Author’s response to reviews

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

Authors:
Izaskun Garcia-Mantrana (igama@iata.csic.es)
Cristina Alcántara (crisalba@iata.csic.es)
Marta Selma-Royo (mselma@iata.csic.es)
Alba Boix-Amorós (albaboix@iata.csic.es)
Majda Dzidic (majda.dzidic@gmail.com)
Jose Gimeno-Alcañiz (jgimeno@iata.csic.es)
Isabel Úbeda-Sansano (isabel.ubeda@gmail.com)
Ignacio Sorribes-Monrabal (isorribesm@gmail.com)
Ramon Escuriet (rescuriet@catsalut.cat)
Fernando Gil Raga (fgil@hospitalmanises.es)
Anna Parra Llorca (annaparrallorca@gmail.com)
Cecilia Martinez-Costa (ceciliam@comv.es)
Maria Carmen Collado (mcolam@iata.csic.es)

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Response to Editor

Editor Comments

Editorial concerns:
We have assessed your responses to the reviewers' comments and feel the following items need to be further addressed:

1. "Further, sampling methods of breastmilk and stool for microbiota analysis are not sufficiently detailed." You have responded with "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."

Study protocols are supposed to provide a detailed and in-depth description of the methodology and processes that are to be applied during a study. This is both to allow other people to exactly replicate your study, but also to ensure that authors cannot change their methodology after a study has already started. Furthermore, the publication of a detailed study protocol means that final study writeups require less methodological information, as you can refer to your study protocol for further details, it is not acceptable to do this the other way around. We feel that without the information requested by the reviewer, your study protocol is incomplete. Please provide further, detailed information regarding sampling methods.

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)”

The information included is: “Faeces, urine and breastmilk were collected in sterile containers of different sizes and shapes. For the standardized milk collection, Breast skin was cleaned with 0.5% chlorhexidine solution and first drops were discarded. Morning collection was recommendable. Then, breast milk was collected by use of a sterile pumper in sterile bottles to normalize the milk collection. Finally, breast milk samples were sent to biobank and then, aliquoted and stored at -80°C until further analysis. Oral sampling consisted of simple swabbing of maternal and infant inner part of the cheek with sterile oral swabs. For the morning saliva collection, the Salivette® was used. All samples were sent to Biobank, and then, 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)”. Once all cohort samples were collected and placed in Biobank, aliquots were shipped and centralized at IATA-CSIC for the analysis.”
2. power calculation

You have provided some information regarding sample size in your response to the reviewers. Please make sure that your sample size reasoning is also clearly described in your study protocol.

Authors answer: Thank you. The information on sample size is included in the manuscript. 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.” (pag 12, lines 363-369 and also, pag. 13, lines 377-379)

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%.

3. During previous review, you have been asked to clarify a number of items. Although you have provided clear clarification in your response to the reviewers, many of these concerns do not appear to have been addressed in your manuscript. Please make sure that your previous responses to the reviewers have also been implemented into your manuscript.

Authors answer: Thank you very much for the comment. We have checked that all our clarifications have been included in the manuscript. Please, if more details are needed or more clarifications, please, let us know where more details are needed.