Review of the doctoral dissertation written by doctor Alicja Harmoza and entitled:

"Variability of the microbiome of pregnant women and its impact on frequency of preterm birth"

Preterm labour is being acknowledged as the leading obstetric pathology. The frequency varies and depends on the area of the world. In Poland, it is estimated at 6-8%. The introduction and presentation of knowledge in the researched area has been precisely and clearly presented in the dissertation. Modern obstetrics does not have any tools that can unambiguously predict the occurrence of preterm birth, though treatment and its effectiveness are often limited. Alicja Harmozy's thesis focuses on new diagnostic methods, which are developing very quickly in the world of science. One of the most frequent issues which becomes the subject of scientific consideration is the issue of the microbiome. Human and his/her body has a non-sterile environment and we are inhabited by billions of microorganisms that perform very diverse functions in our body. The study of the genomes and diversity for many years was unavailable due to technological reasons, the last decade significantly accelerated and improved diagnostic methods, thanks to which the development of microbiome research has become very popular, but above all, it has shown new directions and possibilities. More and more research shows that the quality, nature of flora and number of microorganisms inhabiting us affect the metabolic and health processes of our body. The same can be true for premature birth. One of the main causes, which have been confirmed by previous research on preterm birth, is infection and inflammation. Microbiome disorders, such as abnormal vaginal flora determined by simple diagnostic methods, allow diagnosing so-called Bacterial vaginosis as a known risk factor for premature birth. The technological leap has brought out our, so far, research activities to a completely different analytical level,

which probably in the nearest future will bring additional knowledge and answers on how a detailed analysis of the microbiome can affect both diagnostic, preventive and perhaps also therapeutic processes. To a large extent, the research to-date has been focused on the assessment of vaginal discharge in case of obstetrics complications. This made me even more interested in this work of doctor Alicja Hormeza, who decided to analyse the microbiome of faeces collected from pregnant women. There were analysed faecal samples from women who gave birth prematurely and from those with accurate predicted due date labour. The doctoral student clearly defines the criteria for inclusion and exclusion to the study group. The research methodology is clearly described and explained. The study group consisted of 50 women in total, where 28 gave birth prematurely during the 32-35 weeks of pregnancy, and I will come back to this point again presenting it jointly. The characteristics of the study group did not vary from the control group in terms of age and BMI. In addition, two groups were provided with supplementary indicators of typical parameters such as CRP protein level, white blood cell counts, haemoglobin concentration, platelet count, haematocrit, amount of protein and leukocytes in the urine. The microbiome was analysed using an NGS technique called next-generation sequencing. Out of over 3.5 million sequences, 73.9% were positioned and classified for taxonomic evaluation. 2,315 types of microorganisms have been identified. An additional research procedure arose from the usage of artificial intelligence, evaluating algorithms to select the predictive modelling solutions and features that most likely can affect the occurrence of preterm delivery with the highest probability.

The obtained results' analysis were carried out for two study groups. Dr Harmoza proved that in the group of women who gave birth prematurely, the average number of taxonomic elements was significantly lower than in the group of women who gave birth in due time.

In the analytics using artificial intelligence, the highest predictive values were presented by the hematocrit and CRP.

The results are presented using data descriptions, tables and figures that have been attractively structured. Comments on the methodology and the obtained results will be presented later in this review.

In the discussion, the doctoral student confronts the obtained results with literature data, skilfully trying to emphasize the value of her own observations. The discussion itself is conducted sensibly and supported by extensively quoted literature.

The presented conclusions are correctly edited. The entire work proves the due diligence of the doctoral student and the conduct of the research process.

Every research raises questions, it is the result of interest in the study and may encourage the doctoral student for further work in this area. The following questions appeared to the reviewer while preparing this evaluation:

1. In the table of characteristics of the studied population, the time of preterm birth is defined as minimum-maximum for 32-35 weeks, in the table on page 41 of this dissertation - for 26-35 weeks. Clarification is required.

2. I haven't found in the thesis the description of the moment of collecting the material for examination in relation to childbirth (days from childbirth). An additional study, that is worth carrying out, should answer the question whether the way of delivery affects the result of assessing the mother's microbiome collected in the manner described in the methodology.

3. Whether the variation between the samples could have influenced the ultimate results and conclusions. Based on Table 21 the degree of differentiation was significant.

4. Has the analysed model used by artificial intelligence already been tested in other studies, for example on a larger population?

5. In addition to the differences in the number of taxonomic items of the microbiome and the frequency of occurrence of individual families, did the PhD student consider

measuring the results on microbiome studies using artificial intelligence for the purpose of assessing block of types that could foster or protect preterm birth.

Inquiries and the above-mentioned comments do not affect the high evaluation mark of the conducted research process and, as I mentioned, should be an encouragement for further scientific activities. It should be emphasized that the research methodology, as for the level of doctoral thesis, is very innovative and indicates important areas of research in the near future. The doctoral thesis written by Alicja Harmozy has been planned and carried out correctly. The literature is properly quoted and includes both basic obstetrics titles and the latest world reports. It is worth noting that the doctoral student also uses the Polish reports. I would like to underline that the idea of the research is very interesting in terms of knowledge, and currently is being subject as a vigorous scientific debate.

The author demonstrated the ability to conduct clinical research. In conclusion, I consider the dissertation to be a valuable achievement that meets the criteria of the Act on higher education and science dated on July 20, 2018. Therefore, I have an honour to bring to the Medical Sciences Discipline Council of Collegium Medicum named by Ludwik Rydygier in Bydgoszcz of the Nicolaus Copernicus University in Toruń for admission of Ms. Alicja Harmoza's dissertation to further stages of the doctoral procedure.

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