

# BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email [editorial.bmjopen@bmj.com](mailto:editorial.bmjopen@bmj.com)

# BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-026033
Article Type:	Protocol
Date Submitted by the Author:	15-Aug-2018
Complete List of Authors:	Souza, Renato; Universidade Estadual de Campinas, Obstetrics and Gynecology Galvão, Rafael Leite, Debora; Campinas' State University, Department of Tocogynecology; Universidade Federal de Pernambuco, Passini Jr, Renato; Universidade Estadual de Campinas Faculdade de Ciências Medicas Baker, Philip ; University of Leicester, College of Medicine Cecatti, Jose Guilherme; University of Campinas, Obstetrics and Gynecology
Keywords:	preterm birth, metabolomics, biomarkers, prediction

SCHOLARONE™  
Manuscripts

Peer Review Only

1  
2  
3 **1 The use of metabolomics for predicting spontaneous preterm birth in asymptomatic**  
4  
5 **2 pregnant women: protocol for a systematic review and meta-analysis**  
6  
7  
8  
9

10 4 Renato T. Souza ([renatotsouzasp@gmail.com](mailto:renatotsouzasp@gmail.com))

11 5 Rafael Bessa Freitas Galvão ([rafaelbfg@gmail.com](mailto:rafaelbfg@gmail.com))

12 6 Débora Farias Batista Leite ([deborafariasleite@gmail.com](mailto:deborafariasleite@gmail.com))

13 7 Renato Passini Jr ([passini@caism.unicamp.br](mailto:passini@caism.unicamp.br))

14 8 Philip N. Baker ([philip.baker@leicester.ac.uk](mailto:philip.baker@leicester.ac.uk))

15 9 José Guilherme Cecatti ([cecatti@unicamp.br](mailto:cecatti@unicamp.br))

16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26 11 Department of Obstetrics and Gynaecology, University of Campinas, Campinas, São Paulo,  
27  
28 12 Brazil.

29  
30 13 Clinics Hospital of Federal University of Pernambuco, Recife, Pernambuco, Brazil.

31  
32 14 College of Life Sciences, University of Leicester, England, United Kingdom.

33  
34  
35  
36  
37 16 This systematic review protocol is registered at PROSPERO platform (CRD42018100172). Available  
38  
39 17 from: [http://www.crd.york.ac.uk/PROSPERO/display\\_record.php?ID=CRD42018100172](http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42018100172).

40  
41  
42  
43  
44 19 Corresponding author:

45  
46 20 Renato T Souza

47  
48 21 101 Alexander Fleming, Cidade Universitária, campinas, São Paulo, Brazil.

49  
50 22 ZIPCODE 13083-881

51  
52 23 [renatotsouzasp@gmail.com](mailto:renatotsouzasp@gmail.com)

53  
54  
55 24 Word count: 2,921.  
56  
57  
58  
59  
60

1  
2  
3 25 **ABSTRACT**  
4

5 26 **Introduction:** Preterm birth (PTB) is the leading cause of neonatal mortality and short- and  
6  
7 27 long-term morbidity. The aetiology and pathophysiology of spontaneous PTB are still  
8  
9  
10 28 unclear, which makes the identification of reliable and accurate predictor markers more  
11  
12 29 difficult, particularly for unscreened or asymptomatic women. Metabolomics biomarkers  
13  
14 30 have been demonstrated to be potentially accurate biomarkers for many disorders with  
15  
16 31 complex mechanisms such as PTB. Therefore, we aim to perform a systematic review of  
17  
18 32 metabolomics markers associated with spontaneous PTB. Our research question is “What is  
19  
20 33 the performance of metabolomics for predicting spontaneous preterm birth in  
21  
22 34 asymptomatic pregnant women?”  
23  
24

25  
26 35 **Methods and analysis:** We will focus on studies assessing metabolomics techniques for  
27  
28 36 predicting spontaneous preterm birth in asymptomatic pregnant women. We will conduct a  
29  
30 37 comprehensive systematic review of the literature from the last 10 years. Only  
31  
32 38 observational cohort and case-control studies will be included. Our search strategy will be  
33  
34 39 carried out by two independent reviewers, who will scan title and abstract before carrying  
35  
36 40 out a full review of the article. The scientific databases to be explored include PubMed,  
37  
38 41 MedLine, ScieLo, EMBASE, LILACS, Web of Science, Scopus, and others.  
39  
40

41  
42 42 **Ethics and dissemination:** This systematic review protocol does not require ethical approval.  
43  
44 43 We intend to disseminate our findings in scientific peer-reviewed journal, the Preterm  
45  
46 44 SAMBA study open access website, specialists’ conferences, and to our funding agencies.  
47

48  
49 45 Registration details: This protocol is registered in PROSPERO platform (code  
50  
51 46 CRD42018100172).  
52

53  
54 47 **Keywords:** preterm birth, spontaneous preterm birth, metabolomics, biomarkers,  
55  
56 48 prediction.  
57

1  
2  
3 49 **Strengths and limitations of this study**  
4

- 5 50 ● This systematic review protocol takes into account some important aspects regarding  
6  
7 51 conducting a systematic review about spontaneous preterm birth and metabolomics such as  
8  
9 52 the criteria used for defining spontaneous preterm birth, different population risk  
10  
11 53 stratification, method used to estimate gestational age, and metabolomics techniques  
12  
13  
14 54 details.  
15  
16 55 ● Two independent reviewers are responsible for searching and selecting studies, as also  
17  
18 56 extracting data, and a third reviewer will resolve any disagreement.  
19  
20  
21 57 ● If possible, proper statistical methods will be applied to investigate metabolomics  
22  
23 58 accuracy in predicting spontaneous preterm birth.  
24  
25

26 59  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## 60 INTRODUCTION

61 Spontaneous preterm birth (sPTB) is the leading cause of perinatal mortality and short- and  
62 long-term morbidity [1,2]. It is defined as birth that occurs before 37 weeks gestation due to  
63 spontaneous onset of labour or preterm premature rupture of membranes (pPROM) [3,4].  
64 Several pathways and mechanisms linked with preterm birth have been proposed including,  
65 neuroendocrine, vascular, immune-inflammatory, and behavioural processes [5]. More  
66 specifically, several markers associated with uterine distension/contraction, decidual  
67 inflammation/infection and activation of hypothalamic–pituitary–adrenal axis had been  
68 studied in the past decades [5,6]. However, no single marker or combination of markers has  
69 been found to be accurate enough for predicting sPTB [7–10].

70 Preterm birth is a complex and multifactorial syndrome that possibly has a long pre-clinical  
71 phase, maternal and fetal interactions, genetic and environmental influences, and adaptive  
72 mechanisms [11,12]. These challenging aspects, and the presence of still unknown  
73 underlying mechanisms, are the main limitations for the identification of an accurate  
74 predictor for sPTB [13–15]. None of the predictors used in clinical practice, such as previous  
75 history of preterm birth, infection (vaginal and urinary contaminants), fibronectin and  
76 transvaginal ultrasonography cervical length demonstrated exceptional accuracy for  
77 predicting spontaneous preterm birth [7]. An exploration of innovative approaches is  
78 urgently required.

79 Metabolomics is the study of metabolites, through identification and quantification of low-  
80 weight molecular particles, i.e. tens to hundreds thousands of intermediate products and  
81 substrates of systems biology chemical reactions [16,17]. This novel approach has been  
82 applied for identifying biomarkers and underlying biochemical pathways associated with  
83 complex obstetrical syndromes as preeclampsia, fetal growth restriction, gestational

1  
2  
3 84 diabetes and preterm birth. In contrast to other “*Omic*s Sciences” techniques, metabolomics  
4  
5 85 is more closely associated with the phenotype of the disease and might thus identify a more  
6  
7 86 robust and reliable set of predictors [18]. Importantly, implementing an adequate *Omic*s  
8  
9 87 experimental design is crucial for metabolomics studies.

10  
11  
12 88 Using different baseline population (asymptomatic vs symptomatic or low- vs high-risk  
13  
14 89 women for developing sPTB), study designs (prospective cohorts, case-control or cross  
15  
16 90 sectional studies), sources of samples (amniotic fluid, vaginal fluid, blood, urine, hair, etc)  
17  
18 91 and the timing of sample collection each have significant effects on study findings and the  
19  
20 92 consequent interpretation and contribution to the current gap of knowledge [16].  
21  
22 93 Therefore, we aim to conduct a systematic review of the use of metabolomics biomarkers  
23  
24 94 for predicting spontaneous preterm birth in asymptomatic pregnant women. This protocol  
25  
26 95 describes the methods that will be applied in our systematic review.  
27  
28  
29  
30

## 31 96 **METHODS AND ANALYSIS**

32  
33  
34 97 The current systematic review proposal will be conducted, written and published following  
35  
36 98 the Preferred Reporting Items for Systematics Reviews and Meta-Analyses (PRISMA-P)  
37  
38 99 recommendations [19]. Also, it is properly registered at PROSPERO platform – code  
39  
40 100 CRD42018100172.

### 41 42 43 101 **Review question**

44  
45 102 What is the performance of metabolomics for predicting spontaneous preterm birth in  
46  
47 103 asymptomatic pregnant women?

### 48 49 104 **Eligibility Criteria**

50  
51  
52 105 Original cohort or case-control studies involving asymptomatic pregnant women at the  
53  
54 106 moment of sample collection (exposure) and with samples analysed using metabolomics  
55  
56  
57  
58  
59  
60

1  
2  
3 107 techniques. Studies will be excluded if (1) they are cross-sectional studies, clinical trials,  
4  
5 108 editorials, letter to editors, case reports, expert opinions, commentaries, or any type of  
6  
7 109 review; (2) they describes only experimental studies with animals; or (3) they are duplicated  
8  
9  
10 110 data (e.g. data published in conferences proceedings and, then, published again in scientific  
11  
12 111 journals). In this case, only the most complete publication will be considered, after  
13  
14 112 comparing and confirming that the same technique and metabolites were explored. Studies  
15  
16 113 published from 2008 to 2018 will be considered, and there will be no language restriction.  
17  
18 114 Before submitting this systematic review for publication, we will rerun the search strategy to  
19  
20 115 identify new studies that have been published after performing the first round of search.  
21  
22

## 23 116 **Participants**

24  
25  
26 117 The current review is interested in evaluating the performance of metabolomics biomarkers  
27  
28 118 for spontaneous preterm birth in asymptomatic women, which may contribute to clinical  
29  
30 119 practice, potentially providing information regarding onset of preterm labour. Nevertheless,  
31  
32 120 we aim to identify studies addressing only early predictors collected from asymptomatic  
33  
34 121 women (i.e. women who are in an early preclinical stage), which might contribute to a wider  
35  
36 122 window of opportunity for interventions and also to develop a widely reproducible  
37  
38 123 screening test. Asymptomatic pregnant women should not have regular uterine  
39  
40 124 tightening/contractions or signs of rupture of membranes (i.e. watery discharge). In  
41  
42 125 addition, the study should preferably have a standardized definition of spontaneous  
43  
44 126 preterm birth, the outcome of interest.  
45  
46  
47

## 48 127 **Information Sources**

49  
50  
51 128 The search will be held in the following databases: PubMed, EMBASE, ProQuest, Scopus,  
52  
53 129 CINAHL, and Web of Science, BVS/BIREME, which includes the Latin American and  
54  
55 130 Caribbean Health Sciences Literature (LILACS), Medline and the Scientific Electronic Library  
56  
57



1  
2  
3 131 Online (Scielo). In addition, secondary sources of original studies will be explored such as  
4  
5 132 Google Scholar, hand-held searching of the reference list of eligible studies, conference  
6  
7 133 proceedings, and contact with authors when necessary.  
8

#### 9 134 **Search Strategy**

11 135 The following terms will be used in our search strategy for the different scientific databases:  
12  
13 136 (preterm birth, premature birth, premature infant, premature labor, extremely premature  
14  
15 137 infant, premature obstetric labor, spontaneous preterm birth, extreme preterm birth, late  
16  
17 138 preterm birth, moderate preterm birth, preterm premature rupture of membranes, preterm  
18  
19 139 delivery, PROM, sPTB, preterm PROM, pPROM, p-PROM) AND (metabolomic\*,  
20  
21 140 metabonomic\*, metabolit\*, lipidomic\*, H NMR, proton NMR, proton nuclear magnetic  
22  
23 141 resonance, liquid chromatogra\*, gas chromatogra\*, UPLC, ultra-performance liquid  
24  
25 142 chromatograph\*, ultra performance liquid chromatograph\*, HPLC, high performance liquid  
26  
27 143 chromatograph\*, high-performance liquid chromatograph\*) AND (pregnan\*, antenat\*,  
28  
29 144 ante nat\*, prenat\*, pre nat\*). Respective adaptations in the syntax of search for each  
30  
31 145 database will be applied accordingly. No filters - such as “research in animal’s models” and  
32  
33 146 “reviews” - will be used in our search strategy, as it will be excluded according to eligibility  
34  
35 147 criteria. The complete search strategy, including Boolean terms, is provided as  
36  
37 148 Supplementary Material.  
38  
39

#### 40 149 **Data Management**

41 150 We will export search results to a reference manager (Mendeley®). Then, the following  
42  
43 151 information will be collected from each study using an appropriate form, which will be  
44  
45 152 entered in an Excel® spreadsheet: author’s name, year of publication, country, study design,  
46  
47 153 number of participants with and without spontaneous preterm birth, type of metabolomics  
48  
49 154 analysis technique (liquid or gas chromatography, nuclear resonance), laboratory methods  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 155 for metabolites data acquiring (targeted or untargeted techniques, etc), subtype of preterm  
4  
5 156 birth (spontaneous or pPROM), number of fetuses (singleton vs multiple), gestational age  
6  
7 157 when samples were collected, source of samples (type/site of tissue), low or high-risk for  
8  
9 158 preterm birth (authors criteria used to define the population will be collected) and method  
10  
11 159 applied to estimate gestational age. If possible, additional variables related to spontaneous  
12  
13 160 preterm birth categories (delivery before 28 weeks and before 34 weeks) will be recorded  
14  
15 161 for secondary analyses. Finally, we will check the biochemical class of identified metabolites  
16  
17 162 in Human Metabolome Database (HMDB, version 4.0) to explore and synthesize whether  
18  
19 163 there are common biological pathways associated with spontaneous preterm birth [17].  
20  
21  
22

#### 23 164 **Selection Process**

24  
25 165 Two independent reviewers (RTS and RBF) will be responsible for screening and selecting  
26  
27 166 studies initially according to title or abstract. Full text of non-excluded studies will be read to  
28  
29 167 discriminate eligibility. A third reviewer (DFBL) will consider any disagreement; additional  
30  
31 168 reviewers (RPJ, PNB and JGC) will be responsible for supervising all steps and approving data  
32  
33 169 extraction.  
34  
35  
36

#### 37 170 **Data Collection Process**

38  
39 171 We will extract search results to a reference manager where all studies will be stored. Then,  
40  
41 172 included studies will be placed in a new folder. Finally, we will manually extract data of  
42  
43 173 interest from these included studies to an Excel® file. Each reviewer will have their own  
44  
45 174 reference manager account, file and folder and discrepant results will be discussed together  
46  
47 175 with the third reviewer.  
48  
49

#### 50 176 **Outcomes and Prioritization**

1  
2  
3 177 The primary outcome is spontaneous preterm birth, defined as any birth occurred before 37  
4  
5 178 weeks of gestation due to spontaneous onset of labor or preterm premature rupture of  
6  
7 179 membranes. Secondary outcomes are:

8  
9  
10 180 1) Spontaneous preterm birth before 28 weeks;

11  
12 181 2) Spontaneous preterm birth before 34 weeks;

13  
14 182 The capacity to predict different degrees of sPTB (categories of gestational age) is important  
15  
16 183 as the extreme (<28wks) and non-late preterm (<34wks) newborns have different adverse  
17  
18 184 outcomes compared to non-extreme ( $\geq 28$ wks) or late ( $\geq 34$  wks) preterm newborns.

19  
20  
21 185 Ideally, the method of gestational age estimation should be clearly reported. For instance, it  
22  
23 186 can be reported as estimated by last menstrual period (LMP) and confirmed by an early  
24  
25 187 ultrasound or only by an early ultrasound when LMP is unknown/uncertain.

## 26 27 28 188 **Index test**

29  
30 189 Metabolomics techniques to predict spontaneous preterm birth is the diagnostic test of  
31  
32 190 interest. Metabolomics is a technique to identify and quantify metabolites from biological  
33  
34 191 samples using different type of platforms/equipment. The most common platforms include  
35  
36 192 gas, liquid chromatography or ultra-performance liquid chromatography coupled to a mass  
37  
38 193 spectrometer or a proton nuclear magnetic resonance [20]. If possible, the performance of  
39  
40 194 each metabolomics techniques will be assessed through hierarchical summary receiver  
41  
42 195 operator characteristic curve (HSROC) (meta-analysis).

## 43 44 45 46 196 **Risk of Bias in individual Studies**

47  
48  
49 197 We will apply the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool [21]  
50  
51 198 to assess the risk of bias and applicability of primary diagnostic accuracy studies. Each study  
52  
53 199 will be classified as “low”, “high” or “unclear” regarding risk of bias for each of the four  
54  
55 200 domains of QUADAS tool: Patient Selection, Index Test (metabolomics), Reference Standard

1  
2  
3 201 (occurrence of preterm birth), and Flow and Timing of participant's inclusion and follow-up.  
4  
5 202 For example, studies will be labelled as "low" risk of bias for Reference Standard when  
6  
7 203 definition of spontaneous preterm birth and gestational age estimation are clear; "high" risk  
8  
9 204 of bias would be considered when the moment of sample collection is not well described.

## 11 205 **Data Synthesis**

12 206 We will report details of identification, screening, eligibility and included studies using a flow  
13  
14 207 diagram, according to PRISMA recommendations [19]. Data from included studies will be  
15  
16 208 synthesized into tables according to the variables of interest. If possible, we will present  
17  
18 209 data meta-analysis according to study design, metabolomics technique and type of samples  
19  
20 210 analysed. We intend to perform subgroup analysis according to:

- 21 211 • Different metabolomics methods applied: gas or liquid chromatography coupled  
22  
23 212 with mass spectrometry or proton nuclear magnetic resonance;
- 24 213 • Singleton and multiple pregnancies;
- 25 214 • Low-risk and high-risk women for developing preterm birth;
- 26 215 • Subtype of preterm birth: Spontaneous preterm birth exclusively due to  
27  
28 216 spontaneous onset of labour with intact membranes or sPTB due to premature  
29  
30 217 rupture of membranes.

## 31 218 **Potential anticipated limitations to this review**

32 219 Firstly, although we have not considered any language restriction, we consider that there  
33  
34 220 might be a limitation in studies published entirely in non-English language. However, in the  
35  
36 221 last decade, more than 95% of scientific biomedical literature has been published in English  
37  
38 222 [22], then we consider this a minor selection bias. Secondly, we intend to stratify the groups  
39  
40 223 according to population risk. However, the characterization of low- or high-risk for  
41  
42 224 spontaneous preterm birth is controversial and lacks standardization, which might limit data

1  
2  
3 225 comparison and subgroup analysis. Finally, categorization of sPTB into spontaneous onset of  
4  
5 226 labour or pPROM is another topic of potential limitation - the recognition of the main initial  
6  
7 227 mechanism for preterm delivery might not always be possible. Even when specified, it might  
8  
9 228 provoke uncertainty and could limit further considerations regarding preterm phenotypes.

### 11 229 **Patient and Public Involvement**

12  
13  
14 230 Patients will not be directly involved in the study and no experience or direct impact from  
15  
16 231 their perspective can be discussed.

### 18 232 **ETHICS AND DISSEMINATION**

19  
20  
21 233 This systematic review does not require ethical approval from the Research Council or Ethics  
22  
23 234 board. We intend to disseminate our findings in scientific peer-reviewed journal, general  
24  
25 235 free access website of Preterm Screening and Metabolomics in Brazil and Auckland (Preterm  
26  
27 236 SAMBA) study, specialists' conferences, and to our funding agencies.

### 30 237 **DISCUSSION**

31  
32  
33 238 This systematic review will comprise current knowledge related with metabolomics in the  
34  
35 239 context of preterm birth prediction. Metabolomics science, a resourceful innovative field  
36  
37 240 that allows better understanding on pathophysiology of complex syndromes, may address  
38  
39 241 the main compounds associated with the spontaneous preterm delivery and, therefore,  
40  
41 242 motivate further researchers to validate early measurable predictors of preterm birth.

42  
43 243 Metabolomics performance for predicting sPTB remains unclear and standardized and high-  
44  
45 244 quality studies are needed to clarify the clinical application of metabolites for predicting  
46  
47 245 sPTB. Nevertheless, metabolomics discovery studies commonly requires further validation  
48  
49 246 studies; reproducible methodology is crucial. This systematic review protocol will collate the  
50  
51 247 main potential early biomarkers, subgroup analysis and standardized definition for  
52  
53  
54  
55  
56  
57  
58  
59  
60

248 spontaneous preterm birth to better understand metabolomics performance in predicting  
 249 sPTB and also to show its heterogeneity in terms of methodology (samples used,  
 250 metabolomics technique, definition of SPTB phenotype, etc). High performing predictors of  
 251 preterm birth will help combat this leading cause of neonatal mortality and morbidity.

252

253 **References**

- 254 1 Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: when? Where? Why? *Lancet*  
 255 2005;**365**:891–900. doi:10.1016/S0140-6736(05)71048-5
- 256 2 Howson CP, Kinney M V, McDougall L, *et al.* Born Too Soon: Preterm birth matters.  
 257 *Reprod Health* 2013;**10 Suppl 1**:S1. doi:10.1186/1742-4755-10-S1-S1
- 258 3 Blencowe H, Cousens S, Chou D, *et al.* Born Too Soon: The global epidemiology of 15  
 259 million preterm births. *Reprod Health* 2013;**10**:S2. doi:10.1186/1742-4755-10-S1-S2
- 260 4 Ananth C V, Vintzileos AM. Epidemiology of preterm birth and its clinical subtypes. *J*  
 261 *Matern Fetal Neonatal Med* 2006;**19**:773–82. doi:10.1080/14767050600965882
- 262 5 Behrman R, Butler AS, editors. *Institute of Medicine (IOM). Preterm Birth: Causes,*  
 263 *Consequences, and Prevention.* Washington, D.C.: : National Academies Press 2007.  
 264 doi:10.17226/11622
- 265 6 Manuck TA, Esplin MS, Biggio J, *et al.* The phenotype of spontaneous preterm birth:  
 266 application of a clinical phenotyping tool. *Am J Obstet Gynecol* Published Online First:  
 267 February 2015. doi:10.1016/j.ajog.2015.02.010
- 268 7 Honest H, Hyde CJ, Khan KS. Prediction of spontaneous preterm birth: no good test  
 269 for predicting a spontaneous preterm birth. *Curr Opin Obstet Gynecol* 2012;**24**:422–  
 270 33. doi:10.1097/GCO.0b013e328359823a
- 271 8 Conde-Agudelo A, Papageorghiou A, Kennedy S, *et al.* Novel biomarkers for the  
 272 prediction of the spontaneous preterm birth phenotype: a systematic review and  
 273 meta-analysis. *BJOG An Int J Obstet Gynaecol* 2011;**118**:1042–54. doi:10.1111/j.1471-  
 274 0528.2011.02923.x
- 275 9 Hee L. Likelihood ratios for the prediction of preterm delivery with biomarkers. *Acta*  
 276 *Obstet Gynecol Scand* 2011;**90**:1189–99. doi:10.1111/j.1600-0412.2011.01187.x
- 277 10 Goldenberg RL, Iams JD, Mercer BM, *et al.* The Preterm Prediction Study: toward a  
 278 multiple-marker test for spontaneous preterm birth. *Am J Obstet Gynecol*  
 279 2001;**185**:643–51. doi:10.1067/mob.2001.116752
- 280 11 Di Renzo GC. The great obstetrical syndromes. *J Matern neonatal Med* 2009;**22**:633–

- 1  
2  
3 281 5. doi:10.1080/14767050902866804  
4  
5 282 12 Brosens I, Pijnenborg R, Vercruyssen L, *et al.* The 'Great Obstetrical Syndromes' are  
6 283 associated with disorders of deep placentation. *Am J Obstet Gynecol* 2011;**204**:193–  
7 284 201. doi:10.1016/j.ajog.2010.08.009  
8  
9 285 13 Practice bulletin no. 130: prediction and prevention of preterm birth. *Obstet Gynecol*  
10 286 2012;**120**:964–73. doi:10.1097/AOG.0b013e3182723b1b  
11  
12 287 14 Goldenberg RL, Culhane JF, Iams JD, *et al.* Epidemiology and causes of preterm birth.  
13 288 *Lancet* 2008;**371**:75–84. doi:10.1016/S0140-6736(08)60074-4  
14  
15 289 15 Honest H, Hyde CJ, Khan KS. Prediction of spontaneous preterm birth. *Curr Opin*  
16 290 *Obstet Gynecol* 2012;**24**:422–33. doi:10.1097/GCO.0b013e328359823a  
17  
18 291 16 Horgan RP, Clancy OH, Myers JE, *et al.* An overview of proteomic and metabolomic  
19 292 technologies and their application to pregnancy research. *BJOG* 2009;**116**:173–81.  
20 293 doi:10.1111/j.1471-0528.2008.01997.x  
21  
22 294 17 Wishart DS, Feunang YD, Marcu A, *et al.* HMDB 4.0: the human metabolome database  
23 295 for 2018. *Nucleic Acids Res* 2018;**46**:D608–17. doi:10.1093/nar/gkx1089  
24  
25 296 18 Dettmer K, Hammock BD. Metabolomics--a new exciting field within the omics  
26 297 sciences. *Environ Health Perspect* 2004;**112**:A396-7.  
27  
28 298 19 Shamseer L, Moher D, Clarke M, *et al.* Preferred reporting items for systematic review  
29 299 and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ*  
30 300 2015;**350**:g7647. doi:10.1136/BMJ.G7647  
31  
32 301 20 Zhang A, Sun H, Wang P, *et al.* Modern analytical techniques in metabolomics  
33 302 analysis. *Analyst* 2012;**137**:293–300. doi:10.1039/c1an15605e  
34  
35 303 21 Whiting PF, Rutjes AWS, Westwood ME, *et al.* QUADAS-2: A Revised Tool for the  
36 304 Quality Assessment of Diagnostic Accuracy Studies. *Ann Intern Med* 2011;**155**:529.  
37 305 doi:10.7326/0003-4819-155-8-201110180-00009  
38  
39 306 22 Rosselli D. The language of biomedical sciences. *Lancet* 2016;**387**:1720–1.  
40 307 doi:10.1016/S0140-6736(16)30259-8  
41  
42 308  
43  
44  
45  
46

47 309 **Author's Contributions**

- 48  
49 310 RTS and RFBG will conduct the systematic review as independent first reviewers. JGC, RPJ  
50  
51 311 and DFBL will decide about conflicting decisions regarding papers selections. PNB, RPJ and  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 312 JGC participated in the systematic review conception, methodology and framework,  
4  
5 313 together will all the others co-authors.  
6  
7

#### 8 314 **Funding**

9  
10 315 This research was supported by Brazilian National Research Council (grant number  
11  
12 316 401636/2013-5) and Bill and Melinda Gates Foundation (grant number OPP1107597- Grand  
13  
14 317 Challenges Brazil: Reducing the burden of preterm birth, FIOTEC number 05/2013), which  
15  
16 318 provided funding to PRETERM-SAMBA project ([www.medscinet.com/samba](http://www.medscinet.com/samba)). RTS and DFL  
17  
18 319 have been awarded PhD scholarships from the CAPES Foundation, an agency under the  
19  
20 320 Ministry of Education of Brazil, process 88881.134095/2016-01 and 8881.134512/2016-01  
21  
22 321 respectively. The sponsors played no role on the study design or manuscript writing.  
23  
24  
25  
26

#### 27 322 **Competing interests**

28  
29 323 All authors are carrying original research about metabolomics and presenting conferences  
30  
31 324 about this topic, including spontaneous preterm birth, preeclampsia, gestational diabetes  
32  
33 325 mellitus and fetal growth restriction. Philip N Baker is principal investigator of Metabolomics  
34  
35 326 Diagnostics Ltd, a company dedicated to develop innovative screening tests using  
36  
37 327 metabolomics technology.  
38  
39  
40  
41

#### 42 328 **Acknowledgements**

43  
44 329 Ana Paula de Morais e Oliveira, librarian of University of Campinas – Unicamp, Brazil, for  
45  
46 330 collaborating in developing search strategy and Rachel Hanisch for her suggestions to some  
47  
48 331 sections of the paper.  
49  
50

#### 51 332 **Ethics approval and consent to participate**



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

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

For peer review only

1  
2  
3 Search strategy: #1 AND #2 AND #3  
4  
5

6 preterm birth  
7 premature birth  
8 premature infant  
9 premature labor  
10 extremely premature infant  
11 premature obstetric labor  
12 spontaneous preterm birth  
13 extreme preterm birth  
14 late preterm birth  
15 1 (OR for each  
16 term)  
17 moderate preterm birth  
18 preterm premature rupture of membranes  
19 preterm delivery  
20 PROM  
21 sPTB  
22 preterm PROM  
23 pPROM  
24 p-PROM  
25  
26  
27  
28  
29  
30  
31 metabolomic\*  
32 metabonomic\*  
33 metabolit\*  
34 lipidomic\*  
35 H NMR  
36 proton NMR  
37 proton nuclear magnetic resonance  
38 2 (OR for each  
39 term)  
40 liquid chromatogra\*  
41 UPLC  
42 ultra-performance liquid chromatograph\*  
43 ultra performance liquid chromatograph\*  
44 HPLC  
45 high performance liquid chromatograph\*  
46 high-performance liquid chromatograph\*  
47  
48  
49  
50  
51  
52 pregnan\*  
53 antenat\*  
54 3 (OR for each  
55 term)  
56 ante nat\*  
57 prenat\*  
58 pre nat\*  
59  
60

## PRISMA-P 2015 Checklist

This checklist has been adapted for use with systematic review protocol submissions to BioMed Central journals from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 4:1

An Editorial from the Editors-in-Chief of *Systematic Reviews* details why this checklist was adapted - Moher D, Stewart L & Shekelle P: Implementing PRISMA-P: recommendations for prospective authors. *Systematic Reviews* 2016 5:15

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
<b>ADMINISTRATIVE INFORMATION</b>					
<b>Title</b>					
Identification	1a	Identify the report as a protocol of a systematic review	x	<input type="checkbox"/>	2
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	<input type="checkbox"/>	<input type="checkbox"/>	n/a
<b>Registration</b>	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	x	<input type="checkbox"/>	48-49
<b>Authors</b>					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	x	<input type="checkbox"/>	4-23
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	x	<input type="checkbox"/>	283-287
<b>Amendments</b>	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	<input type="checkbox"/>	<input type="checkbox"/>	n/a
<b>Support</b>					
Sources	5a	Indicate sources of financial or other support for the review	x	<input type="checkbox"/>	275-282
Sponsor	5b	Provide name for the review funder and/or sponsor	x	<input type="checkbox"/>	275-282
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	x	<input type="checkbox"/>	282
<b>INTRODUCTION</b>					
<b>Rationale</b>	6	Describe the rationale for the review in the context of what is already known	x	<input type="checkbox"/>	51-87
<b>Objectives</b>	7	Provide an explicit statement of the question(s) the review will address with reference to	x	<input type="checkbox"/>	94-95

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
		participants, interventions, comparators, and outcomes (PICO)			
<b>METHODS</b>					
<b>Eligibility criteria</b>	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	x	<input type="checkbox"/>	96-107
<b>Information sources</b>	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	x	<input type="checkbox"/>	119-125
<b>Search strategy</b>	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	x	<input type="checkbox"/>	127-136
<b>STUDY RECORDS</b>					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	x	<input type="checkbox"/>	142-155
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	x	<input type="checkbox"/>	157-161
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	x	<input type="checkbox"/>	163-167
<b>Data items</b>	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	x	<input type="checkbox"/>	142-155
<b>Outcomes and prioritization</b>	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	x	<input type="checkbox"/>	169-179
<b>Risk of bias in individual studies</b>	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	x	<input type="checkbox"/>	189-196
<b>DATA</b>					
<b>Synthesis</b>	15a	Describe criteria under which study data will be quantitatively synthesized	x	<input type="checkbox"/>	198-209
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., $I^2$ , Kendall's tau)	x	<input type="checkbox"/>	185-187
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	x	<input type="checkbox"/>	185-187

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	x	<input type="checkbox"/>	198-202
<b>Meta-bias(es)</b>	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	<input type="checkbox"/>	<input type="checkbox"/>	n/a
<b>Confidence in cumulative evidence</b>	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	<input type="checkbox"/>	<input type="checkbox"/>	n/a

For peer review only

# BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-026033.R1
Article Type:	Protocol
Date Submitted by the Author:	16-Nov-2018
Complete List of Authors:	Souza, Renato; Universidade Estadual de Campinas, Obstetrics and Gynecology Galvão, Rafael; Universidade Estadual de Campinas, Obstetrics and Gynecology Leite, Debora; Campinas' State University, Department of Tocogynecology; Universidade Federal de Pernambuco, Passini Jr, Renato; Universidade Estadual de Campinas Faculdade de Ciencias Medicas Baker, Philip ; University of Leicester, College of Medicine Cecatti, Jose; University of Campinas, Obstetrics and Gynecology
<b>Primary Subject Heading</b>:	Obstetrics and gynaecology
Secondary Subject Heading:	Diagnostics
Keywords:	preterm birth, metabolomics, biomarkers, prediction

SCHOLARONE™  
Manuscripts

1  
2  
3 1 **The use of metabolomics for predicting spontaneous preterm birth in asymptomatic**  
4  
5  
6 2 **pregnant women: protocol for a systematic review and meta-analysis**  
7  
8 3

9  
10 4 Renato T. Souza <sup>1</sup> ([renatotsouzasp@gmail.com](mailto:renatotsouzasp@gmail.com))

11  
12 5 Rafael Bessa Freitas Galvão <sup>1</sup> ([rafaelbfg@gmail.com](mailto:rafaelbfg@gmail.com))

13  
14 6 Débora Farias Batista Leite <sup>1,2</sup> ([deborafariasleite@gmail.com](mailto:deborafariasleite@gmail.com))

15  
16 7 Renato Passini Jr <sup>1</sup> ([passini@caism.unicamp.br](mailto:passini@caism.unicamp.br))

17  
18 8 Philip N. Baker <sup>3</sup> ([philip.baker@leicester.ac.uk](mailto:philip.baker@leicester.ac.uk))

19  
20 9 José Guilherme Cecatti <sup>1</sup> ([cecatti@unicamp.br](mailto:cecatti@unicamp.br))  
21  
22

23  
24  
25 10

26  
27 11 <sup>1</sup> Department of Obstetrics and Gynaecology, University of Campinas, Campinas, São Paulo,  
28  
29  
30 12 Brazil.

31  
32 13 <sup>2</sup> Clinics Hospital of Federal University of Pernambuco, Recife, Pernambuco, Brazil.

33  
34 14 <sup>3</sup> College of Life Sciences, University of Leicester, England, United Kingdom.  
35  
36

37  
38 15

39  
40 16 This systematic review protocol is registered at PROSPERO platform (CRD42018100172). Available  
41  
42 17 from: [http://www.crd.york.ac.uk/PROSPERO/display\\_record.php?ID=CRD42018100172](http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42018100172).  
43  
44

45  
46 18

47 19 Corresponding author:

48  
49 20 José Guilherme Cecatti

50  
51 21 101 Alexander Fleming, Cidade Universitária, campinas, São Paulo, Brazil.

52  
53 22 ZIPCODE 13083-881

54  
55 23 [cecatti@unicamp.br](mailto:cecatti@unicamp.br)

56  
57 24 Word count: 2,233.  
58  
59  
60

1  
2  
3 25 **ABSTRACT**  
4

5  
6 26 **Introduction:** Preterm birth (PTB) is the leading cause of neonatal mortality and short- and  
7  
8 27 long-term morbidity. The aetiology and pathophysiology of spontaneous PTB are still  
9  
10 28 unclear, which makes the identification of reliable and accurate predictor markers more  
11  
12 29 difficult, particularly for unscreened or asymptomatic women. Metabolomics biomarkers  
13  
14 30 have been demonstrated to be potentially accurate biomarkers for many disorders with  
15  
16 31 complex mechanisms such as PTB. Therefore, we aim to perform a systematic review of  
17  
18 32 metabolomics markers associated with spontaneous PTB. Our research question is “What is  
19  
20 33 the performance of metabolomics for predicting spontaneous preterm birth in  
21  
22 34 asymptomatic pregnant women?”  
23  
24  
25  
26

27 35 **Methods and analysis:** We will focus on studies assessing metabolomics techniques for  
28  
29 36 predicting spontaneous preterm birth in asymptomatic pregnant women. We will conduct a  
30  
31 37 comprehensive systematic review of the literature from the last 10 years. Only  
32  
33 38 observational cohort and case-control studies will be included. Our search strategy will be  
34  
35 39 carried out by two independent reviewers, who will scan title and abstract before carrying  
36  
37 40 out a full review of the article. The scientific databases to be explored include PubMed,  
38  
39 41 MedLine, ScieLo, EMBASE, LILACS, Web of Science, Scopus, and others.  
40  
41  
42  
43

44 42 **Ethics and dissemination:** This systematic review protocol does not require ethical approval.  
45  
46 43 We intend to disseminate our findings in scientific peer-reviewed journal, the Preterm  
47  
48 44 SAMBA study open access website, specialists’ conferences, and to our funding agencies.  
49

50  
51 45 **Registration details:** This protocol is registered in PROSPERO platform (code  
52  
53 46 CRD42018100172).  
54

55  
56 47 **Keywords:** preterm birth, spontaneous preterm birth, metabolomics, biomarkers,  
57  
58 48 prediction.  
59  
60



## 49 **Strengths and limitations of this study**

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

## 62 INTRODUCTION

63 Spontaneous preterm birth (sPTB) is the leading cause of perinatal mortality and short- and  
64 long-term morbidity [1,2]. It is defined as birth that occurs before 37 weeks gestation due to  
65 spontaneous onset of labour or preterm premature rupture of membranes (pPROM) [3,4].  
66 Several pathways and mechanisms linked with preterm birth have been proposed including,  
67 neuroendocrine, vascular, immune-inflammatory, and behavioural processes [5]. More  
68 specifically, several markers associated with uterine distension/contraction, decidual  
69 inflammation/infection and activation of hypothalamic–pituitary–adrenal axis had been  
70 studied in the past decades [5,6]. However, no single marker or combination of markers has  
71 been found to be accurate enough for predicting sPTB [7–10]. History of previous preterm  
72 birth, cervical length at second trimester and cervico-vaginal fetal fibronectin are the most  
73 promising clinical tests for predicting spontaneous preterm, but they seem not to be  
74 clinically useful for asymptomatic women. Sensitivity of short cervical length (<25mm) and  
75 high cervico-vaginal fFN (>50ng/ml) are around 33-36% and 46%, respectively [11–13].  
76 Preterm birth is a complex and multifactorial syndrome that possibly has a long pre-clinical  
77 phase, maternal and fetal interactions, genetic and environmental influences, and adaptive  
78 mechanisms [14,15]. These challenging aspects, and the presence of still unknown  
79 underlying mechanisms, are the main limitations for the identification of an accurate  
80 predictor for sPTB [16–18]. None of the predictors used in clinical practice, such as previous  
81 history of preterm birth, infection (vaginal and urinary contaminants), fibronectin and  
82 transvaginal ultrasonography cervical length demonstrated exceptional accuracy for  
83 predicting spontaneous preterm birth [7]. An exploration of innovative approaches is  
84 urgently required.

1  
2  
3 85 Metabolomics is the study of metabolites, through identification and quantification of low-  
4  
5 86 weight molecular particles, i.e. tens to hundreds thousands of intermediate products and  
6  
7  
8 87 substrates of systems biology chemical reactions [19,20]. This novel approach has been  
9  
10 88 applied for identifying biomarkers and underlying biochemical pathways associated with  
11  
12  
13 89 complex obstetrical syndromes as preeclampsia, fetal growth restriction, gestational  
14  
15 90 diabetes and preterm birth. In contrast to other “*Omics Sciences*” techniques, metabolomics  
16  
17  
18 91 is more closely associated with the phenotype of the disease and might thus identify a more  
19  
20 92 robust and reliable set of predictors [21]. Importantly, implementing an adequate *Omics*  
21  
22  
23 93 experimental design is crucial for metabolomics studies. Using different baseline population  
24  
25 94 (asymptomatic vs symptomatic or low- vs high-risk women for developing sPTB), study  
26  
27 95 designs (prospective cohorts, case-control or cross sectional studies), sources of samples  
28  
29 96 (amniotic fluid, vaginal fluid, blood, urine, hair, etc) and the timing of sample collection each  
30  
31 97 have significant effects on study findings and the consequent interpretation and  
32  
33 98 contribution to the current gap of knowledge [19].  
34  
35  
36  
37 99 Different reviews collating scientific knowledge regarding preterm birth  
38  
39  
40 100 biomarkers/predictors has been conducted. Different methodology approaches has been  
41  
42 101 applied so far, including narrative, systematic and umbrella reviews, a more comprehensive  
43  
44 102 review that includes not only original studies but also other reviews [7,19,22,23]. At the best  
45  
46  
47 103 of our knowledge, there is no systematic review on metabolomics markers. Therefore, we  
48  
49 104 aim to conduct a systematic review of original studies investigating the use of metabolomics  
50  
51  
52 105 biomarkers for predicting spontaneous preterm birth in asymptomatic pregnant women.  
53  
54 106 This protocol describes the methods that will be applied in our systematic review.  
55  
56  
57

## 58 107 **METHODS AND ANALYSIS**

59  
60

1  
2  
3 108 The current systematic review proposal will be conducted, written and published following  
4  
5 109 the Preferred Reporting Items for Systematics Reviews and Meta-Analyses (PRISMA-P)  
6  
7  
8 110 recommendations [24]. Also, it is properly registered at PROSPERO platform – code  
9  
10 111 CRD42018100172.

### 112 **Review question**

113 What is the performance of metabolomics for predicting spontaneous preterm birth in  
14  
15  
16  
17  
18 114 asymptomatic pregnant women?

### 115 **Eligibility Criteria**

116 Original cohort or case-control studies involving asymptomatic pregnant women at the  
17  
18  
19  
20  
21 117 moment of sample collection (exposure) and with samples analysed using metabolomics  
22  
23  
24  
25  
26  
27 118 techniques. Studies will be excluded if (1) they are cross-sectional studies, clinical trials,  
28  
29  
30 119 editorials, letter to editors, case reports, expert opinions, commentaries, or any type of  
31  
32  
33 120 review; (2) they describes only experimental studies with animals; or (3) they are duplicated  
34  
35 121 data (e.g. data published in conferences proceedings and, then, published again in scientific  
36  
37  
38 122 journals). In this case, only the most complete publication will be considered, after  
39  
40 123 comparing and confirming that the same technique and metabolites were explored. Studies  
41  
42 124 published from 2008 to 2018 will be considered, and there will be no language restriction.  
43  
44  
45 125 Before submitting this systematic review for publication, we will rerun the search strategy to  
46  
47 126 identify new studies that have been published after performing the first round of search.

### 127 **Participants**

128 The current review is interested in evaluating the performance of metabolomics biomarkers  
129  
130 129 for spontaneous preterm birth in asymptomatic pregnant women, which may contribute to  
131  
132  
133 130 clinical practice, potentially providing information regarding onset of preterm labour.  
134  
135  
136 131 Nevertheless, we aim to identify studies addressing only early predictors collected from

1  
2  
3 132 women who are in an early preclinical stage, which might contribute to a wider window of  
4  
5 133 opportunity for interventions and also to develop a widely reproducible screening test.  
6  
7  
8 134 Asymptomatic pregnant women should not have regular uterine tightening/contractions or  
9  
10 135 signs of rupture of membranes (i.e. watery discharge). In addition, the study should  
11  
12  
13 136 preferably have a standardized definition of spontaneous preterm birth, the outcome of  
14  
15 137 interest.

### 18 138 **Information Sources**

19  
20 139 The search will be held in the following databases: PubMed, EMBASE, Scopus, CINAHL, and  
21  
22  
23 140 Web of Science, BVS/BIREME, which includes the Latin American and Caribbean Health  
24  
25 141 Sciences Literature (LILACS), Medline and the Scientific Electronic Library Online (Scielo). In  
26  
27 142 addition, secondary sources of original studies will be explored such as Google Scholar,  
28  
29  
30 143 hand-held searching of the reference list of eligible studies, conference proceedings, and  
31  
32 144 contact with authors when necessary.

### 35 145 **Search Strategy**

36  
37 146 The following terms will be used in our search strategy for the different scientific databases:  
38  
39  
40 147 (preterm birth, premature birth, premature infant, premature labor, extremely premature  
41  
42 148 infant, premature obstetric labor, spontaneous preterm birth, extreme preterm birth, late  
43  
44 149 preterm birth, moderate preterm birth, preterm premature rupture of membranes, preterm  
45  
46 150 delivery, PROM, sPTB, preterm PROM, pPROM, p-PROM) AND (metabolomic\*,  
47  
48 151 metabonomic\*, metabolit\*, lipidomic\*, H NMR, proton NMR, proton nuclear magnetic  
49  
50 152 resonance, liquid chromatogra\*, gas chromatogra\*, UPLC, ultra-performance liquid  
51  
52 153 chromatograph\*, ultra-performance liquid chromatograph\*, HPLC, high performance liquid  
53  
54 154 chromatograph\*, high-performance liquid chromatograph\*) AND (pregnan\*, antenat\*,  
55  
56 155 ante nat\*, prenat\*, pre nat\*) (Supplementary Material). Respective adaptations in the  
57  
58  
59  
60

1  
2  
3 156 syntax of search for each database will be applied accordingly. No filters - such as “research  
4  
5  
6 157 in animal’s models” and “reviews” - will be used in our search strategy, as it will be excluded  
7  
8 158 according to eligibility criteria. The complete search strategy, including Boolean terms, is  
9  
10  
11 159 provided as Supplementary Material.

## 12 13 160 **Data Management**

14  
15 161 We will export search results to a reference manager (Mendeley®). Then, the following  
16  
17 162 information will be collected from each study using an appropriate form, which will be  
18  
19  
20 163 entered in an Excel® spreadsheet: author’s name, year of publication, country, study design,  
21  
22 164 number of participants with and without spontaneous preterm birth, type of metabolomics  
23  
24 165 analysis technique (liquid or gas chromatography, nuclear resonance), laboratory methods  
25  
26 166 for metabolites data acquiring (targeted or untargeted techniques, etc), subtype of preterm  
27  
28 167 birth (spontaneous preterm labour or pPROM), number of fetuses (singleton vs multiple),  
29  
30 168 gestational age when samples were collected, source of samples (type/site of tissue), low or  
31  
32 169 high-risk for preterm birth (authors criteria used to define the population will be collected)  
33  
34 170 and method applied to estimate gestational age. If possible, additional variables related to  
35  
36 171 spontaneous preterm birth categories (delivery before 28 weeks and before 34 weeks) will  
37  
38 172 be recorded for secondary analyses. Original authors will be contacted to clarify data, when  
39  
40 173 needed. Finally, we will check the biochemical class of identified metabolites in Human  
41  
42 174 Metabolome Database (HMDB, version 4.0) to explore and synthesize whether there are  
43  
44 175 common biological pathways associated with spontaneous preterm birth [20].  
45  
46  
47  
48  
49  
50

## 51 176 **Selection Process**

52  
53 177 Two independent reviewers (RTS and RBF) will be responsible for screening and selecting  
54  
55 178 studies initially according to title or abstract. Both researchers will read the full text of non-  
56  
57 179 excluded studies to discriminate eligibility. A third reviewer (DFBL) will consider any  
58  
59  
60

1  
2  
3 180 disagreement; additional reviewers (RPJ, PNB and JGC) will be responsible for supervising all  
4  
5  
6 181 steps and approving data extraction.  
7

### 8 182 **Data Collection Process**

9  
10 183 We will extract search results to a reference manager where all studies will be stored. Then,  
11  
12  
13 184 included studies will be placed in a new folder. Finally, we will manually extract data of  
14  
15 185 interest from these included studies to an Excel® file. Each reviewer will have their own  
16  
17  
18 186 reference manager account, file and folder and discrepant results will be discussed together  
19  
20 187 with the third reviewer.  
21  
22

### 23 188 **Outcomes and Prioritization**

24  
25 189 The primary outcome is spontaneous preterm birth, defined as any birth occurred before 37  
26  
27  
28 190 weeks of gestation due to spontaneous onset of labor or preterm premature rupture of  
29  
30 191 membranes. Secondary outcomes are:

- 32 192 1. Spontaneous preterm birth before 28 weeks;
- 33  
34 193 2. Spontaneous preterm birth before 32 weeks;
- 35  
36  
37 194 3. Spontaneous preterm birth before 34 weeks;
- 38  
39

40 195 The capacity to predict different degrees of sPTB (categories of gestational age) is important  
41  
42 196 as the extreme (<28wks), moderate (<32 weeks) and non-late preterm (<34wks) newborns  
43  
44  
45 197 have different adverse outcomes compared to non-extreme (≥28wks); non-moderate  
46  
47 198 (≥32wks) or late (≥34 wks) preterm newborns.

48  
49  
50 199 Ideally, the method of gestational age estimation should be clearly reported. For instance, it  
51  
52 200 can be reported as estimated by last menstrual period (LMP) and confirmed by an early  
53  
54 201 ultrasound or only by an early ultrasound when LMP is unknown/uncertain.  
55  
56

### 57 202 **Index test**

1  
2  
3 203 Metabolomics techniques to predict spontaneous preterm birth is the diagnostic test of  
4  
5 204 interest. Metabolomics is a technique to identify and quantify metabolites from biological  
6  
7  
8 205 samples using different type of platforms/equipment. The most common platforms include  
9  
10 206 gas, liquid chromatography or ultra-performance liquid chromatography coupled to a mass  
11  
12  
13 207 spectrometer or a proton nuclear magnetic resonance [25]. If possible, the performance of  
14  
15 208 each metabolomics techniques will be assessed through hierarchical summary receiver  
16  
17  
18 209 operator characteristic curve (HSROC) (meta-analysis).

### 20 210 **Risk of Bias in individual Studies**

21  
22  
23 211 We will apply the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool [26]  
24  
25 212 to assess the risk of bias and applicability of primary diagnostic accuracy studies. Each study  
26  
27 213 will be classified as “low”, “high” or “unclear” regarding risk of bias for each of the four  
28  
29  
30 214 domains of QUADAS tool: Patient Selection, Index Test (metabolomics), Reference Standard  
31  
32 215 (occurrence of preterm birth), and Flow and Timing of participant’s inclusion and follow-up.  
33  
34  
35 216 For example, studies will be labelled as “low” risk of bias for Reference Standard when  
36  
37 217 definition of spontaneous preterm birth and gestational age estimation are clear; “high” risk  
38  
39  
40 218 of bias would be considered when the moment of sample collection is not well described.

### 41 42 219 **Data Synthesis**

43  
44  
45 220 We will report details of identification, screening, eligibility and included studies using a flow  
46  
47 221 diagram, according to PRISMA recommendations [24]. Data from included studies will be  
48  
49  
50 222 synthesized into tables according to the variables of interest. If possible, we will present  
51  
52 223 data meta-analysis according to study design, metabolomics technique and type of samples  
53  
54  
55 224 analysed. We intend to perform subgroup analysis according to:

- 56  
57 225 • Different metabolomics methods applied: gas or liquid chromatography coupled  
58  
59 226 with mass spectrometry or proton nuclear magnetic resonance;



- 1  
2  
3 227      ● Singleton and multiple pregnancies;  
4  
5  
6 228      ● Low-risk and high-risk women for developing preterm birth;  
7  
8 229      ● Subtype of preterm birth: Spontaneous preterm birth exclusively due to  
9  
10            spontaneous onset of labour with intact membranes or sPTB due to premature  
11  
12            rupture of membranes.  
13  
14

15 232      Heterogeneity will be assessed by Cochran's Q, Hotelling's T-squared ( $\tau^2$ ) and  $I^2$  tests. Funnel  
16  
17 233      plots and sensitivity and cumulative analyses will be applied for detection of temporal  
18  
19 234      trends and publication bias.  
20  
21  
22

### 23 235      **Potential anticipated limitations to this review**

24  
25 236      Firstly, although we have not considered any language restriction, we consider that there  
26  
27 237      might be a limitation in studies published entirely in non-English language. However, in the  
28  
29 238      last decade, more than 95% of scientific biomedical literature has been published in English  
30  
31 239      [27], then we consider this a minor selection bias. Secondly, we intend to stratify the groups  
32  
33 240      according to population risk. However, the characterization of low- or high-risk for  
34  
35 241      spontaneous preterm birth is controversial and lacks standardization, which might limit data  
36  
37 242      comparison and subgroup analysis. Finally, categorization of sPTB into spontaneous onset of  
38  
39 243      labour or pPROM is another topic of potential limitation - the recognition of the main initial  
40  
41 244      mechanism for preterm delivery might not always be possible. Even when specified, it might  
42  
43 245      provoke uncertainty and could limit further considerations regarding preterm phenotypes.  
44  
45 246      In addition, another limitation is that individual patient data will not be collected.  
46  
47  
48  
49  
50

### 51 247      **Patient and Public Involvement**

52  
53  
54 248      Patients will not be directly involved in the study and no experience or direct impact from  
55  
56 249      their perspective can be discussed.  
57  
58  
59

60 250

1  
2  
3 251 **ETHICS AND DISSEMINATION**  
4

5 252 This systematic review does not require ethical approval from the Research Council or Ethics  
6  
7  
8 253 board. We intend to disseminate our findings in scientific peer-reviewed journal, general  
9  
10 254 free access website of Preterm Screening and Metabolomics in Brazil and Auckland (Preterm  
11  
12  
13 255 SAMBA) study, specialists' conferences, and to our funding agencies.  
14  
15

16 256 **DISCUSSION**  
17

18  
19 257 This systematic review will comprise current knowledge related with metabolomics in the  
20  
21 258 context of preterm birth prediction. Metabolomics science, a resourceful innovative field  
22  
23  
24 259 that allows better understanding on pathophysiology of complex syndromes, may address  
25  
26 260 the main compounds associated with the spontaneous preterm delivery and, therefore,  
27  
28  
29 261 motivate further researchers to validate early measurable predictors of preterm birth.  
30  
31 262 Metabolomics performance for predicting sPTB remains unclear and standardized and high-  
32  
33  
34 263 quality studies are needed to clarify the clinical application of metabolites for predicting  
35  
36 264 sPTB. Nevertheless, metabolomics discovery studies commonly requires further validation  
37  
38  
39 265 studies; reproducible methodology is crucial. This systematic review protocol will collate the  
40  
41 266 main potential early biomarkers, subgroup analysis and standardized definition for  
42  
43  
44 267 spontaneous preterm birth to better understand metabolomics performance in predicting  
45  
46 268 sPTB and also to show its heterogeneity in terms of methodology (samples used,  
47  
48  
49 269 metabolomics technique, definition of SPTB phenotype, etc). High performing predictors of  
50  
51 270 preterm birth will help combat this leading cause of neonatal mortality and morbidity.  
52

53 271  
54  
55  
56  
57  
58  
59  
60

273 **References**

- 274 1 Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: when? Where? Why? *Lancet*  
275 2005;**365**:891–900. doi:10.1016/S0140-6736(05)71048-5
- 276 2 Howson CP, Kinney M V, McDougall L, *et al.* Born Too Soon: Preterm birth matters.  
277 *Reprod Health* 2013;**10 Suppl 1**:S1. doi:10.1186/1742-4755-10-S1-S1
- 278 3 Blencowe H, Cousens S, Chou D, *et al.* Born Too Soon: The global epidemiology of 15  
279 million preterm births. *Reprod Health* 2013;**10**:S2. doi:10.1186/1742-4755-10-S1-S2
- 280 4 Ananth C V, Vintzileos AM. Epidemiology of preterm birth and its clinical subtypes. *J*  
281 *Matern Fetal Neonatal Med* 2006;**19**:773–82. doi:10.1080/14767050600965882
- 282 5 Behrman R, Butler AS, editors. *Institute of Medicine (IOM). Preterm Birth: Causes,*  
283 *Consequences, and Prevention.* Washington, D.C.: National Academies Press 2007.  
284 doi:10.17226/11622
- 285 6 Manuck TA, Esplin MS, Biggio J, *et al.* The phenotype of spontaneous preterm birth:  
286 application of a clinical phenotyping tool. *Am J Obstet Gynecol* 2015;**212**(4):487.e1-  
287 487.e11. doi:10.1016/j.ajog.2015.02.010
- 288 7 Honest H, Hyde CJ, Khan KS. Prediction of spontaneous preterm birth: no good test  
289 for predicting a spontaneous preterm birth. *Curr Opin Obstet Gynecol* 2012;**24**:422–  
290 33. doi:10.1097/GCO.0b013e328359823a
- 291 8 Conde-Agudelo A, Papageorghiou A, Kennedy S, *et al.* Novel biomarkers for the  
292 prediction of the spontaneous preterm birth phenotype: a systematic review and  
293 meta-analysis. *BJOG* 2011;**118**:1042–54. doi:10.1111/j.1471-0528.2011.02923.x
- 294 9 Hee L. Likelihood ratios for the prediction of preterm delivery with biomarkers. *Acta*  
295 *Obstet Gynecol Scand* 2011;**90**:1189–99. doi:10.1111/j.1600-0412.2011.01187.x
- 296 10 Goldenberg RL, Iams JD, Mercer BM, *et al.* The Preterm Prediction Study: toward a  
297 multiple-marker test for spontaneous preterm birth. *Am J Obstet Gynecol*  
298 2001;**185**:643–51. doi:10.1067/mob.2001.116752
- 299 11 Iams JD, Goldenberg RL, Meis PJ, *et al.* The length of the cervix and the risk of  
300 spontaneous premature delivery. National Institute of Child Health and Human  
301 Development Maternal Fetal Medicine Unit Network. *N Engl J Med* 1996;**334**:567–72.  
302 doi:10.1056/NEJM199602293340904
- 303 12 Smith V, Devane D, Begley CM, *et al.* A systematic review and quality assessment of  
304 systematic reviews of fetal fibronectin and transvaginal length for predicting preterm  
305 birth. *Eur J Obstet Gynecol Reprod Biol* 2007;**133**:134–42.  
306 doi:10.1016/j.ejogrb.2007.03.005
- 307 13 Abbott DS, Hezelgrave NL, Seed PT, *et al.* Quantitative fetal fibronectin to predict  
308 preterm birth in asymptomatic women at high risk. *Obstet Gynecol* 2015;**125**:1168–

- 1  
2  
3 309 76. doi:10.1097/AOG.0000000000000754  
4  
5 310 14 Di Renzo GC. The great obstetrical syndromes. *J Matern neonatal Med* 2009;**22**:633–  
6 311 5. doi:10.1080/14767050902866804  
7  
8  
9 312 15 Brosens I, Pijnenborg R, Vercruyssen L, *et al*. The ‘Great Obstetrical Syndromes’ are  
10 313 associated with disorders of deep placentation. *Am J Obstet Gynecol* 2011;**204**:193–  
11 314 201. doi:10.1016/j.ajog.2010.08.009  
12  
13 315 16 Practice bulletin no. 130: prediction and prevention of preterm birth. *Obstet Gynecol*  
14 316 2012;**120**:964–73. doi:10.1097/AOG.0b013e3182723b1b  
15  
16  
17 317 17 Goldenberg RL, Culhane JF, Iams JD, *et al*. Epidemiology and causes of preterm birth.  
18 318 *Lancet* 2008;**371**:75–84. doi:10.1016/S0140-6736(08)60074-4  
19  
20 319 18 Honest H, Hyde CJ, Khan KS. Prediction of spontaneous preterm birth. *Curr Opin*  
21 320 *Obstet Gynecol* 2012;**24**:422–33. doi:10.1097/GCO.0b013e328359823a  
22  
23  
24 321 19 Horgan RP, Clancy OH, Myers JE, *et al*. An overview of proteomic and metabolomic  
25 322 technologies and their application to pregnancy research. *BJOG* 2009;**116**:173–81.  
26 323 doi:10.1111/j.1471-0528.2008.01997.x  
27  
28 324 20 Wishart DS, Feunang YD, Marcu A, *et al*. HMDB 4.0: the human metabolome database  
29 325 for 2018. *Nucleic Acids Res* 2018;**46**:D608–17. doi:10.1093/nar/gkx1089  
30  
31  
32 326 21 Dettmer K, Hammock BD. Metabolomics—a new exciting field within the omics  
33 327 sciences. *Environ Health Perspect* 2004;**112**:A396–7.  
34  
35 328 22 Lucaroni F, Morciano L, Rizzo G, *et al*. Biomarkers for predicting spontaneous preterm  
36 329 birth: an umbrella systematic review. *J Matern Neonatal Med* 2018;**31**:726–34.  
37 330 doi:10.1080/14767058.2017.1297404  
38  
39  
40 331 23 Romero R, Espinoza J, Gotsch F, *et al*. The use of high-dimensional biology (genomics,  
41 332 transcriptomics, proteomics, and metabolomics) to understand the preterm  
42 333 parturition syndrome. *BJOG* 2006;**113**:118–35. doi:10.1111/j.1471-  
43 334 0528.2006.01150.x  
44  
45  
46 335 24 Shamseer L, Moher D, Clarke M, *et al*. Preferred reporting items for systematic review  
47 336 and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ*  
48 337 2015;**350**:g7647. doi:10.1136/BMJ.G7647  
49  
50  
51 338 25 Zhang A, Sun H, Wang P, *et al*. Modern analytical techniques in metabolomics  
52 339 analysis. *Analyst* 2012;**137**:293–300. doi:10.1039/c1an15605e  
53  
54 340 26 Whiting PF, Rutjes AWS, Westwood ME, *et al*. QUADAS-2: A Revised Tool for the  
55 341 Quality Assessment of Diagnostic Accuracy Studies. *Ann Intern Med* 2011;**155**:529.  
56 342 doi:10.7326/0003-4819-155-8-201110180-00009  
57  
58  
59 343 27 Rosselli D. The language of biomedical sciences. *Lancet* 2016;**387**:1720–1.  
60 344 doi:10.1016/S0140-6736(16)30259-8

1  
2  
3 3454  
5 346 **Author's Contributions**

6  
7  
8 347 RTS and RFBG will conduct the systematic review as independent first reviewers. JGC, RPJ  
9  
10 348 and DFBL will decide about conflicting decisions regarding papers selections. PNB, RPJ and  
11  
12 349 JGC participated in the systematic review conception, methodology and framework,  
13  
14  
15 350 together will all the others co-authors.

16  
17  
18 351 **Funding**

19  
20  
21 352 This research was supported by Brazilian National Research Council (grant number  
22  
23 353 401636/2013-5) and Bill and Melinda Gates Foundation (grant number OPP1107597- Grand  
24  
25 354 Challenges Brazil: Reducing the burden of preterm birth, FIOTEC number 05/2013), which  
26  
27 355 provided funding to PRETERM-SAMBA project ([www.medscinet.com/samba](http://www.medscinet.com/samba)). RTS and DFL  
28  
29 356 have been awarded PhD scholarships from the CAPES Foundation, an agency under the  
30  
31 357 Ministry of Education of Brazil, process 88881.134095/2016-01 and 8881.134512/2016-01  
32  
33 358 respectively. The sponsors played no role on the study design or manuscript writing.

34  
35  
36 359 **Competing interests**

37  
38  
39 360 All authors are carrying original research about metabolomics and presenting conferences  
40  
41 361 about this topic, including spontaneous preterm birth, preeclampsia, gestational diabetes  
42  
43 362 mellitus and fetal growth restriction. Philip N Baker is principal investigator of Metabolomics  
44  
45 363 Diagnostics Ltd, a company dedicated to develop innovative screening tests using  
46  
47 364 metabolomics technology.

48  
49  
50 365 **Acknowledgements**

1  
2  
3 366 Ana Paula de Morais e Oliveira, librarian of University of Campinas – Unicamp, Brazil, for  
4  
5  
6 367 collaborating in developing search strategy and Rachel Hanisch for her suggestions to some  
7  
8 368 sections of the paper.  
9

10  
11 369 **Ethics approval and consent to participate**  
12

13  
14 370 This systematic review does not require ethical approval from the Research Council or Ethics  
15  
16 371 board.  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

1  
2  
3 Search strategy: #1 AND #2 AND #3  
4  
5

6 preterm birth  
7 premature birth  
8 premature infant  
9 premature labor  
10 extremely premature infant  
11 premature obstetric labor  
12 spontaneous preterm birth  
13 extreme preterm birth  
14 late preterm birth  
15 1 (OR for each  
16 term)  
17 moderate preterm birth  
18 preterm premature rupture of membranes  
19 preterm delivery  
20 PROM  
21 sPTB  
22 preterm PROM  
23 pPROM  
24 p-PROM  
25  
26  
27  
28  
29

30  
31 metabolomic\*  
32 metabonomic\*  
33 metabolit\*  
34 lipidomic\*  
35 H NMR  
36 proton NMR  
37 proton nuclear magnetic resonance  
38 2 (OR for each  
39 term)  
40 liquid chromatogra\*  
41 UPLC  
42 ultra-performance liquid chromatograph\*  
43 ultra performance liquid chromatograph\*  
44 HPLC  
45 high performance liquid chromatograph\*  
46 high-performance liquid chromatograph\*  
47  
48  
49  
50

51  
52  
53 3 (OR for each  
54 term)  
55 pregnan\*  
56 antenat\*  
57 ante nat\*  
58 prenat\*  
59 pre nat\*  
60

## PRISMA-P 2015 Checklist

This checklist has been adapted for use with systematic review protocol submissions to BioMed Central journals from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 4:1

An Editorial from the Editors-in-Chief of *Systematic Reviews* details why this checklist was adapted - Moher D, Stewart L & Shekelle P: Implementing PRISMA-P: recommendations for prospective authors. *Systematic Reviews* 2016 5:15

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
<b>ADMINISTRATIVE INFORMATION</b>					
<b>Title</b>					
Identification	1a	Identify the report as a protocol of a systematic review	x	<input type="checkbox"/>	2
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	<input type="checkbox"/>	<input type="checkbox"/>	n/a
<b>Registration</b>	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	x	<input type="checkbox"/>	48-49
<b>Authors</b>					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	x	<input type="checkbox"/>	4-23
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	x	<input type="checkbox"/>	283-287
<b>Amendments</b>	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	<input type="checkbox"/>	<input type="checkbox"/>	n/a
<b>Support</b>					
Sources	5a	Indicate sources of financial or other support for the review	x	<input type="checkbox"/>	275-282
Sponsor	5b	Provide name for the review funder and/or sponsor	x	<input type="checkbox"/>	275-282
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	x	<input type="checkbox"/>	282
<b>INTRODUCTION</b>					
<b>Rationale</b>	6	Describe the rationale for the review in the context of what is already known	x	<input type="checkbox"/>	51-87
<b>Objectives</b>	7	Provide an explicit statement of the question(s) the review will address with reference to	x	<input type="checkbox"/>	94-95



Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
		participants, interventions, comparators, and outcomes (PICO)			
<b>METHODS</b>					
<b>Eligibility criteria</b>	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	x	<input type="checkbox"/>	96-107
<b>Information sources</b>	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	x	<input type="checkbox"/>	119-125
<b>Search strategy</b>	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	x	<input type="checkbox"/>	127-136
<b>STUDY RECORDS</b>					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	x	<input type="checkbox"/>	142-155
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	x	<input type="checkbox"/>	157-161
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	x	<input type="checkbox"/>	163-167
<b>Data items</b>	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	x	<input type="checkbox"/>	142-155
<b>Outcomes and prioritization</b>	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	x	<input type="checkbox"/>	169-179
<b>Risk of bias in individual studies</b>	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	x	<input type="checkbox"/>	189-196
<b>DATA</b>					
<b>Synthesis</b>	15a	Describe criteria under which study data will be quantitatively synthesized	x	<input type="checkbox"/>	198-209
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., $I^2$ , Kendall's tau)	x	<input type="checkbox"/>	185-187
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	x	<input type="checkbox"/>	185-187

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	x	<input type="checkbox"/>	198-202
<b>Meta-bias(es)</b>	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	<input type="checkbox"/>	<input type="checkbox"/>	n/a
<b>Confidence in cumulative evidence</b>	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	<input type="checkbox"/>	<input type="checkbox"/>	n/a

For peer review only

# BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-026033.R2
Article Type:	Protocol
Date Submitted by the Author:	20-Dec-2018
Complete List of Authors:	Souza, Renato; Universidade Estadual de Campinas, Obstetrics and Gynecology Galvão, Rafael; Universidade Estadual de Campinas, Obstetrics and Gynecology Leite, Debora; Campinas' State University, Department of Tocogynecology; Universidade Federal de Pernambuco, Passini Jr, Renato; Universidade Estadual de Campinas Faculdade de Ciencias Medicas Baker, Philip ; University of Leicester, College of Medicine Cecatti, Jose; University of Campinas, Obstetrics and Gynecology
<b>Primary Subject Heading</b>:	Obstetrics and gynaecology
Secondary Subject Heading:	Diagnostics
Keywords:	preterm birth, metabolomics, biomarkers, prediction, metabolome

SCHOLARONE™  
Manuscripts

1  
2  
3 1 **The use of metabolomics for predicting spontaneous preterm birth in asymptomatic**  
4  
5  
6 2 **pregnant women: protocol for a systematic review and meta-analysis**  
7  
8 3

9  
10 4 Renato T. Souza <sup>1</sup> ([renatotsouzasp@gmail.com](mailto:renatotsouzasp@gmail.com))

11 5 Rafael Bessa Freitas Galvão <sup>1</sup> ([rafaelbfg@gmail.com](mailto:rafaelbfg@gmail.com))

12 6 Débora Farias Batista Leite <sup>1,2</sup> ([deborafariasleite@gmail.com](mailto:deborafariasleite@gmail.com))

13 7 Renato Passini Jr <sup>1</sup> ([passini@caism.unicamp.br](mailto:passini@caism.unicamp.br))

14 8 Philip N. Baker <sup>3</sup> ([philip.baker@leicester.ac.uk](mailto:philip.baker@leicester.ac.uk))

15 9 José Guilherme Cecatti <sup>1</sup> ([cecatti@unicamp.br](mailto:cecatti@unicamp.br))

16 10

17 11 <sup>1</sup> Department of Obstetrics and Gynaecology, University of Campinas, Campinas, São Paulo,  
18 12 Brazil.

19 13 <sup>2</sup> Clinics Hospital of Federal University of Pernambuco, Recife, Pernambuco, Brazil.

20 14 <sup>3</sup> College of Life Sciences, University of Leicester, England, United Kingdom.

21 15

22 16 This systematic review protocol is registered at PROSPERO platform (CRD42018100172). Available  
23 17 from: [http://www.crd.york.ac.uk/PROSPERO/display\\_record.php?ID=CRD42018100172](http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42018100172).

24 18

25 19 Corresponding author:

26 20 José Guilherme Cecatti

27 21 101 Alexander Fleming, Cidade Universitária, campinas, São Paulo, Brazil.

28 22 ZIPCODE 13083-881

29 23 [cecatti@unicamp.br](mailto:cecatti@unicamp.br)

30 24 Word count: 2,233.

1  
2  
3 25 **ABSTRACT**  
4

5 26 **Introduction:** Preterm birth (PTB) is the leading cause of neonatal mortality and short- and  
6  
7 long-term morbidity. The aetiology and pathophysiology of spontaneous PTB are still  
8  
9 unclear, which makes the identification of reliable and accurate predictor markers more  
10  
11 difficult, particularly for unscreened or asymptomatic women. Metabolomics biomarkers  
12  
13 have been demonstrated to be potentially accurate biomarkers for many disorders with  
14  
15 complex mechanisms such as PTB. Therefore, we aim to perform a systematic review of  
16  
17 metabolomics markers associated with spontaneous PTB. Our research question is “What is  
18  
19 the performance of metabolomics for predicting spontaneous preterm birth in  
20  
21 asymptomatic pregnant women?”  
22  
23  
24  
25  
26

27 35 **Methods and analysis:** We will focus on studies assessing metabolomics techniques for  
28  
29 predicting spontaneous preterm birth in asymptomatic pregnant women. We will conduct a  
30  
31 comprehensive systematic review of the literature from the last 10 years. Only  
32  
33 observational cohort and case-control studies will be included. Our search strategy will be  
34  
35 carried out by two independent reviewers, who will scan title and abstract before carrying  
36  
37 out a full review of the article. The scientific databases to be explored include PubMed,  
38  
39 MedLine, ScieLo, EMBASE, LILACS, Web of Science, Scopus, and others.  
40  
41  
42  
43

44 42 **Ethics and dissemination:** This systematic review protocol does not require ethical approval.  
45  
46 We intend to disseminate our findings in scientific peer-reviewed journal, the Preterm  
47  
48 SAMBA study open access website, specialists’ conferences, and to our funding agencies.  
49

50 44  
51  
52 45 **Registration details:** This protocol is registered in PROSPERO platform (code  
53  
54 CRD42018100172).  
55

56  
57 47 **Keywords:** preterm birth, spontaneous preterm birth, metabolomics, biomarkers,  
58  
59 prediction, metabolome.  
60

1  
2  
3 49 **Strengths and limitations of this study**  
4

- 5 50 • This systematic review protocol takes into account some important aspects  
6  
7  
8 51 regarding conducting a systematic review about spontaneous preterm birth and  
9  
10 52 metabolomics such as the criteria used for defining spontaneous preterm birth,  
11  
12  
13 53 different population risk stratification, method used to estimate gestational age, and  
14  
15 54 metabolomics techniques details.  
16  
17  
18 55 • Two independent reviewers are responsible for searching and selecting studies, as  
19  
20 56 also extracting data, and a third reviewer will resolve any disagreement.  
21  
22  
23 57 • If possible, proper statistical methods will be applied to investigate metabolomics  
24  
25 58 accuracy in predicting spontaneous preterm birth.  
26  
27  
28 59 • Possible limitations to this review include the different criteria applied for defining  
29  
30 60 spontaneous preterm birth, and the diverse population risk stratification.  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## 62 INTRODUCTION

63 Spontaneous preterm birth (sPTB) is the leading cause of perinatal mortality and short- and  
64 long-term morbidity [1,2]. It is defined as birth that occurs before 37 weeks gestation due to  
65 spontaneous onset of labour or preterm premature rupture of membranes (pPROM) [3,4].  
66 Several pathways and mechanisms linked with preterm birth have been proposed including,  
67 neuroendocrine, vascular, immune-inflammatory, and behavioural processes [5]. More  
68 specifically, several markers associated with uterine distension/contraction, decidual  
69 inflammation/infection and activation of hypothalamic–pituitary–adrenal axis had been  
70 studied in the past decades [5,6]. However, no single marker or combination of markers has  
71 been found to be accurate enough for predicting sPTB [7–10]. History of previous preterm  
72 birth, cervical length at second trimester and cervico-vaginal fetal fibronectin are the most  
73 promising clinical tests for predicting spontaneous preterm, but they seem not to be  
74 clinically useful for asymptomatic women. Sensitivity of short cervical length (<25mm) and  
75 high cervico-vaginal fFN (>50ng/ml) are around 33-36% and 46%, respectively [11–13].  
76 Preterm birth is a complex and multifactorial syndrome that possibly has a long pre-clinical  
77 phase, maternal and fetal interactions, genetic and environmental influences, and adaptive  
78 mechanisms [14,15]. These challenging aspects, and the presence of still unknown  
79 underlying mechanisms, are the main limitations for the identification of an accurate  
80 predictor for sPTB [16–18]. None of the predictors used in clinical practice, such as previous  
81 history of preterm birth, infection (vaginal and urinary contaminants), fibronectin and  
82 transvaginal ultrasonography cervical length demonstrated exceptional accuracy for  
83 predicting spontaneous preterm birth [7]. An exploration of innovative approaches is  
84 urgently required.

1  
2  
3 85 Metabolomics is the study of metabolites, through identification and quantification of low-  
4  
5 86 weight molecular particles, i.e. tens to hundreds thousands of intermediate products and  
6  
7  
8 87 substrates of systems biology chemical reactions [19,20]. This novel approach has been  
9  
10 88 applied for identifying biomarkers and underlying biochemical pathways associated with  
11  
12  
13 89 complex obstetrical syndromes as preeclampsia, fetal growth restriction, gestational  
14  
15 90 diabetes and preterm birth. In contrast to other “*Omics Sciences*” techniques, metabolomics  
16  
17  
18 91 is more closely associated with the phenotype of the disease and might thus identify a more  
19  
20 92 robust and reliable set of predictors [21]. Importantly, implementing an adequate *Omics*  
21  
22  
23 93 experimental design is crucial for metabolomics studies. Using different baseline population  
24  
25 94 (asymptomatic vs symptomatic or low- vs high-risk women for developing sPTB), study  
26  
27 95 designs (prospective cohorts, case-control or cross sectional studies), sources of samples  
28  
29 96 (amniotic fluid, vaginal fluid, blood, urine, hair, etc) and the timing of sample collection each  
30  
31 97 have significant effects on study findings and the consequent interpretation and  
32  
33 98 contribution to the current gap of knowledge [19].  
34  
35  
36  
37 99 Different reviews collating scientific knowledge regarding preterm birth  
38  
39  
40 100 biomarkers/predictors has been conducted. Different methodology approaches has been  
41  
42 101 applied so far, including narrative, systematic and umbrella reviews, a more comprehensive  
43  
44 102 review that includes not only original studies but also other reviews [7,19,22,23]. At the best  
45  
46  
47 103 of our knowledge, there is no systematic review on metabolomics markers. Therefore, we  
48  
49 104 aim to conduct a systematic review of original studies investigating the use of metabolomics  
50  
51  
52 105 biomarkers for predicting spontaneous preterm birth in asymptomatic pregnant women.  
53  
54 106 This protocol describes the methods that will be applied in our systematic review.  
55  
56  
57

## 58 107 **METHODS AND ANALYSIS**

59  
60



1  
2  
3 108 The current systematic review proposal will be conducted, written and published following  
4  
5 109 the Preferred Reporting Items for Systematics Reviews and Meta-Analyses (PRISMA-P)  
6  
7  
8 110 recommendations [24]. Also, it is properly registered at PROSPERO platform – code  
9  
10 111 CRD42018100172.

### 112 **Review question**

113 What is the performance of metabolomics for predicting spontaneous preterm birth in  
14  
15  
16  
17  
18 114 asymptomatic pregnant women?

### 115 **Eligibility Criteria**

116 Original cohort or case-control studies involving asymptomatic pregnant women at the  
17  
18  
19  
20  
21 117 moment of sample collection (exposure) and with samples analysed using metabolomics  
22  
23  
24  
25 118 techniques. Studies will be excluded if (1) they are cross-sectional studies, clinical trials,  
26  
27  
28  
29 119 editorials, letter to editors, case reports, expert opinions, commentaries, or any type of  
30  
31  
32 120 review; (2) they describes only experimental studies with animals; or (3) they are duplicated  
33  
34  
35 121 data (e.g. data published in conferences proceedings and, then, published again in scientific  
36  
37  
38 122 journals). In this case, only the most complete publication will be considered, after  
39  
40  
41 123 comparing and confirming that the same technique and metabolites were explored. Studies  
42  
43 124 published from 2008 to 2018 will be considered, and there will be no language restriction.  
44  
45 125 Before submitting this systematic review for publication, we will rerun the search strategy to  
46  
47 126 identify new studies that have been published after performing the first round of search.

### 127 **Participants**

128 The current review is interested in evaluating the performance of metabolomics biomarkers  
129  
130 129 for spontaneous preterm birth in asymptomatic pregnant women, which may contribute to  
131  
132  
133 130 clinical practice, potentially providing information regarding onset of preterm labour.  
134  
135  
136 131 Nevertheless, we aim to identify studies addressing only early predictors collected from

1  
2  
3 132 women who are in an early preclinical stage, which might contribute to a wider window of  
4  
5 133 opportunity for interventions and also to develop a widely reproducible screening test.  
6  
7  
8 134 Asymptomatic pregnant women should not have regular uterine tightening/contractions or  
9  
10 135 signs of rupture of membranes (i.e. watery discharge). In addition, the study should  
11  
12  
13 136 preferably have a standardized definition of spontaneous preterm birth, the outcome of  
14  
15 137 interest.

### 18 138 **Information Sources**

19  
20 139 The search will be held in the following databases: PubMed, EMBASE, Scopus, CINAHL, and  
21  
22  
23 140 Web of Science, BVS/BIREME, which includes the Latin American and Caribbean Health  
24  
25 141 Sciences Literature (LILACS), Medline and the Scientific Electronic Library Online (Scielo). In  
26  
27  
28 142 addition, secondary sources of original studies will be explored such as Google Scholar,  
29  
30 143 hand-held searching of the reference list of eligible studies, conference proceedings, and  
31  
32  
33 144 contact with authors when necessary.

### 35 145 **Search Strategy**

36  
37 146 The following terms will be used in our search strategy for the different scientific databases:  
38  
39  
40 147 (preterm birth, premature birth, premature infant, premature labor, extremely premature  
41  
42 148 infant, premature obstetric labor, spontaneous preterm birth, extreme preterm birth, late  
43  
44  
45 149 preterm birth, moderate preterm birth, preterm premature rupture of membranes, preterm  
46  
47 150 delivery, PROM, sPTB, preterm PROM, pPROM, p-PROM) AND (metabolomic\*,  
48  
49 151 metabonomic\*, metabolit\*, lipidomic\*, H NMR, proton NMR, proton nuclear magnetic  
50  
51  
52 152 resonance, liquid chromatogra\*, gas chromatogra\*, UPLC, ultra-performance liquid  
53  
54 153 chromatograph\*, ultra-performance liquid chromatograph\*, HPLC, high performance liquid  
55  
56  
57 154 chromatograph\*, high-performance liquid chromatograph\*) AND (pregnan\*, antenat\*,  
58  
59 155 ante nat\*, prenat\*, pre nat\*) (Supplementary Material). Respective adaptations in the  
60

1  
2  
3 156 syntax of search for each database will be applied accordingly. No filters - such as “research  
4  
5  
6 157 in animal’s models” and “reviews” - will be used in our search strategy, as it will be excluded  
7  
8 158 according to eligibility criteria. The complete search strategy, including Boolean terms, is  
9  
10  
11 159 provided as Supplementary Material.

## 12 13 160 **Data Management**

14  
15 161 We will export search results to a reference manager (Mendeley®). Then, the following  
16  
17 162 information will be collected from each study using an appropriate form, which will be  
18  
19  
20 163 entered in an Excel® spreadsheet: author’s name, year of publication, country, study design,  
21  
22 164 number of participants with and without spontaneous preterm birth, type of metabolomics  
23  
24 165 analysis technique (liquid or gas chromatography, nuclear resonance), laboratory methods  
25  
26 166 for metabolites data acquiring (targeted or untargeted techniques, etc), subtype of preterm  
27  
28 167 birth (spontaneous preterm labour or pPROM), number of fetuses (singleton vs multiple),  
29  
30 168 gestational age when samples were collected, source of samples (type/site of tissue), low or  
31  
32 169 high-risk for preterm birth (authors criteria used to define the population will be collected)  
33  
34  
35 170 and method applied to estimate gestational age. If possible, additional variables related to  
36  
37 171 spontaneous preterm birth categories (delivery before 28 weeks and before 34 weeks) will  
38  
39 172 be recorded for secondary analyses. Original authors will be contacted to clarify data, when  
40  
41  
42 173 needed. Finally, we will check the biochemical class of identified metabolites in Human  
43  
44 174 Metabolome Database (HMDB, version 4.0) to explore and synthesize whether there are  
45  
46 175 common biological pathways associated with spontaneous preterm birth [20].  
47  
48  
49  
50

## 51 176 **Selection Process**

52  
53 177 Two independent reviewers (RTS and RBF) will be responsible for screening and selecting  
54  
55 178 studies initially according to title or abstract. Both researchers will read the full text of non-  
56  
57 179 excluded studies to discriminate eligibility. A third reviewer (DFBL) will consider any  
58  
59  
60

1  
2  
3 180 disagreement; additional reviewers (RPJ, PNB and JGC) will be responsible for supervising all  
4  
5  
6 181 steps and approving data extraction.

### 8 182 **Data Collection Process**

9  
10 183 We will extract search results to a reference manager where all studies will be stored. Then,  
11  
12  
13 184 included studies will be placed in a new folder. Finally, we will manually extract data of  
14  
15 185 interest from these included studies to an Excel® file. Each reviewer will have their own  
16  
17  
18 186 reference manager account, file and folder and discrepant results will be discussed together  
19  
20 187 with the third reviewer.

### 22 188 **Outcomes and Prioritization**

23  
24  
25 189 The primary outcome is spontaneous preterm birth, defined as any birth occurred before 37  
26  
27 190 weeks of gestation due to spontaneous onset of labor or preterm premature rupture of  
28  
29  
30 191 membranes. Secondary outcomes are:

- 31  
32 192 1. Spontaneous preterm birth before 28 weeks;
- 33  
34 193 2. Spontaneous preterm birth before 32 weeks;
- 35  
36 194 3. Spontaneous preterm birth before 34 weeks;

37  
38  
39 195 The capacity to predict different degrees of sPTB (categories of gestational age) is important  
40  
41 196 as the extreme (<28wks), moderate (<32 weeks) and non-late preterm (<34wks) newborns  
42  
43 197 have different adverse outcomes compared to non-extreme (≥28wks); non-moderate  
44  
45 198 (≥32wks) or late (≥34 wks) preterm newborns.

46  
47  
48 199 Ideally, the method of gestational age estimation should be clearly reported. For instance, it  
49  
50 200 can be reported as estimated by last menstrual period (LMP) and confirmed by an early  
51  
52 201 ultrasound or only by an early ultrasound when LMP is unknown/uncertain.

### 54 202 **Index test**

1  
2  
3 203 Metabolomics techniques to predict spontaneous preterm birth is the diagnostic test of  
4  
5 204 interest. Metabolomics is a technique to identify and quantify metabolites from biological  
6  
7  
8 205 samples using different type of platforms/equipment. The most common platforms include  
9  
10 206 gas, liquid chromatography or ultra-performance liquid chromatography coupled to a mass  
11  
12  
13 207 spectrometer or a proton nuclear magnetic resonance [25]. The performance of the  
14  
15 208 different thresholds of each metabolite will be compared and summarized through  
16  
17  
18 209 hierarchical summary receiver operator characteristic curve (HSROC) (meta-analysis)  
19  
20 210 according to the subgroups described above.

### 211 **Risk of Bias in individual Studies**

212 We will apply the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool [26]  
213 to assess the risk of bias and applicability of primary diagnostic accuracy studies. Each study  
214 will be classified as “low”, “high” or “unclear” regarding risk of bias for each of the four  
215 domains of QUADAS tool: Patient Selection, Index Test (metabolomics), Reference Standard  
216 (occurrence of preterm birth), and Flow and Timing of participant’s inclusion and follow-up.  
217 For example, studies will be labelled as “low” risk of bias for Reference Standard when  
218 definition of spontaneous preterm birth and gestational age estimation are clear; “high” risk  
219 of bias would be considered when the moment of sample collection is not well described.

### 220 **Data Synthesis**

221 We will report details of identification, screening, eligibility and included studies using a flow  
222 diagram, according to PRISMA recommendations [24]. Data from included studies will be  
223 synthesized into tables according to the variables of interest. If possible, we will present  
224 data meta-analysis according to study design, metabolomics technique and type of samples  
225 analysed. We intend to perform subgroup analysis according to:

- 226 • Different metabolomics methods applied: gas or liquid chromatography coupled

227 with mass spectrometry or proton nuclear magnetic resonance;

228 ● Singleton and multiple pregnancies;

229 ● Low-risk and high-risk women for developing preterm birth;

230 ● Subtype of preterm birth: Spontaneous preterm birth exclusively due to  
231 spontaneous onset of labour with intact membranes or sPTB due to premature  
232 rupture of membranes.

233 ● Gestational age interval when samples were collected: 1<sup>st</sup> trimester, 2<sup>nd</sup> trimester  
234 and 3<sup>rd</sup> trimester.

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

### 238 **Potential anticipated limitations to this review**

239 Firstly, although we have not considered any language restriction, we consider that there  
240 might be a limitation in studies published entirely in non-English language. However, in the  
241 last decade, more than 95% of scientific biomedical literature has been published in English  
242 [27], then we consider this a minor selection bias. Secondly, we intend to stratify the groups  
243 according to population risk. However, the characterization of low- or high-risk for  
244 spontaneous preterm birth is controversial and lacks standardization, which might limit data  
245 comparison and subgroup analysis. Finally, categorization of sPTB into spontaneous onset of  
246 labour or pPROM is another topic of potential limitation - the recognition of the main initial  
247 mechanism for preterm delivery might not always be possible. Even when specified, it might  
248 provoke uncertainty and could limit further considerations regarding preterm phenotypes.  
249 In addition, another limitation is that individual patient data will not be collected.

### 250 **Patient and Public Involvement**

1  
2  
3 251 Patients will not be directly involved in the study and no experience or direct impact from  
4  
5  
6 252 their perspective can be discussed.  
7

8 253

9  
10 254 **ETHICS AND DISSEMINATION**

11  
12  
13 255 This systematic review does not require ethical approval from the Research Council or Ethics  
14  
15 256 board. We intend to disseminate our findings in scientific peer-reviewed journal, general  
16  
17  
18 257 free access website of Preterm Screening and Metabolomics in Brazil and Auckland (Preterm  
19  
20 258 SAMBA) study, specialists' conferences, and to our funding agencies.  
21  
22

23  
24 259 **DISCUSSION**

25  
26 260 This systematic review will comprise current knowledge related with metabolomics in the  
27  
28 261 context of preterm birth prediction. Metabolomics science, a resourceful innovative field  
29  
30 262 that allows better understanding on pathophysiology of complex syndromes, may address  
31  
32  
33 263 the main compounds associated with the spontaneous preterm delivery and, therefore,  
34  
35 264 motivate further researchers to validate early measurable predictors of preterm birth.

36  
37  
38 265 Metabolomics performance for predicting sPTB remains unclear and standardized and high-  
39  
40 266 quality studies are needed to clarify the clinical application of metabolites for predicting  
41  
42  
43 267 sPTB. Nevertheless, metabolomics discovery studies commonly requires further validation  
44  
45  
46 268 studies; reproducible methodology is crucial. This systematic review protocol will collate the  
47  
48 269 main potential early biomarkers, subgroup analysis and standardized definition for  
49  
50 270 spontaneous preterm birth to better understand metabolomics performance in predicting  
51  
52  
53 271 sPTB and also to show its heterogeneity in terms of methodology (samples used,  
54  
55 272 metabolomics technique, definition of SPTB phenotype, etc). High performing predictors of  
56  
57  
58 273 preterm birth will help combat this leading cause of neonatal mortality and morbidity.  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

274

For peer review only



276 **References**

- 277 1 Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: when? Where? Why? *Lancet*  
278 2005;**365**:891–900. doi:10.1016/S0140-6736(05)71048-5
- 279 2 Howson CP, Kinney M V, McDougall L, *et al*. Born Too Soon: Preterm birth matters.  
280 *Reprod Health* 2013;**10 Suppl 1**:S1. doi:10.1186/1742-4755-10-S1-S1
- 281 3 Blencowe H, Cousens S, Chou D, *et al*. Born Too Soon: The global epidemiology of 15  
282 million preterm births. *Reprod Health* 2013;**10**:S2. doi:10.1186/1742-4755-10-S1-S2
- 283 4 Ananth C V, Vintzileos AM. Epidemiology of preterm birth and its clinical subtypes. *J*  
284 *Matern Fetal Neonatal Med* 2006;**19**:773–82. doi:10.1080/14767050600965882
- 285 5 Behrman R, Butler AS, editors. *Institute of Medicine (IOM). Preterm Birth: Causes,*  
286 *Consequences, and Prevention*. Washington, D.C.: National Academies Press 2007.  
287 doi:10.17226/11622
- 288 6 Manuck TA, Esplin MS, Biggio J, *et al*. The phenotype of spontaneous preterm birth:  
289 application of a clinical phenotyping tool. *Am J Obstet Gynecol* 2015;**212**(4):487.e1-  
290 487.e11. doi:10.1016/j.ajog.2015.02.010
- 291 7 Honest H, Hyde CJ, Khan KS. Prediction of spontaneous preterm birth: no good test  
292 for predicting a spontaneous preterm birth. *Curr Opin Obstet Gynecol* 2012;**24**:422–  
293 33. doi:10.1097/GCO.0b013e328359823a
- 294 8 Conde-Agudelo A, Papageorghiou A, Kennedy S, *et al*. Novel biomarkers for the  
295 prediction of the spontaneous preterm birth phenotype: a systematic review and  
296 meta-analysis. *BJOG* 2011;**118**:1042–54. doi:10.1111/j.1471-0528.2011.02923.x
- 297 9 Hee L. Likelihood ratios for the prediction of preterm delivery with biomarkers. *Acta*  
298 *Obstet Gynecol Scand* 2011;**90**:1189–99. doi:10.1111/j.1600-0412.2011.01187.x
- 299 10 Goldenberg RL, Iams JD, Mercer BM, *et al*. The Preterm Prediction Study: toward a  
300 multiple-marker test for spontaneous preterm birth. *Am J Obstet Gynecol*  
301 2001;**185**:643–51. doi:10.1067/mob.2001.116752
- 302 11 Iams JD, Goldenberg RL, Meis PJ, *et al*. The length of the cervix and the risk of  
303 spontaneous premature delivery. National Institute of Child Health and Human  
304 Development Maternal Fetal Medicine Unit Network. *N Engl J Med* 1996;**334**:567–72.  
305 doi:10.1056/NEJM199602293340904
- 306 12 Smith V, Devane D, Begley CM, *et al*. A systematic review and quality assessment of  
307 systematic reviews of fetal fibronectin and transvaginal length for predicting preterm  
308 birth. *Eur J Obstet Gynecol Reprod Biol* 2007;**133**:134–42.  
309 doi:10.1016/j.ejogrb.2007.03.005
- 310 13 Abbott DS, Hezelgrave NL, Seed PT, *et al*. Quantitative fetal fibronectin to predict  
311 preterm birth in asymptomatic women at high risk. *Obstet Gynecol* 2015;**125**:1168–

- 1  
2  
3 312 76. doi:10.1097/AOG.0000000000000754  
4  
5 313 14 Di Renzo GC. The great obstetrical syndromes. *J Matern neonatal Med* 2009;**22**:633–  
6 314 5. doi:10.1080/14767050902866804  
7  
8  
9 315 15 Brosens I, Pijnenborg R, Vercruyse L, *et al.* The ‘Great Obstetrical Syndromes’ are  
10 316 associated with disorders of deep placentation. *Am J Obstet Gynecol* 2011;**204**:193–  
11 317 201. doi:10.1016/j.ajog.2010.08.009  
12  
13 318 16 Practice bulletin no. 130: prediction and prevention of preterm birth. *Obstet Gynecol*  
14 319 2012;**120**:964–73. doi:10.1097/AOG.0b013e3182723b1b  
15  
16  
17 320 17 Goldenberg RL, Culhane JF, Iams JD, *et al.* Epidemiology and causes of preterm birth.  
18 321 *Lancet* 2008;**371**:75–84. doi:10.1016/S0140-6736(08)60074-4  
19  
20 322 18 Honest H, Hyde CJ, Khan KS. Prediction of spontaneous preterm birth. *Curr Opin*  
21 323 *Obstet Gynecol* 2012;**24**:422–33. doi:10.1097/GCO.0b013e328359823a  
22  
23  
24 324 19 Horgan RP, Clancy OH, Myers JE, *et al.* An overview of proteomic and metabolomic  
25 325 technologies and their application to pregnancy research. *BJOG* 2009;**116**:173–81.  
26 326 doi:10.1111/j.1471-0528.2008.01997.x  
27  
28 327 20 Wishart DS, Feunang YD, Marcu A, *et al.* HMDB 4.0: the human metabolome database  
29 328 for 2018. *Nucleic Acids Res* 2018;**46**:D608–17. doi:10.1093/nar/gkx1089  
30  
31  
32 329 21 Dettmer K, Hammock BD. Metabolomics—a new exciting field within the omics  
33 330 sciences. *Environ Health Perspect* 2004;**112**:A396–7.  
34  
35 331 22 Lucaroni F, Morciano L, Rizzo G, *et al.* Biomarkers for predicting spontaneous preterm  
36 332 birth: an umbrella systematic review. *J Matern Neonatal Med* 2018;**31**:726–34.  
37 333 doi:10.1080/14767058.2017.1297404  
38  
39  
40 334 23 Romero R, Espinoza J, Gotsch F, *et al.* The use of high-dimensional biology (genomics,  
41 335 transcriptomics, proteomics, and metabolomics) to understand the preterm  
42 336 parturition syndrome. *BJOG* 2006;**113**:118–35. doi:10.1111/j.1471-  
43 337 0528.2006.01150.x  
44  
45  
46 338 24 Shamseer L, Moher D, Clarke M, *et al.* Preferred reporting items for systematic review  
47 339 and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ*  
48 340 2015;**350**:g7647. doi:10.1136/BMJ.G7647  
49  
50  
51 341 25 Zhang A, Sun H, Wang P, *et al.* Modern analytical techniques in metabolomics  
52 342 analysis. *Analyst* 2012;**137**:293–300. doi:10.1039/c1an15605e  
53  
54 343 26 Whiting PF, Rutjes AWS, Westwood ME, *et al.* QUADAS-2: A Revised Tool for the  
55 344 Quality Assessment of Diagnostic Accuracy Studies. *Ann Intern Med* 2011;**155**:529.  
56 345 doi:10.7326/0003-4819-155-8-201110180-00009  
57  
58  
59 346 27 Rosselli D. The language of biomedical sciences. *Lancet* 2016;**387**:1720–1.  
60 347 doi:10.1016/S0140-6736(16)30259-8

1  
2  
3 3484  
5 349 **Author's Contributions**

6  
7  
8 350 RTS and RFBG will conduct the systematic review as independent first reviewers. JGC, RPJ  
9  
10 351 and DFBL will decide about conflicting decisions regarding papers selections. PNB, RPJ and  
11  
12 352 JGC participated in the systematic review conception, methodology and framework,  
13  
14  
15 353 together will all the others co-authors.  
16

17  
18 354 **Funding**

19  
20  
21 355 This research was supported by Brazilian National Research Council (grant number  
22  
23 356 401636/2013-5) and Bill and Melinda Gates Foundation (grant number OPP1107597- Grand  
24  
25 357 Challenges Brazil: Reducing the burden of preterm birth, FIOTEC number 05/2013), which  
26  
27 358 provided funding to PRETERM-SAMBA project ([www.medscinet.com/samba](http://www.medscinet.com/samba)). RTS and DFL  
28  
29 359 have been awarded PhD scholarships from the CAPES Foundation, an agency under the  
30  
31 360 Ministry of Education of Brazil, process 88881.134095/2016-01 and 8881.134512/2016-01  
32  
33 361 respectively. The sponsors played no role on the study design or manuscript writing.  
34  
35  
36  
37  
38

39 362 **Competing interests**

40  
41 363 All authors are carrying original research about metabolomics and presenting conferences  
42  
43 364 about this topic, including spontaneous preterm birth, preeclampsia, gestational diabetes  
44  
45 365 mellitus and fetal growth restriction. Philip N Baker is principal investigator of Metabolomics  
46  
47 366 Diagnostics Ltd, a company dedicated to develop innovative screening tests using  
48  
49 367 metabolomics technology.  
50  
51  
52  
53

54 368 **Acknowledgements**  
55  
56  
57  
58  
59  
60

1  
2  
3 369 Ana Paula de Morais e Oliveira, librarian of University of Campinas – Unicamp, Brazil, for  
4  
5  
6 370 collaborating in developing search strategy and Rachel Hanisch for her suggestions to some  
7  
8 371 sections of the paper.  
9

10  
11 372 **Ethics approval and consent to participate**  
12

13  
14 373 This systematic review does not require ethical approval from the Research Council or Ethics  
15  
16 374 board.  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

1  
2  
3 Search strategy: #1 AND #2 AND #3  
4  
5

6 preterm birth  
7 premature birth  
8 premature infant  
9 premature labor  
10 extremely premature infant  
11 premature obstetric labor  
12 spontaneous preterm birth  
13 extreme preterm birth  
14 late preterm birth  
15 1 (OR for each  
16 term)  
17 moderate preterm birth  
18 preterm premature rupture of membranes  
19 preterm delivery  
20 PROM  
21 sPTB  
22 preterm PROM  
23 pPROM  
24 p-PROM  
25  
26  
27  
28  
29  
30  
31 metabolomic\*  
32 metabonomic\*  
33 metabolit\*  
34 lipidomic\*  
35 H NMR  
36 proton NMR  
37 proton nuclear magnetic resonance  
38 2 (OR for each  
39 term)  
40 liquid chromatogra\*  
41 UPLC  
42 ultra-performance liquid chromatograph\*  
43 ultra performance liquid chromatograph\*  
44 HPLC  
45 high performance liquid chromatograph\*  
46 high-performance liquid chromatograph\*  
47  
48  
49  
50  
51  
52 pregnan\*  
53 antenat\*  
54 3 (OR for each  
55 term)  
56 ante nat\*  
57 prenat\*  
58 pre nat\*  
59  
60

## PRISMA-P 2015 Checklist

This checklist has been adapted for use with systematic review protocol submissions to BioMed Central journals from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 4:1

An Editorial from the Editors-in-Chief of *Systematic Reviews* details why this checklist was adapted - Moher D, Stewart L & Shekelle P: Implementing PRISMA-P: recommendations for prospective authors. *Systematic Reviews* 2016 5:15

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
<b>ADMINISTRATIVE INFORMATION</b>					
<b>Title</b>					
Identification	1a	Identify the report as a protocol of a systematic review	x	<input type="checkbox"/>	2
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	<input type="checkbox"/>	<input type="checkbox"/>	n/a
<b>Registration</b>	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	x	<input type="checkbox"/>	48-49
<b>Authors</b>					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	x	<input type="checkbox"/>	4-23
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	x	<input type="checkbox"/>	283-287
<b>Amendments</b>	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	<input type="checkbox"/>	<input type="checkbox"/>	n/a
<b>Support</b>					
Sources	5a	Indicate sources of financial or other support for the review	x	<input type="checkbox"/>	275-282
Sponsor	5b	Provide name for the review funder and/or sponsor	x	<input type="checkbox"/>	275-282
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	x	<input type="checkbox"/>	282
<b>INTRODUCTION</b>					
<b>Rationale</b>	6	Describe the rationale for the review in the context of what is already known	x	<input type="checkbox"/>	51-87
<b>Objectives</b>	7	Provide an explicit statement of the question(s) the review will address with reference to	x	<input type="checkbox"/>	94-95

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
		participants, interventions, comparators, and outcomes (PICO)			
<b>METHODS</b>					
<b>Eligibility criteria</b>	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	x	<input type="checkbox"/>	96-107
<b>Information sources</b>	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	x	<input type="checkbox"/>	119-125
<b>Search strategy</b>	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	x	<input type="checkbox"/>	127-136
<b>STUDY RECORDS</b>					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	x	<input type="checkbox"/>	142-155
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	x	<input type="checkbox"/>	157-161
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	x	<input type="checkbox"/>	163-167
<b>Data items</b>	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	x	<input type="checkbox"/>	142-155
<b>Outcomes and prioritization</b>	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	x	<input type="checkbox"/>	169-179
<b>Risk of bias in individual studies</b>	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	x	<input type="checkbox"/>	189-196
<b>DATA</b>					
<b>Synthesis</b>	15a	Describe criteria under which study data will be quantitatively synthesized	x	<input type="checkbox"/>	198-209
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., $I^2$ , Kendall's tau)	x	<input type="checkbox"/>	185-187
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	x	<input type="checkbox"/>	185-187

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	x	<input type="checkbox"/>	198-202
<b>Meta-bias(es)</b>	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	<input type="checkbox"/>	<input type="checkbox"/>	n/a
<b>Confidence in cumulative evidence</b>	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	<input type="checkbox"/>	<input type="checkbox"/>	n/a

For peer review only



# BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-026033.R3
Article Type:	Protocol
Date Submitted by the Author:	09-Jan-2019
Complete List of Authors:	Souza, Renato; Universidade Estadual de Campinas, Obstetrics and Gynecology Galvão, Rafael; Universidade Estadual de Campinas, Obstetrics and Gynecology Leite, Debora; Campinas' State University, Department of Tocogynecology; Universidade Federal de Pernambuco, Passini Jr, Renato; Universidade Estadual de Campinas Faculdade de Ciencias Medicas Baker, Philip ; University of Leicester, College of Medicine Cecatti, Jose; University of Campinas, Obstetrics and Gynecology
<b>Primary Subject Heading</b>:	Obstetrics and gynaecology
Secondary Subject Heading:	Diagnostics
Keywords:	preterm birth, metabolomics, biomarkers, prediction, metabolome

SCHOLARONE™  
Manuscripts

1  
2  
3 1 **The use of metabolomics for predicting spontaneous preterm birth in asymptomatic**  
4  
5  
6 2 **pregnant women: protocol for a systematic review and meta-analysis**  
7  
8 3

9  
10 4 Renato T. Souza <sup>1</sup> ([renatotsouzasp@gmail.com](mailto:renatotsouzasp@gmail.com))

11  
12  
13 5 Rafael Bessa Freitas Galvão <sup>1</sup> ([rafaelbfg@gmail.com](mailto:rafaelbfg@gmail.com))

14  
15 6 Débora Farias Batista Leite <sup>1,2</sup> ([deborafariasleite@gmail.com](mailto:deborafariasleite@gmail.com))

16  
17 7 Renato Passini Jr <sup>1</sup> ([passini@caism.unicamp.br](mailto:passini@caism.unicamp.br))

18  
19 8 Philip N. Baker <sup>3</sup> ([philip.baker@leicester.ac.uk](mailto:philip.baker@leicester.ac.uk))

20  
21 9 José Guilherme Cecatti <sup>1</sup> ([cecatti@unicamp.br](mailto:cecatti@unicamp.br))  
22

23  
24  
25 10

26  
27 11 <sup>1</sup> Department of Obstetrics and Gynaecology, University of Campinas, Campinas, São Paulo,  
28  
29  
30 12 Brazil.

31  
32 13 <sup>2</sup> Clinics Hospital of Federal University of Pernambuco, Recife, Pernambuco, Brazil.

33  
34 14 <sup>3</sup> College of Life Sciences, University of Leicester, England, United Kingdom.  
35

36  
37 15

38  
39 16 This systematic review protocol is registered at PROSPERO platform (CRD42018100172). Available  
40  
41  
42 17 from: [http://www.crd.york.ac.uk/PROSPERO/display\\_record.php?ID=CRD42018100172](http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42018100172).  
43

44  
45 18

46  
47 19 Corresponding author:

48  
49 20 José Guilherme Cecatti

50  
51 21 101 Alexander Fleming, Cidade Universitária, campinas, São Paulo, Brazil.

52  
53 22 ZIPCODE 13083-881

54  
55 23 [cecatti@unicamp.br](mailto:cecatti@unicamp.br)

56  
57 24 Word count: 2,233.  
58  
59  
60

1  
2  
3 25 **ABSTRACT**  
4

5  
6 26 **Introduction:** Preterm birth (PTB) is the leading cause of neonatal mortality and short- and  
7  
8 27 long-term morbidity. The aetiology and pathophysiology of spontaneous PTB are still  
9  
10 28 unclear, which makes the identification of reliable and accurate predictor markers more  
11  
12 29 difficult, particularly for unscreened or asymptomatic women. Metabolomics biomarkers  
13  
14 30 have been demonstrated to be potentially accurate biomarkers for many disorders with  
15  
16 31 complex mechanisms such as PTB. Therefore, we aim to perform a systematic review of  
17  
18 32 metabolomics markers associated with spontaneous PTB. Our research question is “What is  
19  
20 33 the performance of metabolomics for predicting spontaneous preterm birth in  
21  
22 34 asymptomatic pregnant women?”  
23  
24  
25  
26  
27

28 35 **Methods and analysis:** We will focus on studies assessing metabolomics techniques for  
29  
30 36 predicting spontaneous preterm birth in asymptomatic pregnant women. We will conduct a  
31  
32 37 comprehensive systematic review of the literature from the last 10 years. Only  
33  
34 38 observational cohort and case-control studies will be included. Our search strategy will be  
35  
36 39 carried out by two independent reviewers, who will scan title and abstract before carrying  
37  
38 40 out a full review of the article. The scientific databases to be explored include PubMed,  
39  
40 41 MedLine, ScieLo, EMBASE, LILACS, Web of Science, Scopus, and others.  
41  
42  
43  
44

45 42 **Ethics and dissemination:** This systematic review protocol does not require ethical approval.  
46  
47 43 We intend to disseminate our findings in scientific peer-reviewed journal, the Preterm  
48  
49 44 SAMBA study open access website, specialists’ conferences, and to our funding agencies.  
50

51  
52 45 **Registration details:** This protocol is registered in PROSPERO platform (code  
53  
54 46 CRD42018100172).  
55

56  
57 47 **Keywords:** preterm birth, spontaneous preterm birth, metabolomics, biomarkers,  
58  
59 48 prediction, metabolome.  
60

1  
2  
3 49 **Strengths and limitations of this study**  
4

- 5 50 • This systematic review protocol takes into account some important aspects  
6  
7  
8 51 regarding conducting a systematic review about spontaneous preterm birth and  
9  
10 52 metabolomics such as the criteria used for defining spontaneous preterm birth,  
11  
12  
13 53 different population risk stratification, method used to estimate gestational age, and  
14  
15 54 metabolomics techniques details.  
16  
17  
18 55 • Two independent reviewers are responsible for searching and selecting studies, as  
19  
20 56 also extracting data, and a third reviewer will resolve any disagreement.  
21  
22  
23 57 • If possible, proper statistical methods will be applied to investigate metabolomics  
24  
25 58 accuracy in predicting spontaneous preterm birth.  
26  
27  
28 59 • Possible limitations to this review include the different criteria applied for defining  
29  
30 60 spontaneous preterm birth, and the diverse population risk stratification.  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## 62 INTRODUCTION

63 Spontaneous preterm birth (sPTB) is the leading cause of perinatal mortality and short- and  
64 long-term morbidity [1,2]. It is defined as birth that occurs before 37 weeks gestation due to  
65 spontaneous onset of labour or preterm premature rupture of membranes (pPROM) [3,4].  
66 Several pathways and mechanisms linked with preterm birth have been proposed including,  
67 neuroendocrine, vascular, immune-inflammatory, and behavioural processes [5]. More  
68 specifically, several markers associated with uterine distension/contraction, decidual  
69 inflammation/infection and activation of hypothalamic–pituitary–adrenal axis had been  
70 studied in the past decades [5,6]. However, no single marker or combination of markers has  
71 been found to be accurate enough for predicting sPTB [7–10]. History of previous preterm  
72 birth, cervical length at second trimester and cervico-vaginal fetal fibronectin are the most  
73 promising clinical tests for predicting spontaneous preterm, but they seem not to be  
74 clinically useful for asymptomatic women. Sensitivity of short cervical length (<25mm) and  
75 high cervico-vaginal fFN (>50ng/ml) are around 33-36% and 46%, respectively [11–13].  
76 Preterm birth is a complex and multifactorial syndrome that possibly has a long pre-clinical  
77 phase, maternal and fetal interactions, genetic and environmental influences, and adaptive  
78 mechanisms [14,15]. These challenging aspects, and the presence of still unknown  
79 underlying mechanisms, are the main limitations for the identification of an accurate  
80 predictor for sPTB [16–18]. None of the predictors used in clinical practice, such as previous  
81 history of preterm birth, infection (vaginal and urinary contaminants), fibronectin and  
82 transvaginal ultrasonography cervical length demonstrated exceptional accuracy for  
83 predicting spontaneous preterm birth [7]. An exploration of innovative approaches is  
84 urgently required.

1  
2  
3 85 Metabolomics is the study of metabolites, through identification and quantification of low-  
4  
5 86 weight molecular particles, i.e. tens to hundreds thousands of intermediate products and  
6  
7  
8 87 substrates of systems biology chemical reactions [19,20]. This novel approach has been  
9  
10 88 applied for identifying biomarkers and underlying biochemical pathways associated with  
11  
12  
13 89 complex obstetrical syndromes as preeclampsia, fetal growth restriction, gestational  
14  
15 90 diabetes and preterm birth. In contrast to other “*Omics Sciences*” techniques, metabolomics  
16  
17  
18 91 is more closely associated with the phenotype of the disease and might thus identify a more  
19  
20 92 robust and reliable set of predictors [21]. Importantly, implementing an adequate *Omics*  
21  
22  
23 93 experimental design is crucial for metabolomics studies. Using different baseline population  
24  
25 94 (asymptomatic vs symptomatic or low- vs high-risk women for developing sPTB), study  
26  
27 95 designs (prospective cohorts, case-control or cross sectional studies), sources of samples  
28  
29 96 (amniotic fluid, vaginal fluid, blood, urine, hair, etc) and the timing of sample collection each  
30  
31 97 have significant effects on study findings and the consequent interpretation and  
32  
33 98 contribution to the current gap of knowledge [19].  
34  
35  
36  
37 99 Different reviews collating scientific knowledge regarding preterm birth  
38  
39  
40 100 biomarkers/predictors has been conducted. Different methodology approaches has been  
41  
42 101 applied so far, including narrative, systematic and umbrella reviews, a more comprehensive  
43  
44 102 review that includes not only original studies but also other reviews [7,22–24]. At the best of  
45  
46  
47 103 our knowledge, there is no systematic review on metabolomics markers. Therefore, we aim  
48  
49 104 to conduct a systematic review of original studies investigating the use of metabolomics  
50  
51  
52 105 biomarkers for predicting spontaneous preterm birth in asymptomatic pregnant women.  
53  
54 106 This protocol describes the methods that will be applied in our systematic review.  
55  
56  
57

## 58 **METHODS AND ANALYSIS**

59  
60

1  
2  
3 108 The current systematic review proposal will be conducted, written and published following  
4  
5 109 the Preferred Reporting Items for Systematics Reviews and Meta-Analyses (PRISMA-P)  
6  
7  
8 110 recommendations [25]. Also, it is properly registered at PROSPERO platform – code  
9  
10 111 CRD42018100172.

### 112 **Review question**

113 What is the performance of metabolomics for predicting spontaneous preterm birth in  
14  
15  
16  
17  
18 114 asymptomatic pregnant women?

### 115 **Eligibility Criteria**

116 Original cohort or case-control studies involving asymptomatic pregnant women at the  
17  
18  
19  
20  
21 117 moment of sample collection (exposure) and with samples analysed using metabolomics  
22  
23  
24  
25  
26 118 techniques. Studies will be excluded if (1) they are cross-sectional studies, clinical trials,  
27  
28  
29  
30 119 editorials, letter to editors, case reports, expert opinions, commentaries, or any type of  
31  
32  
33 120 review; (2) they describes only experimental studies with animals; or (3) they are duplicated  
34  
35 121 data (e.g. data published in conferences proceedings and, then, published again in scientific  
36  
37  
38 122 journals). In this case, only the most complete publication will be considered, after  
39  
40 123 comparing and confirming that the same technique and metabolites were explored. Studies  
41  
42 124 published from 2008 to 2018 will be considered, and there will be no language restriction.  
43  
44  
45 125 Before submitting this systematic review for publication, we will rerun the search strategy to  
46  
47 126 identify new studies that have been published after performing the first round of search.

### 127 **Participants**

128 The current review is interested in evaluating the performance of metabolomics biomarkers  
129  
130 129 for spontaneous preterm birth in asymptomatic pregnant women, which may contribute to  
131  
132  
133 130 clinical practice, potentially providing information regarding onset of preterm labour.  
134  
135  
136 131 Nevertheless, we aim to identify studies addressing only early predictors collected from

1  
2  
3 132 women who are in an early preclinical stage, which might contribute to a wider window of  
4  
5 133 opportunity for interventions and also to develop a widely reproducible screening test.  
6  
7  
8 134 Asymptomatic pregnant women should not have regular uterine tightening/contractions or  
9  
10 135 signs of rupture of membranes (i.e. watery discharge). In addition, the study should  
11  
12  
13 136 preferably have a standardized definition of spontaneous preterm birth, the outcome of  
14  
15 137 interest.

### 18 138 **Information Sources**

19  
20 139 The search will be held in the following databases: PubMed, EMBASE, Scopus, CINAHL, and  
21  
22  
23 140 Web of Science, BVS/BIREME, which includes the Latin American and Caribbean Health  
24  
25 141 Sciences Literature (LILACS), Medline and the Scientific Electronic Library Online (Scielo). In  
26  
27 142 addition, secondary sources of original studies will be explored such as Google Scholar,  
28  
29  
30 143 hand-held searching of the reference list of eligible studies, conference proceedings, and  
31  
32 144 contact with authors when necessary.

### 35 145 **Search Strategy**

36  
37 146 The following terms will be used in our search strategy for the different scientific databases:  
38  
39  
40 147 (preterm birth, premature birth, premature infant, premature labor, extremely premature  
41  
42 148 infant, premature obstetric labor, spontaneous preterm birth, extreme preterm birth, late  
43  
44  
45 149 preterm birth, moderate preterm birth, preterm premature rupture of membranes, preterm  
46  
47 150 delivery, PROM, sPTB, preterm PROM, pPROM, p-PROM) AND (metabolomic\*,  
48  
49 151 metabonomic\*, metabolit\*, lipidomic\*, H NMR, proton NMR, proton nuclear magnetic  
50  
51  
52 152 resonance, liquid chromatogra\*, gas chromatogra\*, UPLC, ultra-performance liquid  
53  
54 153 chromatograph\*, ultra-performance liquid chromatograph\*, HPLC, high performance liquid  
55  
56  
57 154 chromatograph\*, high-performance liquid chromatograph\*) AND (pregnan\*, antenat\*,  
58  
59 155 ante nat\*, prenat\*, pre nat\*) (Supplementary Material). Respective adaptations in the  
60



1  
2  
3 156 syntax of search for each database will be applied accordingly. No filters - such as “research  
4  
5  
6 157 in animal’s models” and “reviews” - will be used in our search strategy, as it will be excluded  
7  
8 158 according to eligibility criteria. The complete search strategy, including Boolean terms, is  
9  
10  
11 159 provided as Supplementary Material.

## 12 13 160 **Data Management**

14  
15 161 We will export search results to a reference manager (Mendeley®). Then, the following  
16  
17 162 information will be collected from each study using an appropriate form, which will be  
18  
19  
20 163 entered in an Excel® spreadsheet: author’s name, year of publication, country, study design,  
21  
22 164 number of participants with and without spontaneous preterm birth, type of metabolomics  
23  
24 165 analysis technique (liquid or gas chromatography, nuclear resonance), laboratory methods  
25  
26 166 for metabolites data acquiring (targeted or untargeted techniques, etc), subtype of preterm  
27  
28 167 birth (spontaneous preterm labour or pPROM), number of fetuses (singleton vs multiple),  
29  
30 168 gestational age when samples were collected, source of samples (type/site of tissue), low or  
31  
32 169 high-risk for preterm birth (authors criteria used to define the population will be collected)  
33  
34  
35 170 and method applied to estimate gestational age. If possible, additional variables related to  
36  
37 171 spontaneous preterm birth categories (delivery before 28 weeks and before 34 weeks) will  
38  
39 172 be recorded for secondary analyses. Original authors will be contacted to clarify data, when  
40  
41  
42 173 needed. Finally, we will check the biochemical class of identified metabolites in Human  
43  
44 174 Metabolome Database (HMDB, version 4.0) to explore and synthesize whether there are  
45  
46 175 common biological pathways associated with spontaneous preterm birth [20].  
47  
48  
49  
50

## 51 176 **Selection Process**

52  
53  
54 177 Two independent reviewers (RTS and RBF) will be responsible for screening and selecting  
55  
56 178 studies initially according to title or abstract. Both researchers will read the full text of non-  
57  
58 179 excluded studies to discriminate eligibility. A third reviewer (DFBL) will consider any  
59  
60

1  
2  
3 180 disagreement; additional reviewers (RPJ, PNB and JGC) will be responsible for supervising all  
4  
5  
6 181 steps and approving data extraction.

### 8 182 **Data Collection Process**

9  
10 183 We will extract search results to a reference manager where all studies will be stored. Then,  
11  
12  
13 184 included studies will be placed in a new folder. Finally, we will manually extract data of  
14  
15 185 interest from these included studies to an Excel® file. Each reviewer will have their own  
16  
17  
18 186 reference manager account, file and folder and discrepant results will be discussed together  
19  
20 187 with the third reviewer.

### 22 188 **Outcomes and Prioritization**

23  
24  
25 189 The primary outcome is spontaneous preterm birth, defined as any birth occurred before 37  
26  
27 190 weeks of gestation due to spontaneous onset of labor or preterm premature rupture of  
28  
29 191 membranes. Secondary outcomes are:

- 32 192 1. Spontaneous preterm birth before 28 weeks;
- 33  
34 193 2. Spontaneous preterm birth before 32 weeks;
- 35  
36 194 3. Spontaneous preterm birth before 34 weeks;

37  
38  
39 195 The capacity to predict different degrees of sPTB (categories of gestational age) is important  
40  
41 196 as the extreme (<28wks), moderate (<32 weeks) and non-late preterm (<34wks) newborns  
42  
43 197 have different adverse outcomes compared to non-extreme (≥28wks); non-moderate  
44  
45 198 (≥32wks) or late (≥34 wks) preterm newborns.

46  
47  
48 199 Ideally, the method of gestational age estimation should be clearly reported. For instance, it  
49  
50 200 can be reported as estimated by last menstrual period (LMP) and confirmed by an early  
51  
52 201 ultrasound or only by an early ultrasound when LMP is unknown/uncertain.

### 54 202 **Index test**

1  
2  
3 203 Metabolomics techniques to predict spontaneous preterm birth is the diagnostic test of  
4  
5 204 interest. Metabolomics is a technique to identify and quantify metabolites from biological  
6  
7  
8 205 samples using different type of platforms/equipment. The most common platforms include  
9  
10  
11 206 gas, liquid chromatography or ultra-performance liquid chromatography coupled to a mass  
12  
13 207 spectrometer or a proton nuclear magnetic resonance [26]. The performance of the  
14  
15 208 different thresholds of each metabolite will be compared and summarized through  
16  
17  
18 209 hierarchical summary receiver operator characteristic curve (HSROC) (meta-analysis)  
19  
20 210 according to the subgroups described above. Considering that the raw data is not available  
21  
22  
23 211 in the majority of the diagnostic test accuracy studies [27] and that metabolites levels are  
24  
25 212 usually reported as continuous variables, we intend to use a meta-analysis model based on  
26  
27  
28 213 ROC curves [28]. Briefly, a two-parameter model, based on the estimation of  $\alpha$  and  $\beta$   
29  
30 214 parameters (using standard errors or maximum likelihood), will be applied as reported by  
31  
32  
33 215 Kester & Buntinx [28]. Therefore, pooled ROC curves can be converted to a estimated ROC  
34  
35 216 curve with 95% confidence interval. This method can also be applied in categorical-ordinal  
36  
37 217 variables tests.

### 218 **Risk of Bias in individual Studies**

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

## 227 **Data Synthesis**

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

- 233 • Different metabolomics methods applied: gas or liquid chromatography coupled  
234 with mass spectrometry or proton nuclear magnetic resonance;
- 235 • Singleton and multiple pregnancies;
- 236 • Low-risk and high-risk women for developing preterm birth;
- 237 • Subtype of preterm birth: Spontaneous preterm birth exclusively due to  
238 spontaneous onset of labour with intact membranes or sPTB due to premature  
239 rupture of membranes.
- 240 • Gestational age interval when samples were collected: 1<sup>st</sup> trimester, 2<sup>nd</sup> trimester  
241 and 3<sup>rd</sup> trimester.

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

### 245 **Potential anticipated limitations to this review**

246 Firstly, although we have not considered any language restriction, we consider that there  
247 might be a limitation in studies published entirely in non-English language. However, in the  
248 last decade, more than 95% of scientific biomedical literature has been published in English  
249 [30], then we consider this a minor selection bias. Secondly, we intend to stratify the groups  
250 according to population risk. However, the characterization of low- or high-risk for

1  
2  
3 251 spontaneous preterm birth is controversial and lacks standardization, which might limit data  
4  
5  
6 252 comparison and subgroup analysis. Finally, categorization of sPTB into spontaneous onset of  
7  
8 253 labour or pPROM is another topic of potential limitation - the recognition of the main initial  
9  
10 254 mechanism for preterm delivery might not always be possible. Even when specified, it might  
11  
12  
13 255 provoke uncertainty and could limit further considerations regarding preterm phenotypes.  
14  
15 256 In addition, another limitation is that individual patient data will not be collected.

### 17 257 **Patient and Public Involvement**

18 258 Patients will not be directly involved in the study and no experience or direct impact from  
19  
20  
21 259 their perspective can be discussed.  
22  
23  
24

25 260

### 27 261 **ETHICS AND DISSEMINATION**

28  
29  
30 262 This systematic review does not require ethical approval from the Research Council or Ethics  
31  
32 263 board. We intend to disseminate our findings in scientific peer-reviewed journal, general  
33  
34 264 free access website of Preterm Screening and Metabolomics in Brazil and Auckland (Preterm  
35  
36  
37 265 SAMBA) study, specialists' conferences, and to our funding agencies.  
38  
39

### 40 266 **DISCUSSION**

41  
42  
43 267 This systematic review will comprise current knowledge related with metabolomics in the  
44  
45 268 context of preterm birth prediction. Metabolomics science, a resourceful innovative field  
46  
47  
48 269 that allows better understanding on pathophysiology of complex syndromes, may address  
49  
50 270 the main compounds associated with the spontaneous preterm delivery and, therefore,  
51  
52  
53 271 motivate further researchers to validate early measurable predictors of preterm birth.  
54  
55 272 Metabolomics performance for predicting sPTB remains unclear and standardized and high-  
56  
57  
58 273 quality studies are needed to clarify the clinical application of metabolites for predicting  
59  
60

1  
2  
3 274 sPTB. Nevertheless, metabolomics discovery studies commonly requires further validation  
4  
5  
6 275 studies; reproducible methodology is crucial. This systematic review protocol will collate the  
7  
8 276 main potential early biomarkers, subgroup analysis and standardized definition for  
9  
10 277 spontaneous preterm birth to better understand metabolomics performance in predicting  
11  
12  
13 278 sPTB and also to show its heterogeneity in terms of methodology (samples used,  
14  
15 279 metabolomics technique, definition of SPTB phenotype, etc). High performing predictors of  
16  
17  
18 280 preterm birth will help combat this leading cause of neonatal mortality and morbidity.  
19  
20 281  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

283 **References**

- 284 1 Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: when? Where? Why? *Lancet*  
285 2005;**365**:891–900. doi:10.1016/S0140-6736(05)71048-5
- 286 2 Howson CP, Kinney M V, McDougall L, *et al*. Born Too Soon: Preterm birth matters.  
287 *Reprod Health* 2013;**10 Suppl 1**:S1. doi:10.1186/1742-4755-10-S1-S1
- 288 3 Blencowe H, Cousens S, Chou D, *et al*. Born Too Soon: The global epidemiology of 15  
289 million preterm births. *Reprod Health* 2013;**10**:S2. doi:10.1186/1742-4755-10-S1-S2
- 290 4 Ananth C V, Vintzileos AM. Epidemiology of preterm birth and its clinical subtypes. *J*  
291 *Matern Fetal Neonatal Med* 2006;**19**:773–82. doi:10.1080/14767050600965882
- 292 5 Behrman R, Butler AS, editors. *Institute of Medicine (IOM). Preterm Birth: Causes,*  
293 *Consequences, and Prevention*. Washington, D.C.: : National Academies Press 2007.  
294 doi:10.17226/11622
- 295 6 Manuck TA, Esplin MS, Biggio J, *et al*. The phenotype of spontaneous preterm birth:  
296 application of a clinical phenotyping tool. *Am J Obstet Gynecol* Published Online First:  
297 February 2015. doi:10.1016/j.ajog.2015.02.010
- 298 7 Honest H, Hyde CJ, Khan KS. Prediction of spontaneous preterm birth: no good test  
299 for predicting a spontaneous preterm birth. *Curr Opin Obstet Gynecol* 2012;**24**:422–  
300 33. doi:10.1097/GCO.0b013e328359823a
- 301 8 Conde-Agudelo A, Papageorghiou A, Kennedy S, *et al*. Novel biomarkers for the  
302 prediction of the spontaneous preterm birth phenotype: a systematic review and  
303 meta-analysis. *BJOG An Int J Obstet Gynaecol* 2011;**118**:1042–54. doi:10.1111/j.1471-  
304 0528.2011.02923.x
- 305 9 Hee L. Likelihood ratios for the prediction of preterm delivery with biomarkers. *Acta*  
306 *Obstet Gynecol Scand* 2011;**90**:1189–99. doi:10.1111/j.1600-0412.2011.01187.x
- 307 10 Goldenberg RL, Iams JD, Mercer BM, *et al*. The Preterm Prediction Study: toward a  
308 multiple-marker test for spontaneous preterm birth. *Am J Obstet Gynecol*  
309 2001;**185**:643–51. doi:10.1067/mob.2001.116752
- 310 11 Iams JD, Goldenberg RL, Meis PJ, *et al*. The length of the cervix and the risk of  
311 spontaneous premature delivery. National Institute of Child Health and Human  
312 Development Maternal Fetal Medicine Unit Network. *N Engl J Med* 1996;**334**:567–72.  
313 doi:10.1056/NEJM199602293340904
- 314 12 Smith V, Devane D, Begley CM, *et al*. A systematic review and quality assessment of  
315 systematic reviews of fetal fibronectin and transvaginal length for predicting preterm  
316 birth. *Eur J Obstet Gynecol Reprod Biol* 2007;**133**:134–42.  
317 doi:10.1016/j.ejogrb.2007.03.005
- 318 13 Abbott DS, Hezelgrave NL, Seed PT, *et al*. Quantitative fetal fibronectin to predict

- 1  
2  
3 319 preterm birth in asymptomatic women at high risk. *Obstet Gynecol* 2015;**125**:1168–  
4 320 76. doi:10.1097/AOG.0000000000000754  
5  
6  
7 321 14 Di Renzo GC. The great obstetrical syndromes. *J Matern neonatal Med* 2009;**22**:633–  
8 322 5. doi:10.1080/14767050902866804  
9  
10 323 15 Brosens I, Pijnenborg R, Vercruyssen L, *et al*. The ‘Great Obstetrical Syndromes’ are  
11 324 associated with disorders of deep placentation. *Am J Obstet Gynecol* 2011;**204**:193–  
12 325 201. doi:10.1016/j.ajog.2010.08.009  
13  
14  
15 326 16 Practice bulletin no. 130: prediction and prevention of preterm birth. *Obstet Gynecol*  
16 327 2012;**120**:964–73. doi:10.1097/AOG.0b013e3182723b1b  
17  
18 328 17 Goldenberg RL, Culhane JF, Iams JD, *et al*. Epidemiology and causes of preterm birth.  
19 329 *Lancet* 2008;**371**:75–84. doi:10.1016/S0140-6736(08)60074-4  
20  
21  
22 330 18 Honest H, Hyde CJ, Khan KS. Prediction of spontaneous preterm birth. *Curr Opin*  
23 331 *Obstet Gynecol* 2012;**24**:422–33. doi:10.1097/GCO.0b013e328359823a  
24  
25 332 19 Horgan RP, Clancy OH, Myers JE, *et al*. An overview of proteomic and metabolomic  
26 333 technologies and their application to pregnancy research. *BJOG* 2009;**116**:173–81.  
27 334 doi:10.1111/j.1471-0528.2008.01997.x  
28  
29  
30 335 20 Wishart DS, Feunang YD, Marcu A, *et al*. HMDB 4.0: the human metabolome database  
31 336 for 2018. *Nucleic Acids Res* 2018;**46**:D608–17. doi:10.1093/nar/gkx1089  
32  
33 337 21 Dettmer K, Hammock BD. Metabolomics—a new exciting field within the omics  
34 338 sciences. *Environ Health Perspect* 2004;**112**:A396–  
35 339 7. <http://www.ncbi.nlm.nih.gov/pubmed/15159211> (accessed 5 Sep 2017).  
36  
37  
38 340 22 Lucaroni F, Morciano L, Rizzo G, *et al*. Biomarkers for predicting spontaneous preterm  
39 341 birth: an umbrella systematic review. *J Matern Neonatal Med* 2018;**31**:726–34.  
40 342 doi:10.1080/14767058.2017.1297404  
41  
42  
43 343 23 Horgan RP, Clancy OH, Myers JE, *et al*. An overview of proteomic and metabolomic  
44 344 technologies and their application to pregnancy research. *BJOG* 2009;**116**:173–81.  
45 345 doi:10.1111/j.1471-0528.2008.01997.x  
46  
47  
48 346 24 Romero R, Espinoza J, Gotsch F, *et al*. The use of high-dimensional biology (genomics,  
49 347 transcriptomics, proteomics, and metabolomics) to understand the preterm  
50 348 parturition syndrome. *BJOG An Int J Obstet Gynaecol* 2006;**113**:118–35.  
51 349 doi:10.1111/j.1471-0528.2006.01150.x  
52  
53  
54 350 25 Shamseer L, Moher D, Clarke M, *et al*. Preferred reporting items for systematic review  
55 351 and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ*  
56 352 2015;**350**:g7647. doi:10.1136/BMJ.G7647  
57  
58 353 26 Zhang A, Sun H, Wang P, *et al*. Modern analytical techniques in metabolomics  
59 354 analysis. *Analyst* 2012;**137**:293–300. doi:10.1039/c1an15605e  
60



- 1  
2  
3 355 27 McGrath TA, Alabousi M, Skidmore B, *et al.* Recommendations for reporting of  
4 356 systematic reviews and meta-analyses of diagnostic test accuracy: a systematic  
5 357 review. *Syst Rev* 2017;**6**:194. doi:10.1186/s13643-017-0590-8  
6  
7  
8 358 28 Kester ADM, Buntinx F. Meta-analysis of ROC Curves. *Med Decis Mak* 2000;**20**:430–9.  
9 359 doi:10.1177/0272989X0002000407  
10  
11 360 29 Whiting PF, Rutjes AWS, Westwood ME, *et al.* QUADAS-2: A Revised Tool for the  
12 361 Quality Assessment of Diagnostic Accuracy Studies. *Ann Intern Med* 2011;**155**:529.  
13 362 doi:10.7326/0003-4819-155-8-201110180-00009  
14  
15  
16 363 30 Rosselli D. The language of biomedical sciences. *Lancet* 2016;**387**:1720–1.  
17 364 doi:10.1016/S0140-6736(16)30259-8  
18  
19  
20 365

### 21 366 **Author's Contributions**

22  
23  
24 367 RTS and RFBG will conduct the systematic review as independent first reviewers. JGC, RPJ  
25  
26 368 and DFBL will decide about conflicting decisions regarding papers selections. PNB, RPJ and  
27  
28  
29 369 JGC participated in the systematic review conception, methodology and framework,  
30  
31 370 together will all the others co-authors.  
32  
33

### 34 371 **Funding**

35  
36  
37 372 This research was supported by Brazilian National Research Council (grant number  
38  
39 373 401636/2013-5) and Bill and Melinda Gates Foundation (grant number OPP1107597- Grand  
40  
41 374 Challenges Brazil: Reducing the burden of preterm birth, FIOTEC number 05/2013), which  
42  
43 375 provided funding to PRETERM-SAMBA project ([www.medscinet.com/samba](http://www.medscinet.com/samba)). RTS and DFL  
44  
45 376 have been awarded PhD scholarships from the CAPES Foundation, an agency under the  
46  
47 377 Ministry of Education of Brazil, process 88881.134095/2016-01 and 8881.134512/2016-01  
48  
49 378 respectively. The sponsors played no role on the study design or manuscript writing.  
50  
51  
52  
53  
54

### 55 379 **Competing interests**

56  
57  
58  
59  
60

1  
2  
3 380 All authors are carrying original research about metabolomics and presenting conferences  
4  
5  
6 381 about this topic, including spontaneous preterm birth, preeclampsia, gestational diabetes  
7  
8 382 mellitus and fetal growth restriction. Philip N Baker is principal investigator of Metabolomics  
9  
10 383 Diagnostics Ltd, a company dedicated to develop innovative screening tests using  
11  
12  
13 384 metabolomics technology.  
14  
15

### 16 385 **Acknowledgements**

17  
18  
19 386 Ana Paula de Moraes e Oliveira, librarian of University of Campinas – Unicamp, Brazil, for  
20  
21 387 collaborating in developing search strategy and Rachel Hanisch for her suggestions to some  
22  
23  
24 388 sections of the paper.  
25  
26

### 27 389 **Ethics approval and consent to participate**

28  
29  
30 390 This systematic review does not require ethical approval from the Research Council or Ethics  
31  
32 391 board.  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Search strategy: #1 AND #2 AND #3

1  
2  
3  
4  
5  
6 preterm birth  
7 premature birth  
8 premature infant  
9 premature labor  
10 extremely premature infant  
11 premature obstetric labor  
12 spontaneous preterm birth  
13 extreme preterm birth  
14 late preterm birth  
15 1 (OR for each  
16 term)  
17 moderate preterm birth  
18 preterm premature rupture of membranes  
19 preterm delivery  
20 PROM  
21 sPTB  
22 preterm PROM  
23 pPROM  
24 p-PROM  
25  
26  
27  
28  
29  
30  
31 metabolomic\*  
32 metabonomic\*  
33 metabolit\*  
34 lipidomic\*  
35 H NMR  
36 proton NMR  
37 proton nuclear magnetic resonance  
38 2 (OR for each  
39 term)  
40 liquid chromatogra\*  
41 UPLC  
42 ultra-performance liquid chromatograph\*  
43 ultra performance liquid chromatograph\*  
44 HPLC  
45 high performance liquid chromatograph\*  
46 high-performance liquid chromatograph\*  
47  
48  
49  
50  
51  
52 pregnan\*  
53 antenat\*  
54 3 (OR for each  
55 term)  
56 ante nat\*  
57 prenat\*  
58 pre nat\*  
59  
60

## PRISMA-P 2015 Checklist

This checklist has been adapted for use with systematic review protocol submissions to BioMed Central journals from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 4:1

An Editorial from the Editors-in-Chief of *Systematic Reviews* details why this checklist was adapted - Moher D, Stewart L & Shekelle P: Implementing PRISMA-P: recommendations for prospective authors. *Systematic Reviews* 2016 5:15

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
<b>ADMINISTRATIVE INFORMATION</b>					
<b>Title</b>					
Identification	1a	Identify the report as a protocol of a systematic review	x	<input type="checkbox"/>	2
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	<input type="checkbox"/>	<input type="checkbox"/>	n/a
<b>Registration</b>	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	x	<input type="checkbox"/>	48-49
<b>Authors</b>					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	x	<input type="checkbox"/>	4-23
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	x	<input type="checkbox"/>	283-287
<b>Amendments</b>	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	<input type="checkbox"/>	<input type="checkbox"/>	n/a
<b>Support</b>					
Sources	5a	Indicate sources of financial or other support for the review	x	<input type="checkbox"/>	275-282
Sponsor	5b	Provide name for the review funder and/or sponsor	x	<input type="checkbox"/>	275-282
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	x	<input type="checkbox"/>	282
<b>INTRODUCTION</b>					
<b>Rationale</b>	6	Describe the rationale for the review in the context of what is already known	x	<input type="checkbox"/>	51-87
<b>Objectives</b>	7	Provide an explicit statement of the question(s) the review will address with reference to	x	<input type="checkbox"/>	94-95

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
		participants, interventions, comparators, and outcomes (PICO)			
<b>METHODS</b>					
<b>Eligibility criteria</b>	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	x	<input type="checkbox"/>	96-107
<b>Information sources</b>	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	x	<input type="checkbox"/>	119-125
<b>Search strategy</b>	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	x	<input type="checkbox"/>	127-136
<b>STUDY RECORDS</b>					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	x	<input type="checkbox"/>	142-155
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	x	<input type="checkbox"/>	157-161
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	x	<input type="checkbox"/>	163-167
<b>Data items</b>	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	x	<input type="checkbox"/>	142-155
<b>Outcomes and prioritization</b>	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	x	<input type="checkbox"/>	169-179
<b>Risk of bias in individual studies</b>	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	x	<input type="checkbox"/>	189-196
<b>DATA</b>					
<b>Synthesis</b>	15a	Describe criteria under which study data will be quantitatively synthesized	x	<input type="checkbox"/>	198-209
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., $I^2$ , Kendall's tau)	x	<input type="checkbox"/>	185-187
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	x	<input type="checkbox"/>	185-187

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	x	<input type="checkbox"/>	198-202
<b>Meta-bias(es)</b>	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	<input type="checkbox"/>	<input type="checkbox"/>	n/a
<b>Confidence in cumulative evidence</b>	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	<input type="checkbox"/>	<input type="checkbox"/>	n/a

For peer review only