longa duração, com maior utilização de serviços de saúde, maior custo da assistência à saúde, perda de anos de vida produtivos, menor qualidade de vida e maiores taxas de mortes preveníveis (11). Mas as repercussões continuam na geração seguinte. É assustador imaginar que eventos que acontecem durante a gestação podem condicionar toda uma sociedade, e perpetuarem desigualdades sociais e econômicas, bem aos moldes do que previu Aldous Huxley (1). Estima-se em mais de 23 milhões o número de recém-nascidos PIG em países em desenvolvimento nos anos de 2010-2012 (40,177), e tal prevalência pode ser ainda maior caso se apliquem as curvas customizadas de peso ao nascer (75,96). As curvas populacionais de peso ao nascer podem mascarar a restrição de crescimento em mulheres com sobrepeso e obesidade (101) que compõem grande parte do público obstétrico no século XXI (6,29); na nossa amostra, alcançou 43%.

Portanto, é possível que um quarto da população de países em desenvolvimento nasça abaixo do percentil 10 de peso (40,177). Mas quantos outros indivíduos falharam em atingir seu ‘potencial ótimo de crescimento’? O percentil 50

de qualquer curva, apenas uma medida matemática, não significa que o feto cresceu de acordo com o que estaria esperado dele (178,179). Por mais que compreendamos o crescimento fetal como adaptativo, existem dificuldades óbvias ao se determinar se um feto é ‘constitucionalmente’ pequeno ou não. A multiplicidade de fatores que podem interferir com o crescimento fetal - barulho (180), contaminantes ambientais (181) e hábitos de vida (130,135) são alguns exemplos de influências ambientais – torna improvável que qualquer um, isoladamente, preencha todos os Critérios de Hill (182) para figurar como causa da RCF.

É verdade que presenciamos mudanças rápidas de conceitos para normalidade e desvio do crescimento fetal. Em cerca de 50 anos, partimos de uma definição teórica de IUGR baseada apenas no peso ao nascer (57,58,63), e hoje ponderamos a pertinência de fatores constitucionais maternos e fetais no crescimento intrauterino (30,32,74,84). Em um futuro próximo, acredito que consideraremos a ‘síndrome da RCF’, cujos espectros são tão graves quanto a necessidade de adaptação fetal a um ambiente intrauterino hostil; talvez caminhemos para uma definição de RCF ou PIG que não dependa apenas de estimativas de medidas biométricas, ou peso. Com o advento de novas tecnologias e possibilidades de descobrirmos novos marcadores de doença, será factível conceituarmos os desvios de crescimento de acordo com a sua fisiopatologia. Em tempos vindouros, os achados do estudo SCOPE (*Screnning for Pregnancy Endpoints*) em que trabalhamos deverão ser validados na coorte do Preterm-SAMBA.

Enquanto essa discussão se refina, divergências quanto às definições e critérios limitam a qualidade da assistência que podemos prestar. Utilizar uma

estratificação de risco para PIG - seja apenas clínico ou associado à ultrassonografia e marcadores laboratoriais – deve ser uma política pública.

Faz-se mister coletar dados pertinentes a nível populacional. No Brasil, a Declaração de Nascido Vivo contém informações úteis para o registro imediato daquele nascimento (peso ao nascer, sexo, paridade), porém não dispomos de muitos outros mecanismos de vigilância em saúde para monitorar eventos a curto (ex., internação em unidade de cuidado intensivo neonatal), médio (aproveitamento escolar) ou longo prazos (cruzamento dos dados ao nascer com a mortalidade) (13). Isso nos deixa apreensivos de que estaremos sempre na dependência científica de outros países, aplicando modelos de rastreio ou tomando decisões quanto ao diagnóstico, por exemplo, em uma população fenotipicamente diferente da nossa. A nível institucional, o estabelecimento de protocolos multidisciplinares e baseados em evidências atualizadas tem o potencial de melhorar os desfechos perinatais imediatos ao tornar a equipe mais atenta à condição, algo comparável ao efeito *Hawthorne*. Além disso, a parceria entre serviços universitários e unidades básicas de saúde, ou prefeituras, pode ser de fundamental apoio matricial para o seguimento a longo prazo dos recém-nascidos PIG.

Portanto, a avaliação precoce de risco de intercorrências gestacionais é de particular importância para o estabelecimento de medidas preventivas e terapêuticas eficazes. Apesar de algumas intervenções terem sido determinadas há alguns anos, a importância relativa de cada uma delas pode variar a depender da interferência de outros fatores na cascata fisiopatológica que culmina na RCF/PIG. Evidência de ensaios clínicos randomizados sugere que o uso diário de baixas doses de aspirina (60-150mg) previna a RCF (RR 0,56, IC 95% 0,44-0,70) (183), especialmente quando coexiste a pré-eclâmpsia, sugerindo que ambas as condições

podem compartilhar alguma via patológica. Ainda, o fato de a aspirina só ser eficaz se implementada antes de 16 semanas (183) chama a atenção de que a RCF ou pré-eclâmpsia são síndromes clinicamente insidiosas, mas presentes desde o início da gestação. Outros estudos apontam para a suplementação de nutrientes, tais como ácido fólico (48,49), sugerindo que o curso natural da síndrome pode ser modificado. Portanto, seguindo a lógica do modelo da 'Pirâmide Invertida' (184) do atendimento pré-natal, estratégias de rastreio, diagnóstico e tratamento devem se concentrar em idades gestacionais cada vez mais precoces.

Curiosamente, apesar de grande parte das mulheres estarem virtualmente captadas para o atendimento pré-natal na metade da gestação, observamos a assistência pública ao pré-natal como fator de risco para PIG. Todas as maternidades participantes do Preterm-SAMBA eram serviços terciários de referência para gestação de alto risco. Naturalmente, eles podem ter atraído mais gestantes de risco social, uma vez que que o Ministério da Saúde (185) sugere o encaminhamento da Unidade Básica de Saúde à atenção especializada. Porém, precisamos ter cuidado ao associar a assistência pública a eventos desfavoráveis na gestação. A assistência abrangente inicial às gestantes do Sistema Único de Saúde deve ser realizada justamente para identificar quem se beneficia do encaminhamento a serviços terciários. Assim como em outras condições gestacionais, um único fator isolado não tem acurácia preditiva suficiente. Também na restrição de crescimento, é necessária a elaboração de um modelo preditor com vários fatores que atendam à complexidade da síndrome. Neste sentido, a avaliação clínica de risco de uma maneira ampla, integrada, deve incluir a prevenção e o manejo das principais condições em obstetrícia, incluindo, pelo menos, avaliação de risco para RCF/PIG, pré-eclâmpsia, prematuridade e diabetes gestacional.

É lamentável constatar, porém, que o rastreio e a prevenção universal para pré-eclâmpsia não parece ser custo-efetivo (186). Infelizmente, se usarmos apenas o desfecho imediato da gestação (ex., diagnóstico de PIG) como ponto de referência, dificilmente tais estratégias serão implementadas. O número de gestantes que necessitariam de tratamento seria alto e, em muitos casos, a terapêutica seria baseada em evidências de baixa ou moderada qualidade. Estatísticas econômicas que contemplem desfechos de médio ou longo prazo poderiam esclarecer melhor a real magnitude das intervenções durante a gravidez.

Análises futuras da coorte poderão elucidar outros questionamentos sobre o manejo dos recém-nascidos PIG. É sabido que cerca de metade dos óbitos fetais ocorre no período anteparto (109). Entretanto, sua frequência na população geral é baixa, e foi de apenas 0,6% no nosso estudo. É razoável supor, então, que o estudo dos recém-nascidos PIG seja, para os óbitos fetais, o mesmo que os casos de *near miss* materno são para as mortes maternas (187). Acreditamos, então, que o estudo do *near miss* neonatal (188), da restrição de crescimento no recém-nascido (9), e dos percentis de peso em que há maiores taxas de morbidade (73), acrescente informações úteis também para o estudo da morte fetal intrauterina. Além disso, a análise do padrão alimentar das participantes do Preterm-SAMBA pode ajudar a esclarecer o eventual papel que o folato e micronutrientes tem em reduzir o risco para PIG em brasileiras.

A identificação precoce de casos suspeitos de RCF é uma das principais estratégias para reduzir a prevalência de óbitos intrauterinos, e cujo impacto a longo prazo nas estatísticas de saúde é incomensurável. Tais estratégias devem ser acompanhadas de monitorização local criteriosa, além de estabelecimento de políticas de saúde pública que garantam referência e contra referência,

prosseguimento da investigação, atendimento especializado para os casos suspeitos e confirmados, não apenas no período neonatal, mas na infância e adolescência.

A restrição de crescimento fetal é uma condição com potencial de interferir no metabolismo do indivíduo durante toda a sua vida, e com prevalência superior à da pré-eclâmpsia ou prematuridade. O estudo Preterm-SAMBA, seguindo os achados de coortes internacionais, apresentou uma prevalência de recém-nascidos PIG (12,8%) superior à de pré-eclâmpsia (7,5%) e de parto prematuro espontâneo (6,7%). Assim, questionamos o rastreio universal durante a gestação, aos moldes do que fazemos com a diabetes gestacional (189), ou de que se propõe com o parto prematuro (190). Em um futuro próximo, a translação dos achados metabolômicos oferecerá biomarcadores viáveis e custo-efetivos.

A experiência do doutorado

Há bastante tempo, Campinas significa uma cidade de desafios e oportunidades. E nos últimos quatro anos, não foi diferente. Nos idos de 2009, assim que me graduei em medicina, fui convidada pela Prof. Melania Amorim para participar como coordenadora local de um estudo sobre vigilância de morbidade materna grave. Até então, não conhecia o restante do grupo, muito menos pensei que estaria nele até hoje. Esta participação se continuou no Estudo Multicêntrico de Investigação de Prematuridade (EMIP) e, na reunião final, eu e o Prof. Guilherme conversamos para que eu fizesse pós-graduação na UNICAMP. E foi uma das decisões mais acertadas que já tomei.

O doutorado foi difícil; acho que é dessa forma para todos os alunos. Mas tenho certeza de que em qualquer outra instituição não seria tão especial como foi aqui. Além de ganhar experiência como pesquisadora clínica, ao participar do Preterm-SAMBA, tive oportunidade de experimentar novamente como é estar na

bancada. Durante o período em que estive na República da Irlanda, aprender normas de comportamento e conduta em laboratórios de pesquisa e reaprender procedimentos básicos, como pipetar, refinaram a minha técnica e a minha dedicação como pesquisadora. Estudar em uma universidade secular (a *University College Cork* foi fundada em 1845) me fez tentar ser uma discente mais engajada e interessada. Estagiar em um centro de pesquisas de renome internacional me fez imaginar poder, um dia, replicar essa experiência na instituição onde trabalho. Comunicar-me em outra língua me tornou uma pessoa (apenas um pouco) menos introvertida.

Retornei do doutorado sanduíche e continuei meu período ‘sabático’ - afastada de quaisquer compromissos empregatícios - cumprindo os créditos acadêmicos necessários à pós-graduação. Eis que encontro velhos amigos e crio novos vínculos. O *brainstorming* diário permitiu que eu me conhecesse e me compreendesse melhor. Participei de várias disciplinas, voltei a estudar morbidade materna, achei que estava pronta para o desafio científico de escrever uma Carta ao Editor. Muita coisa aconteceu, e sou grata a todas elas.

Sou orgulhosa e extremamente honrada de ter tido o Prof. Guilherme como meu orientador; levarei para sempre, com muito carinho, a responsabilidade desse cartão de visitas. Criei raízes aqui, não apenas laços.

7. CONCLUSÕES

7.1 O rastreio para recém-nascidos PIG deve ser integrado ao rastreio de demais eventos adversos da gestação, compondo uma avaliação multidimensional e compreensiva do binômio materno-fetal.

7.2 A incidência de recém-nascidos PIG foi de 12,8% na coorte do Preterm-SAMBA. O atendimento público de pré-natal pode ser considerado um *proxy* para o baixo nível socioeconômico de gestantes brasileiras, nulíparas, de risco obstétrico habitual. O estímulo a hábitos de vida saudáveis e ao início precoce do pré-natal poderá reduzir o risco das infecções na primeira metade da gestação para o desfecho PIG.

7.3 A validação dos achados de estudos metabolômicos do tipo *untargeted* tem o potencial de diferenciar os recém-nascidos sob maior risco (a curto, médio e longo prazos) dos que são constitucionalmente pequenos. Cientistas e pesquisadores clínicos devem aproximar os discursos, para que o conhecimento sobre rastreio e diagnóstico de RCF/PIG chegue à população.

7.4 Os estudos em metabolômica do tipo *untargeted* no segundo trimestre da gestação demonstraram melhor acurácia preditiva quando comparados aos desenhado para avaliação de um metabólito em específico. A cromatografia líquida acoplada a espectrometria de massa demonstrou melhor performance para a análise do sangue materno, enquanto a cromatografia gasosa foi melhor utilizada para a avaliação do cabelo materno.

7.5 Compostos relacionados ao metabolismo lipídico, p.ex. ácidos graxos e fosfoesfingolipídeos, foram os marcadores mais relacionados à predição do recém-nascidos PIG.

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APÊNDICES

APÊNDICE A – Approaching literature review for academic purposes: the Literature Review Checklist.

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Approaching literature review for academic purposes: the Literature Review Checklist

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Abstract:	A sound and sophisticated literature review precedes a robust dissertation or thesis. It is a welcoming chapter, scrutinizes the main problem of that academic work and anticipates research hypothesis, methods and results. It presents the student as a scholar, keeps the audience interested in how that dissertation/thesis will answer the current gaps in that field. Although it is an integral part of an academic text, there is little guidance for students on elaborating it. Writing the literature review is not a linear process. It translates students' abilities in information literacy, language domain, and critical writing, which should be trained in a systematic way in college. Therefore, this paper discusses what is the purpose of literature review in the context of dissertations and thesis. Secondly, it considers Five Steps of how to develop it: (i) Defining your main topic; (ii) Searching literature; (iii) Analyzing your results; (iv) Writing; and (v) Reflecting on your writing. Ultimately, it proposes a twelve-item Literature Review Checklist – based on Boote & Beile (2005) Scoring Rubric. By clearly stating which are the desired achievements, this Checklist allows young masters and Ph.D students to continuously assess their own progress on literature review elaboration. This tool should be used both by students and institutions, aiming to strengthen the necessary skills for critical academic writing and offering a new perspective on that field of knowledge.	
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Approaching literature review for academic purposes: The Literature Review Checklist

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ABSTRACT

A sound and sophisticated literature review uncovers a robust dissertation or thesis. It is a welcoming chapter, scrutinizes the main problem of that academic work and anticipates research hypothesis, methods and results. It presents the student as a scholar, keeps the audience interested in how that dissertation/thesis will answer the current gaps in that field. Although it is an integral part of an academic text, there is little guidance for students on elaborating it. Writing the literature review is not a linear process. It translates students' abilities in information literacy, language domain, and critical writing, which should be trained in a systematic way in college. Therefore, this paper discusses what is the purpose of literature review in the context of dissertations and thesis. Secondly, it considers Five Steps of how to develop it: (i) Defining your main topic; (ii) Searching literature; (iii) Analyzing your results; (iv) Writing; and (v) Reflecting on your writing. Ultimately, it proposes a twelve-item Literature Review Checklist – based on Boote & Beile (2005) Scoring Rubric. By clearly stating which are the desired achievements, this Checklist allows young masters and Ph.D students to continuously assess their own progress on literature review elaboration. This tool should be used both by students and institutions, aiming to strengthen the necessary skills for critical academic writing and offering a new perspective on that field of knowledge.

KEYWORDS: literature review, checklist, thesis, dissertation, scientific writing.

INTRODUCTION

The literature review (LR) is often seen as a hard work and can be an example of writer's block and procrastination (Fitzmaurice and O'Farrell n.d.) in postgraduate life. Disagreements on LR definition or classification (Grant and Booth 2009) confuse students about its purpose, scope and how to perform it. Conversely, at many universities, the LR is still an important element for any academic work, even considering the more recent trend of producing scientific articles other than classical thesis.

The LR is not an isolated section, neither a copy of the research proposal's background. It identifies the state-of-art in that field, clarifies what is already known, elucidates implications of that problem, links theory and practice (Hart 1998; Kumar 2011; Rowley and Slack 2004), highlights gaps in current literature, and places the dissertation/thesis in the research agenda of that field. Additionally, the postgraduate student comprehends the subject's structure and elaborates cognitive connections (Hart 1998) while analyzing and synthesizing data with increasing maturity.

Then, at the same time, the LR transforms the student and anticipates the other chapters to the reader. Firstly, the LR explains the research question; secondly, it supports hypothesis, objectives, and methods of that research; finally, it facilitates student's interpretation of results and conclusions. For scholars, the LR is a welcoming chapter (Randolph 2009): if it is well written, it demonstrates student's understanding and maturity on that topic. A sound and sophisticated LR uncovers a robust dissertation/thesis.

The best way to elaborate the dissertation/thesis is not unanimous. The LR can be a distinct chapter; be included in different sections; or be part of the introduction chapter, of each research topic, or of each published paper (Paltridge 2002). However, scholars comprehend this is an integral part of an academic work main body: it is intrinsically connected to other sections (Figure 1), and frequently is present. Its structure depends on discipline's orientations, own department's rules, and student's and supervisor's expertise, needs and interest.

Interestingly, many postgraduate students choose to submit their LR to peer-reviewed journals. As LR are critical evaluations of current knowledge, they are indeed publishable material, even as narrative or systematic reviews. However, systematic reviews have specific patterns[1] (Harris et al. 2014) that may not fit entirely dissertation/thesis question. Additionally, its scope may be too narrow, and the strict criteria for studies inclusion may omit important information for the dissertation/thesis. Therefore, this Essay discusses what is and how to

develop the LR in the context of academic dissertations/thesis. Finally, we suggest a Literature Review Checklist to evaluate it.

WHAT IS A THESIS LITERATURE REVIEW?

Conducting a research and writing a dissertation/thesis translates rational thinking and enthusiasm (Evans et al. 2014). While there is strong literature to instruct research methodology and analysis, or to write scientific papers, there is little guidance on performing the LR. The LR is a unique opportunity to assess and contrast various arguments and theories, not just summarize them. The research results should not be discussed within the LR, but the postgraduate student is more prone to write a comprehensive LR while reflecting from own findings (Meth and Williams 2006).

Many people understand that writing the LR is a lonely and linear process: it is assumed that the Ph.D. student dominates techniques and subject's vocabulary and has self-reflection about what has been written. While elaborating the LR, indeed, the student should aggregate diverse skills, which rely mostly on his/her own commitment to master them. Thus, minor supervision should be required (Boote and Beile 2005). However, it must not be the case for many students (Boote and Beile 2005; Granello 2001), and the lack of formal and systematic training to write the LR is an important concern (Boote and Beile 2005).

A sound LR translates the postgraduate student's expertise in academic and scientific writing: it expresses how comfortable he/she is in synthesizing ideas (Boote and Beile 2005). Surely, it demonstrates how well the postgraduate has proceeded in three domains: effective literature search, language domain, and critical writing.

Effective literature search

All students should be trained in gathering appropriate data for specific purposes, and information literacy skills are cornerstones. They are defined as "an individual's ability to know when they need information, to identify information that can help them address the issue or problem at hand, and to locate, evaluate, and use that information effectively" (National Forum On Information Literacy 1999–2000 Report, 2000). Librarians support is of vital

importance in coaching the adequate use of the Boolean logic or other tools for highly efficient literature search, and the appropriate management of electronic databases.

Language domain

The academic writing must be concise and precise: unnecessary words distract the reader from the essential content (Patience et al. 2013). In this context, reading about issues distant from the research topic (Robbins 2016) may enhance student's general vocabulary and familiarity with grammar. Ultimately, it facilitates and encourages the writing itself.

Critical writing

Critical judgment includes critical reading, thinking and writing: it supposes student's analytical reflection about what he/she has read. The student should delineate the basic elements of that topic; characterize the most relevant claims; identify relationships; and, finally, contrast them (Torraco 2005). Each scientific document brings its author's perspective, and, as much as students read, the more they are confident to judge its supporting evidence, underlying premises, and to make their own counter-argument. Paucity of integration or of contradictory points of view demonstrates lower levels of cognitive complexity (Granello 2001).

Thus, while elaborating the LR, the postgraduate student should achieve the highest category of Bloom's cognitive skills: evaluation (Granello 2001). The writer not only summarizes data and understands each topic, but can make judgments, based on objective criteria; compare resources and findings, then identify discrepancies due to methodology; and is capable of constructing his/her own argument (Granello 2001). As a result, students would be confident to show their own *voice*.

Writing a consistent LR is an intense and complex activity, revealing trained and long-lasting writer's academic skills. It is not a lonely or linear process. However, it is unlikely that students are prepared to write the LR

if they do not master the aforementioned domains (Meth and Williams 2006). An institutional environment that supports student's learning is crucial.

Different institutions have distinct ways to promote student's learning process. In the first place, many universities propose modules to develop these *behind the scenes* activities, enhancing self-reflection about general skills (e.g., what we are good at, and what we need to develop), behaviors that should be incorporated (e.g., self-criticism about own thoughts), and each student's role in advancements in his/her field. Lectures or workshops about the LR itself are useful, demonstrating LR's purposes, and how it fits in the whole picture of student's work. They can explain what type of discussion LR must involve, the importance of defining the correct scope, reasons to include a resource, and the main role of critical reading.

Equally important are some pedagogic services which promote continuous study enhancement and improvement of academic skills. Workshops about time management, accomplishment of personal objectives, active learning, or foreign languages for non-native speakers, are examples. Additionally, being in contact with other students make them aware of others' experiences and difficulties. Ultimately, the supervisor's role in providing feedback and setting deadlines is crucial in developing student's abilities and in strengthening writing quality (Granello 2001).

HOW TO DEVELOP THE LITERATURE REVIEW?

There is no consensus about how to elaborate the LR, but four main steps are considered: defining the main topic, searching literature, analyzing your results, and writing (Randolph 2009). We suggest a fifth step, reflecting on what has been written (Figure 2).

First Step: Defining your main topic

Planning the LR is directly linked to the thesis' research main question and occurs in parallel to students' training in the three domains discussed previously. It helps to organize ideas, delimits the LR's scope (Boote and Beile 2005), and avoids waste of time in the process. It includes:

- Reflecting about LR's scope: postgraduate students will have assumptions about what needs to be addressed and what is not essential to the LR (Cooper 1988; Montuori 2005). The Cooper's Taxonomy of Literature Reviews[2] systematizes the writing through six characteristics and not mutually exclusive categories. The *focus* refers to the reviewer's most important interest, while the *goals* concern to what students want to achieve with the LR. The *perspective* assumed answers to student's own point of view on LR, and how he/she presents an issue. The *coverage* defines how comprehensive the student is in presenting the literature, and the *organization* assigns which is the sequence of arguments. The *audience* regards to whom the LR is written for.
- Designating sections and subsections: Headings and subheadings should be specific, explanatory, with a coherent sequence throughout the text (Kumar 2011). They simulate an inverted pyramid, with increasing reflection and arguing deepness.
- Identifying keywords: for each LR section, the relevant keywords should be listed, to guide the literature search. This list mirrors what Hart (1998) advocates as *subject vocabulary*. They will be also useful while writing the LR since they guide the reader through the text.
- Delineating time interval and language of documents to be retrieved in the Second Step. The most recent published documents should be considered, but relevant texts published before a predefined cutoff year can be included if they are classic documents in that field. Extra care should be taken when translating documents.

Second Step: Searching literature

Ability to gather adequate information from literature must be addressed in postgraduate programs; librarian's support is important, especially to access difficult texts. This step comprises:

- Searching the literature itself: it consists of defining which databases (electronic, dissertation/thesis repositories), official documents, books, will be searched, and actively doing so. Information literacy skills have a central role in this stage. While searching electronic databases, may be necessary to apply controlled vocabulary (e.g., Medical Subject Headings, MeSH, for PubMed database) or specific standardized syntax rules.

Besides this, two other approaches are suggested. At first place, checking each document reference list might be useful to find relevant works to be included, and important opinions to be assessed. This is also relevant to reference the original studies and leading authors in that field. Moreover, students can contact directly the experts in that topic to count on their experience, or on a source for additional unpublished documents.

Before dissertation/thesis submission, it is recommended to rerun the electronic search strategy, at least. This will ensure that the most recently published papers will be considered to the LR.

- Selecting documents for inclusion: Generally, the most recent literature will be obtained from published peer-reviewed papers. It is also important to assess books and unpublished material, such as conference abstracts, academic texts or government reports, since the grey literature offers valuable pieces of information. However, once they are not peer-reviewed, it is suggested consciousness when adding it to the LR.

  This task is an important time management tool. Firstly, students can read title and abstract, to understand if that document suits their purposes, addresses the research question, and helps to increasingly develop the topic of interest. Then, they can scan it, check how it is structured, group it with similar documents, and verify if other arguments might be considered (Rowley and Slack 2004).

Third Step: Analyzing your results

Here, critical reading and thinking take place. This step consists of:

- Reading documents: The student may read texts in depth according to LR sections and subsections (*Defining your main topic*), for example; and this is not a passive activity (Fitzmaurice and O'Farrell n.d.). To practice critical analysis, some questions should emerge, such as: Is the research question evident and articulated with previous knowledge? What are authors' research and theoretical orientation, and how do they interact? Are the authors' claims related to another scholars' research? Do the authors consider different points of view? Was the research correctly designed and conducted? Are the results and discussion plausible? Are they in accordance with research objectives and methodology? What are the strengths and

limitations of this work? How do the authors support their findings? How does it contribute to my research topic? (Fitzmaurice and O'Farrell n.d.; Taylor 2007)

- Taking notes: systematically taking notes of each document helps to establish similarities or differences with other documents, and to highlight personal observations. This reinforces student's ideas for the next step, and to develop his/her own academic *voice* (Fitzmaurice and O'Farrell n.d.; Montuori 2005). Voice recognition software (Robbins 2016), mind maps (Rowley and Slack 2004), flowcharts, tables, spreadsheets, personal comments in main texts, or note taking apps, are all available tools to manage these observations, and the student him/herself should perform what better improves his/her learning. Additionally, considering submitting the LR to a peer-reviewed journal, it is advisable to take notes about activities performed in all five steps, so they can be replicated.

Fourth Step: Writing

It is probably difficult to recognize when the student is able and ready to write, after enough reading and thinking. Some students can produce a review in a single long journey session. However, as discussed before, writing is not a linear process, and students do not need to write the LR following the sections' sequence. Writing the LR is a time-consuming task, and some believe that at least six months should be sufficient (Randolph 2009). The LR, and academic writing in general, refers to writer's proper thoughts, conclusions about other's works (Meth and Williams 2006; Montuori 2005; Randolph 2009; Robbins 2016), and how to progress in the chosen field of knowledge. Thus, it is expected that each student presents different learning and writing trajectories.

In this step, writing methods should be taken into consideration; then, editing, citing and referencing correctly, should complete this stage, at least temporarily. Freewriting technique can be a good starting point to brainstorm ideas and to improve understanding of what has been read (Fitzmaurice and O'Farrell n.d.). To put the LR in the agenda, students can determine: two-hour writing sections (in minimum), with pre-specified tasks possible to be reached in one section; short (minutes) and long breaks (days, weeks), to allow sufficient time for mental rest and reflection; and short and long-term goals, to motivate the writing itself (Kotz and Cals 2013). With growing experience, this scheme can vary widely, and it is not a straightforward rule. Importantly, each discipline has a

different way of writing (Fitzmaurice and O'Farrell n.d.), and each department has its own preferred styles for citation and referencing.

Fifth Step: Reflecting on your writing

In this step, the postgraduate student is supposed to ask him/herself the same questions as in *Analyzing your results* step, and this can take more time than anticipated. Ambiguities, repeated ideas, lack of coherence, may not be noted when the student is immersed in this task for so long. Probably, this whole effort will be a work in progress, and refinements in the written material will be done constantly, once started.

LITERATURE REVIEW CHECKLIST

Differently from review papers, the dissertation/thesis LR should not be a stand-alone piece or work. Conversely, it should present the student as a scholar, and should keep the audience interested in how that dissertation/thesis will answer the current gaps in that field.

In a continuous student's academic development and research transparency, a checklist for LR evaluation is convenient, as it clearly states which are the desired achievements for dissertation/thesis LR. Here, we present a Literature Review Checklist, developed from the Literature Review Scoring Rubric (Boote and Beile 2005). To critically analyze the LR, we maintain the five categories but offer twelve criteria, which are not scaled (Figure 3). They all have the same importance and are not mutually exclusive.

First category: Coverage

1. There are justified criteria for inclusion and exclusion of literature from the review.

This criterion builds on LR main topic and coverage (Cooper 1988). We suppose experts would be confident in retrieving and selecting literature. However, postgraduate students must convince their audience about the adequacy of their search strategy and reasons to intentionally select what to cover (Boote and Beile 2005). References from

different fields of knowledge provide distinct points of view but narrowing the coverage may be important in areas with a large body of existing knowledge.

Second category: Synthesis

2. There is a critical examination of the state of the field.

Critical examination means assessment of distinct aspects in that field (Fitzmaurice and O'Farrell n.d.), with a constructive argument. It is not a negative criticism, but an understanding of how other scholars have added to the topic (Fitzmaurice and O'Farrell n.d.), and the student should analyze and contextualize contradictory statements. The writer's personal bias (beliefs, political involvement) can influence how the document is structured and written: cultural and paradigmatic background guide how the theories are revised and presented (Montuori 2005). However, it is important to assume an honest judgment in considering different points of view.

3. The topic or problem is clearly situated in broader scholarly literature.

The broader scholarly literature should be related to the chosen main topic for the LR (*How to develop the Literature Review* section). The LR can cover the literature from one or various disciplines, depending on its scope, but always offering a new perspective. Besides that, students should be careful in citing and referencing. As a rule, it is advisable to assess original studies and primary references. Systematic and narrative reviews show summarized data and might be important to be cited - especially for issues that need to be understood but not to be detailed. Similarly, quotation marks highlight exactly what has been said. However, excessive referencing may disclose lower student's levels of analysis and synthesis.

4. The LR is critically placed in the historical context of the field.

Situating the LR in the historical context shows how much comfortable the student is in addressing that topic. Instead of only presenting statements and theories in a temporal approach - sometimes following a linear timeline - the LR

should characterize student's academic work authenticity in the state-of-art of that field of knowledge. Thus, it reinforces why that dissertation/thesis represents originality in research.

5. Ambiguities in definitions are taken into consideration and resolved.

Different disciplines may have distinct theories on the same topic, and one discipline may consider distinct concepts to explain one topic. These misunderstandings should be addressed and contemplated. The LR should not bring together all theories or concepts, at the same time. Although this could demonstrate in-depth reading about that topic, it can reveal student's ineptitude to comprehend and to synthesize his/her research problem.

6. Important variables and phenomena relevant to the topic are articulated.

The LR is a unique opportunity to articulate ideas and arguments, and to purpose new relationships between them (Boote and Beile 2005; Meth and Williams 2006). More importantly, a sound LR will anticipate to the audience how these important variables and phenomena will be dealt with in that academic work. Indeed, the LR should build a bidirectional link with the remaining sections, but it also grounds connections of all sections to each other (Figure 1).

7. There is a synthesized new perspective on the literature.

The LR is a 'creative inquiry' (Montuori 2005), in which the student elaborates own discourse, building on previous knowledge in that field, and demonstrates own point of view while interpreting other's work (Montuori 2005; Torraco 2005). Thus, students should articulate current knowledge, do not accept results at face value (Boote and Beile 2005; Montuori 2005; Torraco 2005), and improve cognitive abilities (Granello 2001).

Third category: Methodology

8. The main methodologies and research techniques that have been used in the field are identified, and their advantages and disadvantages are discussed.

It is expected that the LR distinguishes what has been done from what needs to be performed - addressing benefits and drawbacks of the main methods applied so far, and considering strategies described to handle (not) expected limitations. While placing his/her research in the methodological context of that topic, the LR will justify study's methodology and substantiate interpretations.

9. Ideas and theories in the field are related to research methodologies.

The audience awaits writer's analysis and synthesis of methodological approaches in the field. Findings should be explained according to the strengths and limitations of previous research methods, and students must avoid interpretations not supported by the analyzed literature. This criterion translates student's comprehension about applicability and type of answer provided by different research methodologies, even with quantitative or qualitative research approach.

Fourth category: Significance

10. The scholarly significance of the research problem is rationalized.

The LR is a welcoming dissertation/thesis section and will present the postgraduate student as a scholar in that field (Boote and Beile 2005). Therefore, the LR should discuss how the research problem is currently addressed in that discipline, or in different disciplines, depending on the LR scope. The LR rationalizes what are the academic paradigms in that topic (Montuori 2005), and how to advance in the field from these starting points. However, too many personal citations - own student's or his/her research team's - can demonstrate a narrow literature search and lack of a comprehensive synthesis of ideas and arguments.

11. The practical significance of the research problem is rationalized.

This means student's comprehensive understanding about research terminology (e.g., risk *versus* associated factor), methodology (e.g., efficacy *versus* effectiveness) and plausible interpretations in the field context. Notably, the academic argumentation about a topic may not always reflect the debate in real life terms. For a quantitative

approach in epidemiology, for example, statistical differences between groups do not explain all multiple factors involved with that problem (Halsey et al. 2015). Therefore, excessive faith in *p*-values can demonstrate lower levels of student's critical evaluation of the research problem's context and implications.

Fifth category: Rhetoric

12. The LR was written with a coherent, clear structure that supported the review.

It strictly relates to the language domain: the text should be coherent, and be presented in a logical sequence, whichever organization (Cooper 1988) approach is chosen. The beginning of each section/subsection should state what themes will be addressed; paragraphs should be carefully linked to each other (Meth and Williams 2006); and the first sentence of each paragraph generally summarizes its contents. Additionally, student's statements are clear and sound, linked to other scholar's works; and there is precise and concise language, with a standardized writing (e.g., active/passive voice and verb tenses). Attention to grammar issues, such as orthography and punctuation, expresses prudence, and anticipates a robust dissertation/thesis. Ultimately, all these strategies provide fluency and consistency to the text.

Although the Scoring Rubric was initially proposed for postgraduate programs in education research, we strongly believe this Checklist is a valuable tool for all academic areas. For students, it is possible to follow own learning curve and to concentrate efforts in not yet achieved criterion. For institutions, it is a guide to support supervisor's feedback, to improve student's writing skills, and to highlight each program's learning goals. These criteria do not present a linear sequence, but, ideally, all twelve achievements should be perceived in the LR.

CONCLUSION

There is no correct rule to classify, evaluate or guide how to elaborate the LR. In this Essay, we have suggested directions to plan, structure and critically evaluate the LR. Planning the LR scope and how to achieve it is a valuable effort, and the Five Steps can be a rational starting point. An institutional environment devoted to active learning will

support students to continuously reflect about LR, which will be a dialogue between the writer and the current literature in that field (Montuori 2005).

Performing the LR itself is a challenging work, but a necessary process to understand our own field of expertise. Knowledge is always transitory, but our responsibility, as scholars, is to contribute in a critical way to our field, allowing others to think through our work. Good researchers are grounded in sophisticated LR, which uncovers trained and long-lasting writer's academic skills. We recommend the Literature Review Checklist as a tool for strengthening the necessary skills for critical academic writing.

Figure captions:

Fig 1 The literature review (LR) chapter is an elemental component of thesis and dissertations, and it is directly connected to other sections. Assessing the LR chapter, the reader might anticipate what to expect from that academic text

Fig 2 The Five Steps to perform a solid literature review for dissertations or thesis. The first three steps are divided in subsections; the fourth, suggests writing strategies; and the fifth, comprises some signaling questions to practice and evaluate critical writing. This is not a straightforward rule and returning to previous steps may be necessary to improve literature review (LR) LR quality

Fig 3 Literature Review Checklist. It comprises 12 criteria that should ideally be present in the literature review section on the dissertation or thesis. Below each criterion there are some signaling questions (SQ) to facilitate judgment if that item was achieved. It represents the literature review learning outcomes

Notes

[1]The systematic reviews questions usually follow the 'PICOS' acronym: Population, Intervention, Comparison, Outcomes, Study design.

[2]In 1988, Cooper has proposed a Taxonomy, which aims to facilitate student's and institution's understanding about reviewing the literature. There are six characteristics with specific categories, briefly described below.

- Focus: Research outcomes; Research methodologies; Theories; Practices or applications.
- Goals: Integration (Generalization, Conflict resolution, Linguistic bridge-building), Criticism, Identification of Central Issues.
- Perspective: Neutral representation, Espousal of position.
- Coverage: Exhaustive, Exhaustive with selective citation, Representative, Central or pivotal.
- Organization: Historical, Conceptual, Methodological.
- Audience: Specialized scholars, General scholars, Practitioners or policymakers, the General public.

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APÊNDICE B – Carta ao Editor do *Journal of Pediatrics*

LETTERS TO THE EDITOR www.jpeds.com · THE JOURNAL OF PEDIATRICS

Fetal and neonatal growth restriction: new criteria, renew challenges

To the Editor:

Beune et al published a statement on growth restriction in the newborn, building on a similar initiative in obstetrics.[1,2] These statements press experts in both fields to critically evaluate their current diagnostic practices regarding fetal growth restriction.

Importantly, both statements agree that clinicians need more than birth weight alone to diagnose growth restriction and endorse a multidimensional approach that considers functional parameters and biometric measurements.[1,2] The goal is to better identify infants who are truly growth restricted and therefore at risk of poor immediate- and long-term health outcomes.[3-5] However, the neonatology statements still lack key considerations before its criteria can be systematically and effectively applied.

First, the lack of experts from Latin America and Africa raises concerns about its global applicability. Epidemiologic and developmental biology research has shown that genetic inheritance involves more than gene coding.[6-9] It is reasonable to expect that epigenetic factors contribute to distinct clinical phenotypes of fetal or neonatal growth worldwide. Second, the absence of gestational age as a criterion encumbers clinical care coordination between obstetricians and neonatologists. Infants born at term and infants born preterm who are growth restricted present distinct clinical outcomes and need to be treated as such.[10-12] Finally, a history of pregnancy complications and antenatal suspicion are imprecise criteria, and their presence could confuse neonatal management. The majority of infants who are small for gestational age cannot be accurately predicted and usually present uneventful pregnancies.[13,14]

Growth restriction is a heterogeneous syndrome; its impact varies according to regional setting, gestational age, and pregnancy features. Therefore, the diagnostic criteria need to reflect this. Obstetricians and neonatologists should collaborate to validate both statements and to propose alternative criteria that encompass these aforementioned critical points.

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All authors perform research in the reproductive field, specifically about fetal growth restriction and preterm birth. J.C. and L.K. participated as subjects on the Gordijn et al survey (doi: 10.1002/uog.15884.) and, thus, have taken a role in the consensus definition of fetal growth restriction in obstetrics.

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Reply

To the Editor:

Leite et al raise concerns regarding the consensus definition of growth restriction in the newborn. We kindly thank them for their feedback and the chance to put these issues in perspective.

First, we agree that global representation is important to come to a reliable consensus definition that also can be successfully implemented globally. We acknowledge the fact that our efforts at incorporating publishing experts from Latin America and Africa were unsuccessful. The essential phenotype of fetal growth restriction is universal as far as we know, and the use of local reference charts is included. Furthermore, the experts from other low- and middle-income countries that participated in the Delphi procedures voted similarly to the rest of the panel. We therefore believe that the definition is applicable in the mentioned areas.

Second, the authors point out that gestational age influences clinical outcomes of fetuses and newborn infants who are growth restricted. We agree, and would like to point out that the Delphi procedure set out to define growth restriction, not to write a management protocol. Gestational age is vital information in the consultation between neonatologists/pathologists or other medical professionals as it affects prognosis and management.

Third, antenatal suspected (fetal) growth restriction was defined in this Delphi procedure according to the previously developed international consensus definition by the same group.[1] For maternal pregnancy complications, examples were given during the consensus procedure and include hypertension and pre-eclampsia. Both variables are included as contributory variables; thus, also without antenatal information the diagnosis of growth restriction in the newborn can be made.

We thank Leite et al for stimulating the discussion on defining growth restriction in the newborn.

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Applying the neonatal Early-Onset Sepsis calculator in cases of clinical chorioamnionitis at or after 34 weeks of gestation

To the Editor:

Carola et al questioned the diagnostic utility and hence the safety of the neonatal Early-Onset Sepsis (EOS) calculator.[1] In a retrospectively studied cohort of 1159 infants born to mothers with clinical chorioamnionitis, the calculator would have missed 2 of 5 infants with culture-proven, early-onset sepsis.

I consider the neonatal EOS calculator to be evidence-based. It is not, however, a diagnostic tool. The calculator estimates the probability of early-onset sepsis based on maternal risk factors and the clinical presentation of the newly born infant.[2,3] Puopolo et al left out the subjective "physician diagnosis of chorioamnionitis" as a variable in the logistic regression model.[3] The clinical recommendations accept a small risk (<1 in 3000) of overlooking, and hence not treating with antibiotics, a baby who will develop early-onset sepsis.[4]

Although the authors consider the risk of early-onset sepsis in neonates born to mothers with clinical chorioamnionitis low (4.3‰), it is 7.5 times greater than the Centers for Disease Control and Prevention national incidence (0.5‰).[5] Methodologically, the EOS calculator should not be used in a (sub)population with a different baseline sepsis prevalence without correcting the constant term (b_0) for the relevant prevalence of 4.3‰ in the logistic regression equation. Unfortunately, the EOS calculator provided only a pretest probability of 0.3‰-0.6‰ in increments of 0.1‰. In the Supplemental Information, Puopolo et al describe in detail how to adjust the intercept to match a given population prevalence, but individual study patient data are required to make the described calculations.[3] There is, however, an alternative calculation for prior correction available[6,7]:

$$\beta_0 = \beta_0 - ln\left|\frac{(1-\tau)\,\bar{y}}{\tau\,(1-\bar{y})}\right|$$

where τ is the fraction of sepsis in the (sub)population and $\bar{y}$ is the fraction of sepsis in the case-control study

APÊNDICE C – Termo de Consentimento Livre e Esclarecido

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Título de projeto: "Utilização da metabolômica para identificação e validação de biomarcadores para parto pré-termo".

Investigador responsável: Dr José Guilherme Cecatti

Instituição a que pertence o investigador responsável: UNICAMP

Eu_____, fui convidada a participar do estudo acima e fui esclarecida que para minha participação, serei submetida à coleta de sangue (aproximadamente 20ml) e de fios de cabelo (20 a 30 fios) com aproximadamente 20 semanas de gravidez e que durante o pré-natal e após o parto, darei algumas informações sobre mim, sobre minha saúde, sobre o pré-natal que estou realizando, sobre o meu parto, sobre minha recuperação após o parto e sobre o meu bebê.

Fui esclarecida que esse projeto está relacionado com a descoberta de substâncias no sangue e no cabelo que, junto com as informações que eu relatei, poderiam prever o risco de apresentar parto prematuro. Sei que prematuridade é o nascimento do bebê antes do tempo previsto (9 meses) e que é um problema muito sério, pois quanto mais prematuro, maiores os riscos de complicações por imaturidade e maior a chance de morte do bebê. Fui orientada que a coleta de sangue é uma picada na veia que poderá trazer um leve desconforto no local e raramente está relacionada a sérias complicações (pode causar vermelhidão e inchaço). Os fios de cabelo serão retirados da região da nuca, através do corte com tesoura.

Fui esclarecida que meu sangue e os fios de cabelo serão analisados por laboratórios que utilizam alta tecnologia chamada Metabolômica, sendo enviados para o exterior - Nova Zelândia, onde existe um laboratório específico com ampla experiência nessa área. Todos os dados e as amostras de sangue e cabelo que serão coletados serão armazenados por um período indeterminado, mas não por menos de 5 (cinco) anos após o fim do estudo. Fui orientada que essa tecnologia (Metabolômica) usada para analisar o sangue e cabelo é uma ciência relativamente nova e que oferece muitas oportunidades de pesquisa no futuro. Declaro que fui informada da possibilidade de meus dados e de minhas amostras serem utilizados para estudos futuros e que concordo com essa utilização futura sem a necessidade de novos consentimentos para cada uma dessas pesquisas.

Os investigadores só obterão os resultados desse estudo meses após o fim da minha gravidez atual. Entendo que os benefícios potenciais desses resultados só estarão disponíveis no futuro próximo. Portanto não haverá um benefício imediato para essa minha gestação. Entretanto, caso eu seja identificada como de alto risco para parto pré-termo, os investigadores entrarão em contato para me informar disso, propiciando a devida atenção nas próximas gestações. Fui informada que terei acesso gratuito a informações sobre o armazenamento e os resultados das minhas amostras e que posso obter essas informações através do contato telefônico.

Os riscos de se participar são mínimos, pois minha participação envolve a coleta de sangue e cabelo além da participação em entrevista para coleta de informações sobre mim e meu bebê. As minhas amostras e meus dados não serão identificados em nenhum momento, mantendo-se o sigilo, inclusive na publicação dos resultados obtidos. Receberei uma cópia desse termo assinado pelo investigador.

A participação nesse estudo é voluntária e eu poderei desistir de participar a qualquer momento, sendo retirado o meu consentimento. Devo explicar, em caso de desistência, se minhas amostras já coletadas poderão ser utilizadas ou não para o estudo. Não haverá nenhum prejuízo no meu atendimento no serviço em que estou sendo atendida, sendo garantida a continuidade do meu tratamento em caso de desistência.

A equipe de investigadores está à disposição para esclarecimentos ou questionamentos, caso seja solicitado por mim. Receberei um número de telefone celular próprio do estudo para contato com os investigadores, inclusive para ter conhecimento dos resultados obtidos com a utilização do meu material e receber instruções sobre as implicações desses resultados.

Todos aqueles que participarem do estudo e seus responsáveis legais, terão direito à informação quanto ao andamento do estudo, assim como ter acesso aos resultados, quando eles estiverem disponíveis.

Além disso, o Comitê de Ética em Pesquisa da Faculdade de Ciências Médicas/FCM - UNICAMP pode ser consultado no telefone (19) 3521-8936.

Eu,_____, declaro ter sido informada e concordo em participar, como voluntária, do projeto acima descrito.

(em caso de responsável legal):

Eu, _____, sou responsável por _____, participante do estudo, e declaro ter sido informado e concordo com a sua participação, como voluntária, no projeto de pesquisa acima descrito.

Campinas, _____de_____de _____

_____ _____

Nome e assinatura da paciente ou responsável legal **Nome e assinatura do responsável por obter consentimento**

_____

Nome e assinatura do pesquisador responsável

ANEXOS

ANEXO A – Protocolo do estudo 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

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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.

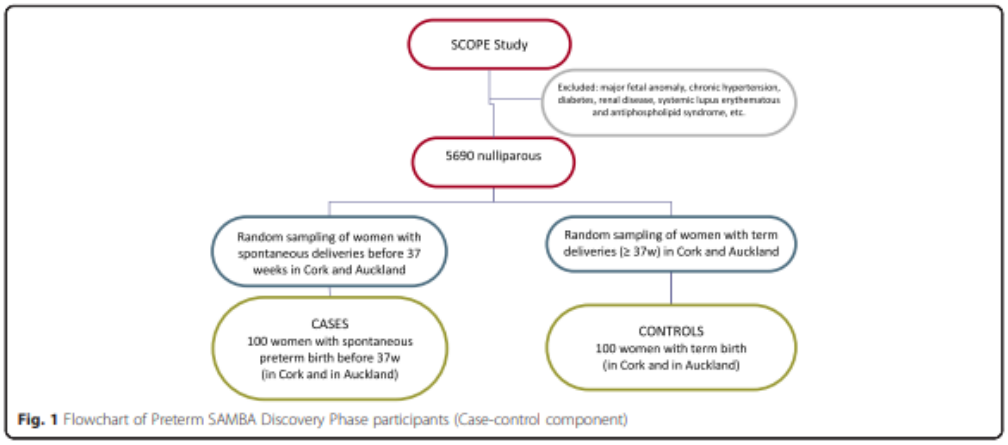

Fig. 1 Flowchart of Preterm SAMBA Discovery Phase participants (Case-control component)

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. Pseudo-anonymised 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

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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.

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ANEXO B – Carta de aprovação do Comitê de Ética em Pesquisa da UNICAMP

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

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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.

1.1) Informar endereço e e-mail do CEP, não somente o telefone. PENDÊNCIA RESPONDIDA.
1.2) 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)