# PEER REVIEW HISTORY

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# ARTICLE DETAILS

| TITLE (PROVISIONAL) | Measuring the Outcomes of Maternal COVID-19-related Prenatal Exposure (MOM-COPE): Study protocol for a multicentric longitudinal project |
|---|---|
| AUTHORS | Provenzi, Livio; Grumi, Serena; Giorda, Roberto; Giacomo, Biasucci; Bonini, Renza; Cavallini, Anna; Decembrino, Lidia; Drera, Bruno; Falcone, Rossana; Fazzi, Elisa; Gardella, Barbara; Giacchero, Roberta; Nacinovich, Renata; Pisoni, C; Prefumo, Federico; Scelsa, Barbara; Spartà, Maria; Veggiotti, Pierangelo; Orcesi, Simona; Borgatti, Renato |

# VERSION 1 – REVIEW

| REVIEWER | T. Gildner<br>Dartmouth College, USA |
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| REVIEW RETURNED | 16-Sep-2020 |

| GENERAL COMMENTS | Comments to authors<br>A very interesting and important study overall! I look forward to seeing what you find. I appreciate your efforts to publish the study protocol. Unfortunately I think the article has some limitations that must be addressed before it is read for publication.<br><br>General comments<br>- Throughout the paper there are some minor typos and grammatical issues (e.g., pg 5 line 90, should say "launched" not "lunched", and pg 12 line 178 I believe you mean z-score not z-point). Please edit a little more thoroughly. It would also be easier to read if the new paragraphs were indented.<br><br>Introduction<br>- Please expand your introduction a bit to discuss why the study of epigenetics is of interest in this context; for example, how epigenetic modifications caused by early life stress may influence long-term physical and mental health in future generations. See for example:<br><br>Conching, Andie Kealohi Sato, and Zaneta Thayer. 2019. "Biological Pathways for Historical Trauma to Affect Health: A Conceptual Model Focusing on Epigenetic Modifications." Social Science and Medicine 230: 74–82.<br><br>Jylhävä, Juulia, Nancy L Pedersen, and Sara Hägg. 2017. "Biological Age Predictors." EBioMedicine 21: 29–36. https://doi.org/https://doi.org/10.1016/j.ebiom.2017.03.046.<br><br>Ryan, Calen P. 2020. "'Epigenetic Clocks': Theory and Applications in Human Biology." American Journal of Human Biology n/a (n/a): e23488. https://doi.org/10.1002/ajhb.23488. |

DeSocio, J. E. (2018). Epigenetics, maternal prenatal psychosocial stress, and infant mental health. Archives of psychiatric nursing, 32(6), 901-906.

Entringer, S., Buss, C., & Wadhwa, P. D. (2010). Prenatal stress and developmental programming of human health and disease risk: concepts and integration of empirical findings. Current opinion in endocrinology, diabetes, and obesity, 17(6), 507.

Babenko, O., Kovalchuk, I., & Metz, G. A. (2015). Stress-induced perinatal and transgenerational epigenetic programming of brain development and mental health. Neuroscience & Biobehavioral Reviews, 48, 70-91.

- In addition, you state "Nonetheless, there is no evidence of the role played by epigenetic
mechanisms in the immediate effects on infants' behavioral and socio-emotional outcomes during the first year of life, a critical time window highly sensitive to alterations of the caregiving environment". This is an inaccurate statement. There are several studies documenting links between prenatal stress, resulting epigenetic modifications, and infant behavior (e.g., fearfulness or stress reactivity). Please do a more thorough job reviewing this literature and including it in the introduction. See for example:

Tollenaar, M. S., Beijers, R., Jansen, J., Riksen-Walraven, J. M. A., & De Weerth, C. (2011). Maternal prenatal stress and cortisol reactivity to stressors in human infants. Stress, 14(1), 53-65.

Ostlund, B. D., Conradt, E., Crowell, S. E., Tyrka, A. R., Marsit, C. J., & Lester, B. M. (2016). Prenatal stress, fearfulness, and the epigenome: exploratory analysis of sex differences in DNA methylation of the glucocorticoid receptor gene. Frontiers in behavioral neuroscience, 10, 147.
Oberlander, T. F., Weinberg, J., Papsdorf, M., Grunau, R., Misri, S., & Devlin, A. M. (2008). Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. Epigenetics, 3(2), 97-106.

Van den Bergh, B. R., Mulder, E. J., Mennes, M., & Glover, V. (2005). Antenatal maternal anxiety and stress and the neurobehavioural development of the fetus and child: links and possible mechanisms. A review. Neuroscience & Biobehavioral Reviews, 29(2), 237-258.

Methods
- In addition to specific aim, it might be worthwhile to outline some possible study hypotheses consistent with the literature you've presented in the introduction, since study hypotheses will likely inform data collection and analysis protocols. For instance, in aim 1 how specifically do you expect prenatal stress to be related to infant behavior and socio-emotional outcomes? (e.g., more maternal stress is expected to correspond with more fearful behavior?)
- How do you plan to distinguish methylation linked with COVID-19-related stress from other stressors (e.g., low income even prior to the pandemic, living in an unsafe environment, experiences of discrimination, non-pandemic traumatic events, etc.)? Please address this in the methods, for example describing any potential

confounders you plan to include in data collection and detailing how these factors will be accounted for during data analysis.
- Please provide a little more information on how the MCPS scale was developed. How were the final items included determined? Would also be helpful to include the instrument in the supplementary materials.
- You mention the study will measure short- and long-term effects, yet it seems you're only collecting data for the first year of life. The term "long-term" led me to believe this might become a cohort study where you follow the children for years. If this is not the case, I might rephrase this to simply say you plan to assess immediate stress effects at birth and collect follow-up data throughout the first year of life.
- How will you recruit participants? Will you practice random sampling? How will you ensure the sample is heterogenous (i.e., not all participants of similar educational and socioeconomic backgrounds)?

Discussion
- You mention the pandemic will especially impact "fragile and exposed populations". What exactly do you mean by that? How will you ensure these individuals will be represented in your sample? This information should be included in the methods section.
- This section is very thin, I would recommend adding additional citations and details to more clearly demonstrate the importance of this exciting project. For instance, you could cite recent work exploring how the pandemic may impact long-term health and how your study will contribute some of the first data assessing these effects. See for example:

Bogin, B., & Varea, C. (2020). COVID-19, crisis, and emotional stress: A biocultural perspective of their impact on growth and development for the next generation. American Journal of Human Biology.

Study limitations
- Please also include the study limitations in the discussion section.
- The use of retrospective data has many limits, including biased recall by participants. In addition, you should mention here as you do at the beginning that an additional limitation may be the prolonged duration of the pandemic (making is difficult to enroll NEP participants). It seems like you can distinguish between women who were pregnant while the area was an epicenter for the pandemic (vs. those who became pregnant while the pandemic was ongoing but transmission was contained in the area)… these later women may still be stressed, but you could hypothesize not as much as those facing active transmission. Also, you might also detail how you plan to deal with the uncertainty of the pandemic in the future (e.g., how you would alter the study design if there was a resurgence of cases in the area). Finally, the use of retrospective data may make it difficult to determine the exact level of stress experienced at particularly vulnerable prenatal periods (e.g., the first trimester). One possible way to partly address this might be to recruit women who were in their third trimester when the pandemic hit the area, women who were in their second trimester when the pandemic hit, and women who were in their first trimester when the pandemic hit or who became pregnant while the pandemic was spreading in the area. This might help to account for COVID-19-related stressors that participants may have encountered at different prenatal timepoints (i.e., across different trimesters).

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| | - An additional limitation is that mothers may respond differently to their babies than normal during the maternal sensitivity test, as they know they are being watched. This should be noted.<br><br>Figures<br>- In Figure 1 please expand the caption to describe what the various colors represent. |
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| **REVIEWER** | Vera Trocado<br>Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal.<br><br>ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal.<br><br>Department of Obstetrics and Gynecology, Unidade Local de Saúde do Alto Minho, Viana do Castelo, Portugal. |
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| **REVIEW RETURNED** | 29-Sep-2020 |

| **GENERAL COMMENTS** | Congratulatios for the study protocol. |
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## VERSION 1 – AUTHOR RESPONSE

**REVIEWER 1**

1. A very interesting and important study overall! I look forward to seeing what you find. I appreciate your efforts to publish the study protocol. Unfortunately I think the article has some limitations that must be addressed before it is ready for publication.

**Reply.** Thanks for your appreciation and for the useful comments. As you can imagine this project was developed during a period in which the reality of the COVID-19 emergency was rapidly changing in Europe and – especially – in Italy, where we live and work. This is also reflected by methodological choices that were aimed at maximizing the beneficial compromises between ideal goals, available resources and methodological rigor. Thanks to your comments we were now able to better reflect our methodological choices in the revised version of the manuscript.

2. Throughout the paper there are some minor typos and grammatical issues (e.g., pg 5 line 90, should say "launched" not "lunched", and pg 12 line 178 I believe you mean z-score not z-point). Please edit a little more thoroughly. It would also be easier to read if the new paragraphs were indented.

**Reply.** Thanks. We edited the revised manuscript for these and other typos and issues with grammar. Please, find below a list of changes:
   - pg 4 line 130: "highlighted" instead of "pointed out"
   - pg 6 line 169: "launched" instead of "lunched"

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- pg 8 line 285: "lifestyle" instead of "life style"
- pg 10 line 302: "z-score" instead of "z-point"
- pg 11 line 406: "which measures" instead of "which measure"
- pg 11 line 410: "questionnaire" instead of "questionaire"

3. Introduction - Please expand your introduction a bit to discuss why the study of epigenetics is of interest in this context; for example, how epigenetic modifications caused by early life stress may influence long-term physical and mental health in future generations. See for example:

- Conching, Andie Kealohi Sato, and Zaneta Thayer. 2019. "Biological Pathways for Historical Trauma to Affect Health: A Conceptual Model Focusing on Epigenetic Modifications." Social Science and Medicine 230: 74–82.
- Jylhävä, Juulia, Nancy L Pedersen, and Sara Hägg. 2017. "Biological Age Predictors." EBioMedicine 21: 29–36. https://doi.org/https://doi.org/10.1016/j.ebiom.2017.03.046.
- Ryan, Calen P. 2020. "'Epigenetic Clocks': Theory and Applications in Human Biology." American Journal of Human Biology n/a (n/a): e23488. https://doi.org/10.1002/ajhb.23488.
- DeSocio, J. E. (2018). Epigenetics, maternal prenatal psychosocial stress, and infant mental health. Archives of psychiatric nursing, 32(6), 901-906.
- Entringer, S., Buss, C., & Wadhwa, P. D. (2010). Prenatal stress and developmental programming of human health and disease risk: concepts and integration of empirical findings. Current opinion in endocrinology, diabetes, and obesity, 17(6), 507.
- Babenko, O., Kovalchuk, I., & Metz, G. A. (2015). Stress-induced perinatal and transgenerational epigenetic programming of brain development and mental health. Neuroscience & Biobehavioral Reviews, 48, 70-91.

**Reply.** We have now improved the Introduction section focused on prenatal stress, methylation and outcomes and we have cited appropriate research as suggested (see page 4-5, lines 141-159). The literature on the epigenetic clock was not included as it refers to different mechanisms that may be misleading for the reader who is not experienced in behavioral epigenetics.

4. In addition, you state "Nonetheless, there is no evidence of the role played by epigenetic mechanisms in the immediate effects on infants' behavioral and socio-emotional outcomes during the first year of life, a critical time window highly sensitive to alterations of the caregiving environment". This is an inaccurate statement. There are several studies documenting links between prenatal stress, resulting epigenetic modifications, and infant behavior (e.g., fearfulness or stress reactivity). Please do a

more thorough job reviewing this literature and including it in the introduction. See for example:

- Tollenaar, M. S., Beijers, R., Jansen, J., Riksen-Walraven, J. M. A., & De Weerth, C. (2011). Maternal prenatal stress and cortisol reactivity to stressors in human infants. Stress, 14(1), 53-65.
- Ostlund, B. D., Conradt, E., Crowell, S. E., Tyrka, A. R., Marsit, C. J., & Lester, B. M. (2016). Prenatal stress, fearfulness, and the epigenome: exploratory analysis of sex differences in DNA methylation of the glucocorticoid receptor gene. Frontiers in behavioral neuroscience, 10, 147.
- Oberlander, T. F., Weinberg, J., Papsdorf, M., Grunau, R., Misri, S., & Devlin, A. M. (2008). Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. Epigenetics, 3(2), 97-106.
- Van den Bergh, B. R., Mulder, E. J., Mennes, M., & Glover, V. (2005). Antenatal maternal anxiety and stress and the neurobehavioural development of the fetus and child: links and possible mechanisms. A review. Neuroscience & Biobehavioral Reviews, 29(2), 237-258.

**Reply.** The Rev 1 is correct. We have "softened" our sentence to reflect that literature exists, nonetheless, in many of these studies only retrospective collections were possible. We also highlighted this issue to reflect the unique advantage of the present study protocol compared to existing literature.

5. Methods - In addition to specific aim, it might be worthwhile to outline some possible study hypotheses consistent with the literature you've presented in the introduction, since study hypotheses will likely inform data collection and analysis protocols. For instance, in aim 1 how specifically do you expect prenatal stress to be related to infant behavior and socio-emotional outcomes? (e.g., more maternal stress is expected to correspond with more fearful behavior?)

**Reply.** We have now included hypotheses for the main goals of the study (see the newly added paragraph "Specific aims and hypotheses". Nonetheless, no specific hypotheses were reported for secondary aims which have an exploratory nature.

6. How do you plan to distinguish methylation linked with COVID-19-related stress from other stressors (e.g., low income even prior to the pandemic, living in an unsafe environment, experiences of discrimination, non-pandemic traumatic events, etc.)? Please address this in the methods, for example describing any potential confounders you plan to include in data collection and detailing how these factors will be accounted for during data analysis.

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**Reply.** Of course, distinguishing additional sources of maternal stress was one of the issues while planning this study. We opted to remove the most obvious stressors for mothers, by selecting a sample of women who were living with the father of the baby, no single mothers, 18-year-old or more – and by including only full-term healthy deliveries (no preterm babies included). Moreover, due to the complexity of the study and the multiple data collection points during the first year of life, we also opted to include only Italian mothers or those who have clear and adequate comprehension of Italian language. While these are limitations to the study, they also allow us to exclude confounders that we were not able to manage. Other sources of distress – such as low socio-economic conditions or traumatic events unrelated to the pandemic – will be assessed using the self-report questionnaires. These variables will be controlled as covariates in the statistical model. This information is now included in the revised version of the manuscript. See "Population" and "Plan of statistical analyses" paragraphs.

7. Please provide a little more information on how the MCPS scale was developed. How were the final items included determined? Would also be helpful to include the instrument in the supplementary materials.

**Reply.** We have now included a schematic version of the MCPS tool as a supplementary material to this submission. Also, we have better explained how these items were selected based on existing literature and our expertise in the field.

8. You mention the study will measure short- and long-term effects, yet it seems you're only collecting data for the first year of life. The term "long-term" led me to believe this might become a cohort study where you follow the children for years. If this is not the case, I might rephrase this to simply say you plan to assess immediate stress effects at birth and collect follow-up data throughout the first year of life.

**Reply.** Correct. Being myself involved in clinical practice and research with very young infants (generally, 0-to-6 months) I often consider long-term something that probably is not. We have now rephrased this sentence as suggested.

9. How will you recruit participants? Will you practice random sampling? How will you ensure the sample is heterogenous (i.e., not all participants of similar educational and socioeconomic backgrounds)?

**Reply.** Sampling is consecutive. The inclusion criteria allow us to collect a reasonably non-heterogenous sample that reflects the compromises of enrolling families during a challenging period, engaging neonatal units who were struggling with the COVID-19 restrictions and reducing risks of potential bias due to uncontrolled sources of stress. At the same time, we did not want to overwhelm parents with too long or massive data collection. As such, the defined inclusion criteria were discussed and agreed by all the participating units and they

reflect a co-constructed methodological choice. The criteria tend to define a medium-high socio-economical portion of the eligible population. We have included this in the limits.

10. Discussion - You mention the pandemic will especially impact "fragile and exposed populations". What exactly do you mean by that? How will you ensure these individuals will be represented in your sample? This information should be included in the methods section.

**Reply.** Sorry, this was wrong. We have rephrased this sentence highlighting that pregnancy may be a specific sensitive window in early development during which plasticity is heightened thus making both the mother and the newborn more susceptible to adverse environmental exposures (see Discussion).

11. This section is very thin, I would recommend adding additional citations and details to more clearly demonstrate the importance of this exciting project. For instance, you could cite recent work exploring how the pandemic may impact long-term health and how your study will contribute some of the first data assessing these effects. See for example:

   - Bogin, B., & Varea, C. (2020). COVID-19, crisis, and emotional stress: A biocultural perspective of their impact on growth and development for the next generation. American Journal of Human Biology.

**Reply.** We have now included references that allowed us to provide a broader discussion framework for the potential implications of this project (Bogin & Varea, 2020; Gildner & Thayer, 2020).

12. Study limitations - Please also include the study limitations in the discussion section. The use of retrospective data has many limits, including biased recall by participants. In addition, you should mention here as you do at the beginning that an additional limitation may be the prolonged duration of the pandemic (making is difficult to enroll NEP participants). It seems like you can distinguish between women who were pregnant while the area was an epicenter for the pandemic (vs. those who became pregnant while the pandemic was ongoing but transmission was contained in the area)… these later women may still be stressed, but you could hypothesize not as much as those facing active transmission. Also, you might also detail how you plan to deal with the uncertainty of the pandemic in the future (e.g., how you would alter the study design if there was a resurgence of cases in the area). Finally, the use of retrospective data may make it difficult to determine the exact level of stress experienced at particularly vulnerable prenatal periods (e.g., the first trimester). One possible way to partly address this might be to recruit women who were in their third trimester when the pandemic hit the area, women who were in their second trimester

when the pandemic hit, and women who were in their first trimester when the pandemic hit or who became pregnant while the pandemic was spreading in the area. This might help to account for COVID-19-reated stressors that participants may have encountered at different prenatal timepoints (i.e., across different trimesters). An additional limitation is that mothers may respond differently to their babies than normal during the maternal sensitivity test, as they know they are being watched. This should be noted.

**Reply.** We have now included a specific limitation paragraph in which we report a number of limitations suggested by the reviewer. Additionally, the investigation of the effects of COVID-19-related stress during different trimesters of pregnancy is a relevant potential goal of this project. Nonetheless, as we were not able to forecast the rates of enrollment in the different neonatal units involved in the study, we were not sure of how many mothers we are going to have for each trimester exposure. As such, this may be another exploratory goal of the study, provided that the distribution of mothers enrolled across the enrolment months will allow us to have comparable subgroups. Consistently, we prefer not to mention this goal in the protocol (-indeed it was not mentioned in the protocol accepted by the Ethics Committee and registered to the NIH Trials repository).

13. Figures - In Figure 1 please expand the caption to describe what the various colors represent.

**Reply.** Figure 1 has no color code. The different colors are just for graphical presentation.

**REVIEWER 2**

1. Congratulations for the study protocol.

**Reply.** Many thanks.

**VERSION 2 – REVIEW**

| REVIEWER | Theresa Gildner<br>Dartmouth College, USA |
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| REVIEW RETURNED | 29-Oct-2020 |

| GENERAL COMMENTS | You did a great job addressing my concerns from the first submission, I have nothing more to add. I look forward to learning what you discover in this study! |
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