

Dear Editor,

We sincerely thank you and the reviewers for the careful evaluation of our manuscript and for the constructive comments that helped us improve the quality and clarity of our study. In this revised version, we carefully addressed all reviewer comments and revised the manuscript accordingly. We clarified the study design and terminology, corrected inconsistencies in tables and numerical reporting, improved the description of the study population and outcome definitions, and strengthened the discussion and limitations regarding potential confounding and selection bias. All changes have been detailed in the point-by-point response document and incorporated into the revised manuscript.

We appreciate the time and effort invested by the reviewers and hope that the revised manuscript will now be suitable for publication.

Sincerely,

Prof. Burak Bayraktar, M.D.

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Reviewer 2:

I still have a few comments on the manuscript:

1. In the Methods, the authors state that they excluded, among others, “cases who gave birth in another hospital”, but a few sentences later they say that miscarriage and IUD were analyzed in the whole cohort (7668 vs 2044), regardless of place of delivery, while only the perinatal/neonatal outcomes were restricted to “in-center” births (2063 vs 377). This is logically inconsistent. The Methods section should clearly describe two analytical populations, not just one list of exclusion criteria.

Answer: In the revised manuscript, we clarified that two analytical populations were used. The overall cohort consisted of all eligible pregnancies undergoing amniocentesis (n = 2044) or NIPT (n = 7668), and this population was used for the analysis of pregnancy loss outcomes (miscarriage and intrauterine fetal demise), which were captured through the institutional registry regardless of the place of delivery. To avoid misunderstanding, the sentence in the exclusion criteria referring to deliveries in another hospital has been revised, and the Methods section now clearly describes these two analytical populations.

In Materials and Methods:

Exclusion criteria included multiple pregnancies, fetal structural or genetic anomalies, suspected fetal infection, parental genetic abnormalities, maternal chronic disease, and cases with missing data.” Pregnancies delivered at other hospitals were not excluded from the overall cohort; however, perinatal and neonatal outcome analyses were restricted to cases with delivery data available at our center. Perinatal and neonatal outcomes were evaluated for 2063 pregnant women in the NIPT group and 377 pregnant women in the amniocentesis group whose birth data were available.

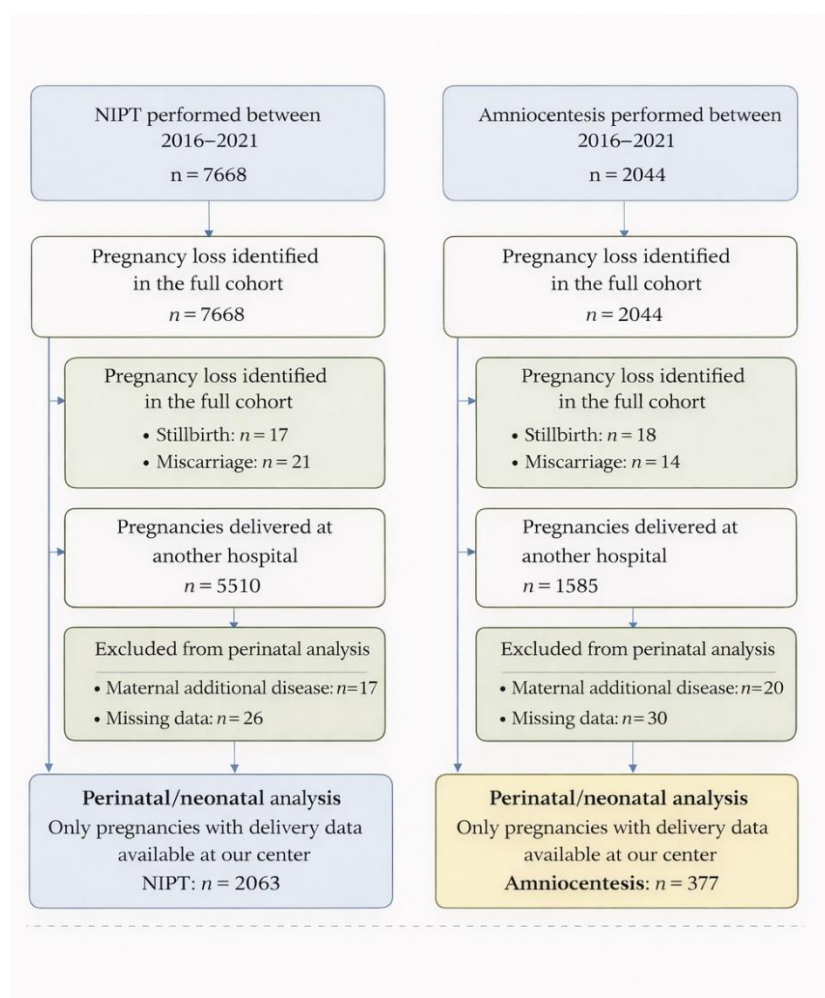
In Table 4 and 5 footnotes:

Miscarriage and intrauterine fetal demise analyses were performed in the full cohort (NIPT n = 7668; amniocentesis n = 2044).

2. In the flowchart for the NIPT group, there are 17 stillbirths, 21 miscarriages, 5510 births at another hospital, 17 maternal additional disease, and 26 missing data, yet the final number is 2063. If these are separate, sequential exclusions as shown in the diagram, the math gives 2077, not 2063.

Answer: We thank the reviewer for carefully checking the flowchart. In fact, the categories shown in the flowchart were derived from overlapping case classifications rather than strictly sequential exclusions. We have therefore revised the flowchart and its legend to clearly distinguish the overall cohort, pregnancy-loss outcomes assessed in the full cohort, and the subset with available in-center delivery data used for perinatal and neonatal analyses. This revision eliminates the apparent numerical inconsistency and better reflects the actual analytical structure of the study.

Figure 1. The flow chart of the study



3. In the flowchart, the starting boxes are labeled “NIPT with normal results” and “Amniocentesis with normal results”. This limits generalizability and changes the interpretation of the comparison. The title and abstract should clearly state that the analysis concerns pregnancies with normal/reportable results, not all women referred for diagnostic testing.

Answer: We thank the reviewer for this important observation. The reviewer is correct that the study population was restricted to pregnancies with normal/reportable test results after exclusion of structural or genetic abnormalities, and this should be more explicitly reflected in the manuscript. In response, we have revised the title/abstract wording and clarified in the Methods section that the analysis was limited to pregnancies with normal/reportable results and without structural or genetic anomalies. This clarification improves the precision of the study population definition and the interpretation of the findings.

In Title:

Association between amniocentesis and adverse pregnancy outcomes in pregnancies with normal/reportable test results: an indication-based comparison with non-invasive prenatal testing

In Abstract:

The analysis was restricted to pregnancies with normal/reportable test results and without structural or genetic anomalies.

In Materials and Methods:

... who underwent reportable/normal NIPT results..

4. In the Methods, the authors write that “Newborns with APGAR scores below 7 at both the 1st and 5th minutes were considered to have low APGAR scores”, while in the Results/Tables they analyze 1st-minute APGAR <7 and 5th-minute APGAR <7 separately, and the composite includes either one of these components. So the current definition in the Methods is incorrect, or at least confusing.

Answer: The Methods section has been revised for clarity. In the analyses we evaluated APGAR scores at the 1st and 5th minutes separately, and the composite neonatal outcome included an APGAR score <7 at either time point. The Methods section has now been corrected to reflect this definition.

In Materials and Methods:

APGAR scores were evaluated separately at the 1st and 5th minutes. Newborns with APGAR scores <7 at the 1st minute or at the 5th minute were considered to have low APGAR scores for the respective analysis.

5. Table 5 is still methodologically unclear. There is a separate row “No **” next to “Miscarriage” and “Intrauterine fetal demise”. For a standard logistic regression, such a “No” row does not really make presentational sense and looks as if a multcategory analysis was being mixed with a binary one.

Answer: The presentation of the “No” row in Table 5 was methodologically inappropriate for logistic regression analysis, as it represents the reference category. Accordingly, the “No” row has been removed and the table has been revised to present only the outcome categories analyzed in the logistic regression models. This modification improves the clarity and methodological consistency of the table.

6. In the Introduction, the authors say that this design “ensured a strong comparison ... while controlling for potential confounding factors”, and in the strengths/limitations section they write that the project was “minimizing potential confounding and enhancing the reliability of the findings”. This is too strong, considering that they themselves acknowledge missing data

on severity of screening risk, biochemical markers, smoking, ART, chronic diseases, etc., and the models are adjusted only for age and parity.

Answer: Specifically, statements suggesting that confounding was minimized or controlled have been replaced with wording indicating that the study design allowed comparison between groups with similar clinical indications, while acknowledging that residual confounding cannot be excluded due to the retrospective design and the absence of detailed data on several potential confounders.

In Introduction:

“This design allowed a comparison of outcomes between women undergoing amniocentesis and those opting for NIPT within a cohort with similar clinical indications, partially addressing potential confounding factors.”

In Limitation:

This design allowed for a more balanced comparison between groups with similar clinical indications. However, residual confounding cannot be completely excluded due to the retrospective design and the absence of detailed data on several potential confounders.

Overall, these are rather smaller issues to correct now.

Answer: We thank the reviewer for the careful re-evaluation of our manuscript and for the positive assessment of the revised version. All comments have been carefully addressed, and the manuscript has been revised accordingly to further improve clarity and methodological transparency.