Author’s response to reviews

Title: MAMI: a birth cohort focused on maternal-infant microbiota during early life

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Author’s response to reviews:

Response to Editor

Editor Comments

Editorial concerns:
1. Section headers

Please ensure that your section headers adhere to our submission guidelines

- Change "Introduction" into "Background"
- Change "Methodology" into "Methods"

Authors answer: Thank you very much for the comment. We have checked the manuscripts and “Background” and “Methods” are included.

2. Sample size

We usually require authors to include a sample size justification in the form of a power calculation in their manuscript. We understand that attrition rate may be low for our study, but please provide an indication of the minimum required number of participants in order to be able to assess significance. If you do not feel that this number can/is likely to be reached, please discuss this as a limitation to your study.

Authors answer: MAMI cohort study is an observational longitudinal study in a healthy mother-infant pairs during early life. In randomized clinical trials, the sample size calculation of patients is generally based on a comparison between the treatment groups under investigation (or prevalence of a disease). However, in observational and longitudinal studies, this requirement is not needed although specific numbers according to the objectives should be also justified. In our observational study, conducted in a sample of healthy pairs mother-infants (as we do not estimate based on the prevalence of a disease), it could be justified based on the recruitment capacity and statistical power. In our case, and also, based on our capacity and also, on the Hospital’s experience (the drop out/exclusion rate is near 25% in the follow-ups), we will initially recruit for the study 250-200 pregnant women, in order to complete the study with a minimum of 100 mother-infant pairs at follow-up. We are glad to inform that we reached the numbers and the drop-off has been close to 10%.

We have included a sentence in the manuscript in order to provide an indication of the minimum required number of participants “We started with a recruitment of 250 pregnant women in order to complete the study with a minimum of 100 mother-infant pairs.”
3. Funding

Please provide a clarification of the role of your funding body with regards to design, execution and analysis of your study.

Authors answer: The funding supported the birth cohort development. The ERC project was applied to develop the cohort study and understand the role of maternal microbes in infant health.

4. MAMI Investigators

To avoid additional delay during the production, please add the list of the MAMI investigators to the "acknowledgments" subsection.

Authors answer: Thank you very much for the comment. MAMI investigators have been listed in “acknowledgements” subsection.

5. Clean version

At this stage, please upload a clean version of your manuscript, without any tracked changes.

Authors answer: We have also uploaded a clean version without any tracked changes.

Response to Reviewers

Reviewer #1

The authors describe their prospective observational, longitudinal cohort study including 185 mother infant pairs. The aim of this birth cohort study is to better understand the influence of nutrition on the maternal microbiome during the last trimester of pregnancy, the seeding of maternal microbiome to the infants and the influence of feeding type on the infant microbiome maturation, as well as the impact of the early life microbiome maturation for health of the infant.

These are relevant aims of utmost importance. However, it is not clear whether the proposed methods will be sufficiently specific to answer all questions.
Authors answer: Thank you very much for the comment. Our proposal is aimed to understand the impact of maternal diet and lactation in the microbiota and microbial transfer to the neonates. We know that different methods would be use to analyze all the biological samples collected in the cohort and also, bioinformatics and regression models and/or machine learning would also provide new insights on the questions raised in the study. This study protocol is aimed to the microbiological aspect in the birth cohort and the microbiota composition is our main outcome.

Further, sampling methods of breastmilk and stool for microbiota analysis are not sufficiently detailed. To fully appreciate the value of any association to health measures in the infants, it seems important to provide details on the health assessments especially related to infections and metabolic health.

Authors answer: Thank you very much for the comment. The reviewer is right and we did not include the detailed info on sample processing. This is a study protocol where the general overview as well as whole study is explained without data and results. We are going to include detailed info in the papers published with the data obtained in the MAMI cohort.

Several typos, incomplete or unclear sentences and the use of past and present tense need attention.

Authors answer: Thank you very much for the comment. We have carefully revised and checked the manuscript to correct all typos and also, to use the verbal time properly (at the time of the initial submission we were completing the follow-up but now, it is completed; just some analysis are still on-going).

L 98, sentence incomplete
Authors answer: We have completed the sentence.

L 132, 'infant' seems missing in the overall objective. Rephrase.
Authors answer: Thank you very much for the comment. We have included “infant”. Now the sentence is “The overall objective of this study is to analyze early microbial exposition focusing on maternal microbiota, including breast milk, shaping the neonatal oral and intestinal microbiota development and immune system maturation”
L 132, 'and' is double
Authors answer: We have removed one “and”.

L144, 'Drop out' instead of 'drop off'
Authors answer: We have corrected the typo.

L 146, use past tense when something was already done
Authors answer: Thank you very much for the comment. We have corrected the sentence.

L 179, detail maternal stool, milk collection
Authors answer: Thank you very much for the comment. We have included brief information on biological samples’s collection.

L 185, detail urine, stool collection
Authors answer: Thank you very much for the comment. We have included a paragraph with a brief description on sampling, management and storage. In general, oral sampling consisted of simple swabbing of maternal and infant inner part of the cheek with sterile oral swabs. Faeces, urine and breastmilk were collected in sterile containers of different sizes and shapes. Once all biological samples arrived to Biobank, biological samples were managed and stored in sterile cryovials under specific standardized protocols at Biobank “Biobanco para la Investigación Biomédica y en Salud Pública de la Comunidad Valenciana (IBSP-CV)”

L 222, detail health outcome measures (how, when, retrospective parent reported or physician diagnosed etc.)
Authors answer: Health outcome measurement is provided by the physician diagnosed and it is collected in the infant records (“cartilla”- “‘card”) at the time that happen. Then, all those info is shared within MAMI team to combine with the other data.
L 237, detail on food intake questions during pregnancy (when) how close to stool sampling and how close to birth was stool sampling? Etc)

Authors answer: The FFQ are collected within the 24h after birth, this info represent the maternal diet during pregnancy. Furthermore, other types of questionnaires, such as a 3-day food records questionnaire and 14-item questionnaire, PREDIMED validated test to evaluate the adherence to the Mediterranean diet were collected in the specific sampling times to pair the biological samples with food diet and also, clinical data in both mothers and infants.

L 248, detail on type of formula, with probiotics, prebiotics, HMOs, etc.

Authors answer: Type of formula and also, if it contains probiotics and prebiotic and which type are collected.

L 256, details on determination of infections

Authors answer: Clinicians and Pediatricians involved in MAMI determine and identify the infections (cause/origin and treatment). All those info is collected in specific clinical records at hospital and primary health centers. Those info is accessible for MAMI team clinician members.

L 267, detail diagnosis of infections

Authors answer: Diagnosis of the infections are recorded by the MAMI pediatrics team when infants are going to the health care center due health-related problems. Clinicians had the tools to diagnose the infections by collecting and analyzing samples and also, to ship them to the hospital for further analysis (as virus and bacteria info). We have included a sentence clarifying that infections were recorded and diagnosed by clinicians (avoiding parent-self reporting)

L 318, detail for which sample this analysis is done, (blood sampling not mentioned in the sample collection, clarify)

Authors answer: Immune markers as cytokines, hormones and other compounds are going to be analyzed mainly in milk although fecal supernatants and oral samples would be also included.

L 325, no power calculation is described, title is misleading, correct/clarify.
Authors answer: MAMI cohort study is an observational longitudinal study in a healthy mother-infant pairs during early life. In randomized clinical trials, the sample size calculation of patients is generally based on a comparison between the treatment groups under investigation (or prevalence of a disease). However, in observational and longitudinal studies, this requirement is not needed although specific numbers according to the objectives should be also justified. In our observational study, conducted in a sample of healthy pairs mother-infants (as we do not estimate based on the prevalence of a disease), it could be justified based on the recruitment capacity and statistical power. In our case, and also, based on our capacity and also, on the Hospital’s experience (the drop out/exclusion rate is near 25% in the follow-ups), we will initially recruit for the study 250-200 pregnant women, in order to complete the study with a minimum of 100 mother-infant pairs at follow-up. We are glad to inform that we reached the numbers and the drop-off has been close to 10%.

We have included a sentence in the manuscript in order to provide an indication of the minimum required number of participants “‘We started with a recruitment of 250 pregnant women in order to complete the study with a minimum of 100 mother-infant pairs.’’”

L 370, states that study ended, earlier the MS says the follow-up is still ongoing, clarify and correct.

Authors answer: Thank you for the comment. The reviewer is right as we did not clarify well the study. MAMI recruitment and follow-up until 2 years of life is already completed (ended early 2019). Bio-samples are ready to be analyzed but the microbiota and metabolites analysis are still on-going.

L 371, strengths and weaknesses not clearly elaborated, present first strengths , then weaknesses.

Authors answer: Thank you very much for the comment. We have modified the strengths and weaknesses section.

L 388, how is metabolic health assessed

Authors answer: The metabolic health is assessed by body compositions data as the use of weight, length, cranial perimeter and others during early life in order to obtain the z-cores according to WHO and check the percentiles, growth trajectories and determine the risk of overweight and obesity. Our ethical committees are not allowing us to collect blood samples from healthy infants and then, other parameters as cholesterol, lipids, etc.. are not able to be analyzed.
The manuscript 'MAMI: a birth cohort focused on maternal-infant microbiota during early life' by Garcia-Mantrana et al. describes an ongoing study aimed at identifying impact of the maternal microbiota, birth mode, diet and lifestyle on the children gut, urine and oral microbiota development and early life health programming in a large cohort of over 150 mother-children pairs followed up during the first 2 years of life. The study is very interesting and aims at assessing very important and yet scarcely addressed issues. For example, I find it very intriguing to look at the effect of place of delivery on infant microbiota development; are there many women in Spain who choose to give birth outside hospital - is there a sufficient number of women in this cohort to address this question?

Authors answer: Thank you very much for the comment. In the Mediterranean area, most of the deliveries happen at the hospital, although, it is becoming popular to have home birth as it is recognized as a safe alternative for low-risk women. Currently there is limited investigation of how the neonatal microbiome develops in the absence of all interventions, including hospitalization. We hypothesize that hospital exposure at birth affects the neonate intestinal microbiota.

This study cohort definitely should be published, but the manuscript needs to be polished before to improve clarity. For example, there are number of typos in the manuscript (see minor comments), and I also think Figure1 should be modified to make it clearer which sample types are collected from mothers and which from children. It is stated in the text, but I think the figure would benefit if this is also made clear there. Also, how many breast milk samples are collected? Until the mother selects to stop breastfeeding? Or is there a unified time point after which breast milk samples are not collected from women?

Authors answer: Thank you very much for the comment. We collected breastmilk samples from mothers who breastfed their infants (approximately 70% of the mothers in the cohort) at the designated timepoints.

We collected breast milk samples in all time points in mother following breastfeeding practices and sample was available. Once, the mothers decide to stop breastfeeding, there would be not available sample. In addition, the information on exclusive and/or partially-breastfeeding practices was also collected.
Authors have to check the tenses used throughout the text - as it reads right now, it seems that DNA extraction, sequencing and data analysis is -already- ongoing; although not all samples are collected yet. If this is true and each time point is processed independently of others, then batch to batch variation has to be taken into account and samples should be spiked with a known mixture of bacteria to correct for this variation.

Authors answer: Thank you very much for the comment. As we mentioned above, recruitment and follow-up at 24 months of life is completed. Bio-samples and information related to them are already collected and processed. We are now working on the first data obtained with microbiota and metabolite profile from mothers and neonates. Our purpose is to refer to the study protocol when the results from MAMI will be published during next years.

Otherwise, please state explicitly that samples are sent to IATA-CSIC laboratories for storage until further analyses.

Authors answer: Thank you very much for the comment. As we comment before, every time cohort samples are collected, first they are sent to Biobank for management, processing and storage. An aliquot of bio-samples will be sent to IATA-CSIC laboratories for further centralized analysis. We changed the sentence in the text to clarify this.

I also think that the abstract should stress more on that this paper describes the study design and does not include any of the results yet, it might be just me, but my first impression after reading the abstract was that the paper includes results on qPCR and sequencing.

Authors answer: Thank you very much for the comment. We have modified the abstract. This paper is a study protocol where the general overview as well as whole study is explained without data and results. Future works related to MAMI would refer this study protocol.

Minor comments:

1. Line 132 - double 'and'

Authors answer: Removed one “and”

2. Line 137 - write out what MAMI stands for; typo in 'clinicians'
Authors answer: Thank you very much for the comment. We have included what MAMI stands for: “Maternal MImicrobes”. We have corrected the typo in “clinicians”.

3. Line 146 - Mothers -were- recruited
Authors answer: We have modified the sentence

4. Line 149,150 - the MAMI project was disseminated; typo in 'midwives'
Authors answer: We have modified the typos.

5. Line 151 - what do authors mean by 'mothers by team members’’?
Authors answer: Thank you very much for the comment. The information habe been given by MAMI team members to the mothers involved in the study. We have modified the sentence in order to clarify the info and also, to avoid confusion.

6. Line 151 - Midwives and doctors are also asked to propagate the information; - midwives were already mentioned in the previous sentence.
Authors answer: Thank you very much for the comment. This sentence would repeat information mentioned before. Then, this sentence has been removed.

7. Line 169 - such -as- diabetes
Authors answer: We have included “as”.

8. Lines 168/171 - in line 168 authors state that inclusion criteria, among others, included 'non-complicated pregnancy’. And then later on, in line 171 - they state that exclusion criteria were non-compliance with any of the inclusion criteria or suffering chorioamionitis; - is that not included into the 'complicated pregnancy’? seems like repeating information
Authors answer: Thank you very much for the comment. You are right and we are repeating information. We have modified the sentence and now it is “The inclusion criteria required the mother to be older than 18 years old, healthy pregnancy and to be able to understand written and
spoken Spanish. The exclusion criteria were non-compliance with any of the inclusion criteria, use of medications or drugs, suffering chorioamnionitis or health complications during the gestational period, as well as experiencing any chronic disease or taking medication for any chronic pathology such as diabetes or pre-gestational thyroid problems.”

9. Line 222 - throughout study completion
Authors answer: We have modified the sentence.

10. Line 227 - will not -be- made public
Authors answer: We have included “be”

11. Line 256 - typo in 'infections'
Authors answer: We have corrected the typo in “infections”

12. Line 370 - 'thus we ended the study with 185 mother-infants' - but the collection is still ongoing… Furthermore, in line 381 - 'Currently we are working on improving cohort retention': the study was targeting first 1000 days, so 2 years, right now 2nd year samples are being collected and the drop-out was 26 %, the cohort will not be followed up further… do authors mean that they are developing new strategies for improving cohort retention in future study cohorts?
Authors answer: Thank you for the comment. We have modified the information on numbers and also, we have clarified the recruitment and follow-up status. As we expect, based on the Hospital’s experience, a drop out/exclusion rate, we will initially recruit for the study 200-250 pregnant women, in order to complete the study with a minimum of 100 mother-infant pairs. This information has been stated in the study protocol.

In addition, MAMI team aims to run an extended follow-up (5 years old recall and then, 10-12 years recall) and we are going to apply for different funding sources (local and national) to cover the extension period. To retain the cohort and also, the interest of the families, we have organized a “MAMI families event” last 2018 to explain the first results we obtained and also, to encourage the families to participate in the follow-up and we are planning to organize a new event at the end of 2020 (when ERC funding ends) to reinforce the adherence to the cohort.