PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Cohort profile: The Swedish Maternal Microbiome project (SweMaMi) - assessing the dynamic associations between the microbiome and maternal and neonatal adverse events
AUTHORS	Fransson, Emma; Gudnadottir, Unnur; Hugerth, Luisa; Itzel, Eva; Hamsten, Marica; Boulund, Fredrik; Pennhag, Alexandra; Du, Juan; Schuppe-Koistinen, Ina; Brusselaers, Nele; Engstrand, Lars

VERSION 1 – REVIEW

REVIEWER	O' Sullivan, Anthony St. George and Sutherland Clinical School and Program Authority for UNSW Medicine, Medicine
REVIEW RETURNED	22-Jul-2022

GENERAL COMMENTS	Over the past decade, it has been established that the human microbiome interacts with many body systems and contributes significantly to health, and dysbiosis of the microbiome can impact on wellbeing and be associated with poor health. Moreover, the microbiome during pregnancy can potentially impact on maternal and foetal health. For these reasons, this research study is timely and the results will significantly contribute to pregnancy and foetal health. A large prospective study was performed with samples being taken twice in pregnancy and once post-partum. The research questions are clear and the methods set out well. The sample population is generally reflective of the Swedish population although they smoked less and had lower BMIs suggesting the sample population is a healthier cohort as the authors acknowledge. **Major points** 1) The introduction is well written and provides a strong basis for why this research should be conducted, however the authors should also provide some discussion on the impact on the microbiome of obesity in pregnancy, excessive gestational weight gain and gestational DM (1-3). 2) The second (lines 20-22) and third (lines 22-27) sentences of the second paragraph should be referenced. 3) The sentence of lines 47-52 needs to be re-written for clarity. Clarify how the “oral bacterial abundance increases and seems to remain stable”. Are the authors referring to different times points in the pregnancy? 4) The first sampling point in between 10-20 weeks into the pregnancy and the microbiome may have already significantly changed by then (3). 5) The subjects self collect specimens. Do the authors have any data on the correlation and accuracy between self-collected specimens and laboratory-collected specimens? The demographic and health data are also self-reported, part of which has been validated in the literature.

	6) Another limitation is that no blood samples were taken. **References** 1. The characteristics of intestinal flora in overweight pregnant women and the correlation with gestational diabetes mellitus; Endocrine Connections 2021; 10: 1366. 2. Cross-Talk Between Gut Microbiota and Adipose Tissues in Obesity and Related Metabolic Diseases; doi: 10.3389/fendo.2022.908868 3. Association of Gut Microbiota during Early Pregnancy with Risk of Incident Gestational Diabetes Mellitus; doi:10.1210/clinem/dgab346

REVIEWER	Susic, Daniella University of New South Wales, Women's and Children's Health
REVIEW RETURNED	31-Jul-2022

GENERAL COMMENTS	In answering this question: 13. Is the supplementary reporting complete (e.g. trial registration; funding details; CONSORT, STROBE or PRISMA checklist)? Can you please just ensure that that information is clear. I can see the ethics approval number and its' compliance with the Declaration of Helsinki and GDPR regulations. Is that all that is required in Sweden?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Prof. Anthony O' Sullivan, St. George and Sutherland Clinical School and Program Authority for UNSW Medicine
Comments to the Author:
Over the past decade, it has been established that the human microbiome interacts with many body systems and contributes significantly to health, and dysbiosis of the microbiome can impact on wellbeing and be associated with poor health. Moreover, the microbiome during pregnancy can potentially impact on maternal and foetal health. For these reasons, this research study is timely and the results will significantly contribute to pregnancy and foetal health. A large prospective study was performed with samples being taken twice in pregnancy and once post-partum. The research questions are clear and the methods set out well. The sample population is generally reflective of the Swedish population although they smoked less and had lower BMIs suggesting the sample population is a healthier cohort as the authors acknowledge.
>> Reply: thank you for this constructive feedback. Please find our detailed responses to your queries below.

Major points
1) The introduction is well written and provides a strong basis for why this research should be conducted, however the authors should also provide some discussion on the impact on the microbiome of obesity in pregnancy, excessive gestational weight gain and gestational DM (1-3).
>> Reply: We thank the reviewer for this suggestion. We have added the following sentences about this in the Introduction on page 4 (marked copy) “Obesity, excessive weight gain and gestational diabetes, all leave an apparently distinct signature on the microbiome (Stanislawski, Dabelea et al. 2017, Hasain, Mokhtar et al. 2020, Yang, Guo et al. 2020, Hu, Chen et al. 2021). There seems to be a crosstalk between the host metabolism, microbiome and adipose tissue, which seems particularly important during pregnancy (Hu, Chen et al. 2021, Su, Chen et al. 2021, Wu, Wang et al. 2022).”
2) The second (lines 20-22) and third (lines 22-27) sentences of the second paragraph should be referenced.

>> Reply: We have added references as suggested.
3) The sentence of lines 47-52 needs to be re-written for clarity. Clarify how the “oral bacterial abundance increases and seems to remain stable”. Are the authors referring to different times points in the pregnancy?
>> Reply: Thank you for pointing this out. We have re-written this section and updated the references as suggested: “Gingival inflammation is known to increase and saliva pH to decrease during pregnancy (Silva de Araujo Figueiredo, Gonçalves Carvalho Rosalem et al. 2017) The gingival and saliva microbiome have been shown to differ significantly between pregnant and non-pregnant women, with an increase in evenness and total diversity amongst pregnant women (Paropkari, Leblebicioglu et al. 2016, Kato, Nagasawa et al. 2022)”
4) The first sampling point in between 10-20 weeks into the pregnancy and the microbiome may have already significantly changed by then (3).
>> Reply: While we agree with the reviewer in principle, sample collection at an earlier time-point would not be possible in the current set-up. By emphasizing home-sampling and a general population, we could not sample earlier than the time it takes for the subjects to identify pregnancy, find the study, respond to questionnaire and receive the home-sampling kit by post. Still, we believe that the microbiome in the early second trimester could be a potentially useful biomarker for pregnancy complications, and therefore that this study set-up can present invaluable health information.
5) The subjects self collect specimens. Do the authors have any data on the correlation and accuracy between self-collected specimens and laboratory-collected specimens? The demographic and health data are also self-reported, part of which has been validated in the literature.
>> Reply: Most often, saliva and fecal samples are self-collected. Here we also use vaginal self-sampling. We show data for self-collected versus. midwife-collected vaginal swabs in this pre-print (Hugerth, Seifert et al. 2018) and we refer to this study as well as to a study from the Ravel group (Forney, Gajer et al. 2010) on page 9: “Self-collected samples have previously been shown to match the quality of physician-collected samples.”
Regarding the demographic data, it is correct that participants have been self-reporting. However, the cohort will be linked to national registers, and we have already collected data on pregnancy length from quality registers, so we do not have to only rely on self-reports.
6) Another limitation is that no blood samples were taken.
>> Reply: This limitation is included in the text and now also added among the bullet points inserted after the abstract. With the current set-up with home-sampling, it was not possible to collect blood, and requiring blood-sampling would likely have resulted in a lower number of participating women.
References
1. The characteristics of intestinal flora in overweight pregnant women and the correlation with gestational diabetes mellitus; Endocrine Connections 2021; 10: 1366.
2. Cross-Talk Between Gut Microbiota and Adipose Tissues in Obesity and Related Metabolic Diseases; doi: 10.3389/fendo.2022.908868
3. Association of Gut Microbiota during Early Pregnancy with Risk of Incident Gestational Diabetes Mellitus; doi:10.1210/clinem/dgab346

Reviewer: 2
Dr. Daniella Susic, University of New South Wales
Comments to the Author:
In answering this question:
13. Is the supplementary reporting complete (e.g. trial registration; funding details; CONSORT, STROBE or PRISMA checklist)? Can you please just ensure that that information is clear. I can see the ethics approval number and its' compliance with the Declaration of Helsinki and GDPR regulations. Is that all that is required in Sweden?
>> Reply: Please see the list below:
• Trial registration – Not applicable, this is not a clinical trial
• Funding details – Provided at the end of the manuscript under the sub heading Funding declaration

• Checklists – We agree that such checklists are important tools. CONSORT is for RCT, PRISMA for systematic reviews and meta-analysis so these are not relevant. To our knowledge, there are only 2 widely used reporting-checklists for protocols, the PRISMA-P for systematic reviews/Meta-analysis, and the SPIRIT for clinical trials (see www.equator-network.org) which are both unsuitable for the present study. The STROBE checklist is most relevant – as it is developed for observational studies - but this is a protocol. It will be useful in future reports of actual findings from the SweMaMi cohort.
• Ethics – On page 8 we describe the recruitment when informed consent is obtained and the voluntary nature of participating was communicated with participants. All studies approved by an ethical board have to submit information about all procedures including detailed description about samples, questionnaires etc and descriptions about potential harm, how to deal with confidentiality of the participants etc. All of which is included in the Helsinki declaration.

References

Hugerth, L. W., M. Seifert, A. A. L. Pennhag, J. Du, M. C. Hamsten, I. Schuppe-Koistinen and L. Engstrand (2018). "A comprehensive automated pipeline for human microbiome sampling, 16S rRNA gene sequencing and bioinformatics processing." bioRxiv: 286526.

VERSION 2 – REVIEW

REVIEWER	O' Sullivan, Anthony St. George and Sutherland Clinical School and Program Authority for UNSW Medicine, Medicine
REVIEW RETURNED	13-Sep-2022

GENERAL COMMENTS	The authors have included references in the introduction which are not more appropriate. The sentences that needed re-writing are now much clearer. The limitations section now contains the limitation I was concerned about.