Peer Review Report

Review Report on Clinical study on the difference in intestinal microecology between patients with preeclampsia and pregnant women at different stages of pregnancy

Original Research, Acta Biochim. Pol.

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EVALUATION

Q 1 **Please summarize the main findings of the study.**

In this manuscript the Authors aimed to analyze the gut microbiota in pregnant women with preeclampsia and healthy pregnant women at different stages of pregnancy. In addition the Authors provided characteristics of blood cell count and biochemical analyses on the lipid profile and renal function. The Authors' concluded that "intestinal flora of pre-eclampsia pregnant women may participate in the regulation of blood cells, lipid metabolism and renal function during pregnancy".

Q 2 **Please highlight the limitations and strengths.**

Limitations: lack of clear conclusion, incomplete descriptions of the methods and data interpretation, figures of inadequate quality. The strengths: unique clinical samples.

Q 3 **Please comment on the methods, results and data interpretation. If there are any objective errors, or if the conclusions are not supported, you should detail your concerns.**

Materials and Methods section in several places is written in a form of a protocol (e.g. collect, centrifuge, use, adopt) rather than it provides a description of what and how has been done.
It lacks essential details (highlighted below).
Inconsistency in the number of analyzed samples.
Inconsistency in the names of the groups.

Check List

Q 4 **Please provide your detailed review report to the editor and authors (including any comments on the Q4 Check List)**

The conclusion is mainly based on the correlation analysis and this, in my opinion, should be stated more clearly, because there is no functional analysis in the manuscript which would support such conclusion.
Apart from this I have several concerns, which are as follows.

Major issues:
1) Materials and Methods (M&M) section in several places is written in a form of a protocol (e.g. collect, centrifuge, use, adopt) rather than it provides a description of what and how has been done. Please use the simple past tense.
2) M&M section, line 104: "(...) preeclampsia group (n=5)", whereas there are 6 dots on bars in Figures 1-4.
3) M&M section, lines 105-106 - healthy pregnant women are divided in 3 groups: early, middle and late pregnancy. But, there is a discrepancy with the Figures 1-4 where the first bar shows data for "pre-pregnancy". Also, in Figures 5-8 the group names are: first, second and third trimester. Please unify.
4) M&M section lacks essential details: how the fecal samples were prepared for the sequencing analysis; what equipment was used for sequencing, more details on the bioinformatic analysis regarding the phylogenetic

affiliation should be provided; explain what is LEfSe analysis (please mind the large and small letters and correct it in the manuscript).
5) Results section, line 155: why is the mean age of pregnant women in the third trimester (29.21 ± 2.58 years old) lower than the age of women in the second trimester (30.50 ± 3.15 years old)?
6) Why in figure 7D, 8D, and 8E only the results from the samples of the first and second trimester (but not the third one) are shown?
7) Results, lines 217–218: "In Lefse analysis, there is a significant difference between Bifidobacterium and Actinobacteria (Figure 7D)". What does it mean? Such result requires a comment/interpretation.
8) Hypertension is one of the main symptoms of preeclampsia. However, Figure 9 does not involve SBP/DBP. Was there any correlation between analyzed bacteria abundance and blood pressure?
9) What is "Bateroides' in Figure 9?
10) Discussion, lines 282–285: "The lipid peroxidation of pregnant women is significantly enhanced, which can further promote the production and secretion of inflammatory factors, cause vascular endothelial damage, and promote the occurrence and development of preeclampsia". There is no reference, thus it is not clear if this observation was made by the Authors? If yes, it should be supported by the data.
11) Discussion, lines 321–323 – "Bifidobacterium, Lactobacillus and Enterococcus have been proved to be related to dyslipidemia and renal dysfunction (Sun et al.,2020). This is consistent with the results of this study." – First of all, I do not find any information about detection and abundance of Lactobacillus and Enterococcus in the Authors manuscript. And the second thing – aren't the Bifidobacterium and Lactobacillus the probiotic (beneficial) bacteria?
12) In my opinion the paper lacks clear conclusion whether the obtained data demonstrate that preeclampsia may be a result of simply dysregulated gut microbiome or some specific bacteria may cause it.
13) Figures description should be more informative.
14) A reference list should be ordered alphabetically by author's surname.
15) The Authors definitely should consult an expert in English grammar and syntax, because in many places the manuscript is very difficult to follow.

Minor issues:
1) Several abbreviations , for e.g. N, L count, or Pro should be explained when first used.
2) Page 3, lines 83–88: "This study takes pre-eclampsia pregnant women as the research object, and healthy pregnant women as the control, carries out macrogenome sequencing of intestinal microflora in stool samples, analyzes the structure, species and functional composition, metabolic pathway, etc. of intestinal microflora, and speculates the important role of key species and key gene functions in pre-eclampsia, further explores the pathogenesis of pre-eclampsia, and provides the prediction of intestinal microflora macrogenome in pre-eclampsia". This sentence is far too long and difficult to understand. What do the Authors mean by saying that the study: "provides the prediction of intestinal microflora macrogenome in pre-eclampsia"? It is also incorrect to write "etc." in a serious research paper.
3) Line 225: should be Bacteroides uniformis (not Uniforms) and Ruminococcus bromii (not Ruminocus Bromii). Also, Line 233: should be Bacteroides uniformis (not Uniforms). Please, pay attention to the correct bacteria nomenclature and use the large and small letters when appropriate (e.g. actinomycetes).
4) Why the titles of Figures 5–8 show "Analysis of (…) pregnant women at different stages of pre-eclampsia and pregnancy"? I believe it should be stated: different stages of pregnancy and pre-eclampsia?
5) Please increase the font size in the center of Fig. 6C and Fig. 7C because it is not very readable.
6) Discussion section, lines 250–252: "This hypoxia state can induce inflammation by releasing chemokines, promoting cytokines, anti-angiogenesis factors, neutrophils, etc;" – please avoid "etc." in a serious research paper.
7) Discussion, line 299: "(…) and affect the circulatory function of the ventilator in pregnant women". – what is ventilator?
8) Discussion, lines 300–301: "(…) hypoproteinemia can also stimulate the mother to increase the synthesis of lipids and lipoproteins". – please provide appropriate citation.

Q 5 **Is the English language of sufficient quality?**

No.

Q 6 Is the quality of the figures and tables satisfactory?

No.

Q 7 Does the reference list cover the relevant literature adequately and in an unbiased manner?

No.

Q 8 Are the statistical methods valid and correctly applied? (e.g. sample size, choice of test)

No.

Q 9 Are the methods sufficiently documented to allow replication studies?

No.

Q 10 Are the data underlying the study available in either the article, supplement, or deposited in a repository? (Sequence/expression data, protein/molecule characterizations, annotations, and taxonomy data are required to be deposited in public repositories prior to publication)

No.

Q 11 Does the study adhere to ethical standards including ethics committee approval and consent procedure?

Yes.

Q 12 Have standard biosecurity and institutional safety procedures been adhered to?

Yes.

QUALITY ASSESSMENT

Q 13 Originality

Q 14 Rigor

Q 15 Significance to the field

Q 16	Interest to general audience	
Q 17	Quality of the writing	
Q 18	Overall quality of the study