GOCM Gynecology and Obstetrics Clinical Medicine

PEER REVIEW HISTORY

Gynecology and Obstetrics Clinical Medicine publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Integrating MicroRNA Profiling and Protein Markers: A Novel Diagnostic Approach for Ectopic Pregnancy
AUTHORS	Dr. Sathya Selvarajan Dr. Jothi Malar Ramalingam Ms. Dhivya Senthil Kumar

VERSION 1 - REVIEW

REVIEWER NAME	***Dr. Jiasong Cao***
REVIEWER AFFILIATION	
REVIEWER CONFLICT OF INTEREST	No competing interests.
DATE REVIEW RETURNED	08-May-2024

GENERAL COMMENTS	I have completed the review of the manuscript entitled "Integrating MicroRNA Profiling and Protein Markers: A Novel Diagnostic Approach for Ectopic Pregnancy" submitted to Gynecology and Obstetrics Clinical Medicine (Manuscript ID: gocm-2024-000034). This study investigates a significant issue in obstetrics and provides intriguing insights into the use of microRNAs and protein markers for diagnosing ectopic pregnancy. I am delighted to review this article, but before considering its acceptance, the authors need to address the following issues: 1. The authors effectively highlight the limitations of conventional clinical techniques, such as transvaginal ultrasound, which often fails to detect small or premature ectopic pregnancies. They propose an intriguing alternative approach utilizing a combination of microRNA profiling and protein markers. This method is promising and could potentially offer a significant improvement in the detection rates of these challenging cases. However, it remains unclear from the manuscript how significantly this novel approach enhances the detection efficiency specifically for small or premature ectopic pregnancies compared to the traditional methods. 2. In relation to the quantitative detection of maternal serum β-hCG, progesterone, and sFLT-1 levels, microRNAs require higher precision in detection. How did the authors ensure the accuracy of microRNA detection in this study?

	3. While sFLT-1 is recognized for its predictive value in pre-eclampsia, its application in early pregnancy screening, particularly for the differential diagnosis between pre-eclampsia and ectopic pregnancy, warrants further elucidation. The manuscript commendably addresses the use of sFLT-1 alongside other biomarkers but could benefit significantly from a more detailed discussion on how to distinguish these two critical conditions effectively during early pregnancy. 4. In fact, there are many published miRNAs that can be used for screening and diagnosis of ectopic pregnancy. The authors have chosen to focus on eight specific miRNAs in this study. It would be beneficial for the manuscript if the authors could elaborate on the rationale and criteria behind the selection of these particular miRNAs. 5. The manuscript provides valuable insights into the use of miR-519d as a diagnostic marker for ectopic pregnancy. However, to enhance the depth and applicability of the findings, it would be beneficial if the authors could include results from combination tests involving other miRNAs beyond miR-519d. 6. The authors present an intriguing analysis of miRNA roles in diagnosing ectopic pregnancies. However, to provide a more complete scientific context and enhance the manuscript's utility, it would be beneficial if the authors could include a comprehensive list of miRNA target genes in the supplementary materials. 7. Please highlight the functions of different miRNAs in Table 2, especially the potential downstream signaling and biological functions mediated by miR-519d.

REVIEWER NAME	***Santhi Silambanan***
REVIEWER AFFILIATION	Sri Ramachandra Institute of Higher Education and Research (Deemed to be University)
REVIEWER CONFLICT OF INTEREST	NIL.
DATE REVIEW RETURNED	25-Jul-2024

GENERAL COMMENTS	NIL.

VERSION 1 – AUTHOR RESPONSE

Dear Reviewer,

We are pleased to resubmit our revised manuscript titled "Integrating MicroRNA Profiling and Protein Markers: A Novel Diagnostic Approach for Ectopic Pregnancy" for your consideration. We have carefully considered the insightful comments and suggestions provided by you and the other reviewers. Based on this valuable feedback, we have made the necessary revisions to improve the clarity, accuracy, and overall quality of our manuscript.

Attached, you will find a detailed response to each of the reviewers' comments, outlining the changes made and how these have enhanced our work. We believe these revisions have significantly strengthened our manuscript and addressed all of the concerns raised during the review process.

We sincerely appreciate the time and effort you have dedicated to reviewing our manuscript, and we look forward to your positive feedback on this revised version.

Regards,
Dr Sathya Selvarajan

To,

Jiasong Cao (Reviewer-1),

Thank you for your thoughtful feedback and for highlighting an important aspect of our work. We appreciate your recognition of the potential of our proposed approach combining microRNA profiling and protein markers as a novel diagnostic method for ectopic pregnancy.

Reply to Comments:

1. The authors effectively highlight the limitations of conventional clinical techniques, such as transvaginal ultrasound, which often fails to detect small or premature ectopic pregnancies. They propose an intriguing alternative approach utilizing a combination of microRNA profiling and protein markers. This method is promising and could potentially offer a significant improvement in the detection rates of these challenging cases. However, it remains unclear from the manuscript how significantly this novel approach enhances the detection efficiency specifically for small or premature ectopic pregnancies compared to the traditional methods.

We acknowledge that our current study does not specifically focus on small or premature ectopic pregnancies. Our aim was to assess the overall diagnostic efficacy of the combined markers for ectopic pregnancy, regardless of gestational age. However, we agree with the reviewer that evaluating the performance of this approach for smaller and earlier-stage ectopic pregnancies would provide valuable insights, especially given the limitations of traditional methods like transvaginal ultrasound in such cases. As such, we have now explicitly mentioned this as a limitation of the current study in the Limitations section of the manuscript. Future studies will investigate the diagnostic utility of this method in detecting small or premature ectopic pregnancies, with a focused cohort study providing more targeted insights.

2. In relation to the quantitative detection of maternal serum β-hCG, progesterone, and sFLT-1 levels, microRNAs require higher precision in detection. How did the authors ensure the accuracy of microRNA detection in this study?

The study focused on the accuracy of microRNA detection using several key strategies.

- The microRNA was extracted from serum samples using the miRNeasy Serum/Plasma Kit (QIAGEN), ensuring minimal RNA degradation and high yield.

- The RT-PCR protocol was performed using a two-step setup, including reverse transcription using the miScript II RT Kit and real-time PCR using specific microRNA primers and the Rotor-Gene Q 5PLEX HRM platform.

- Caenorhabditis elegans miR 39.1 was used as a spike-in control to monitor RNA isolation and reverse transcription efficiency across samples. RNU6 was used as an endogenous control to normalize microRNA expression levels.

- The QuantiTect SYBR Green PCR Kit (QIAGEN) was used for PCR amplification, providing accurate quantification with minimal non-specific amplification. All RT-PCR reactions were performed in triplicate to ensure precision and minimize experimental variability. These methodological controls and protocols were carefully selected to meet the precision requirements for quantitative studies.

3. While sFLT-1 is recognized for its predictive value in pre-eclampsia, its application in early pregnancy screening, particularly for the differential diagnosis between pre-eclampsia and ectopic pregnancy, warrants further elucidation. The manuscript commendably addresses the use of sFLT-1 alongside other biomarkers but could benefit significantly from a more detailed discussion on how to distinguish these two critical conditions effectively during early-pregnancy.

We would like to clarify that this study focuses on the role of sFLT-1 in ectopic pregnancy and does not explore its application in pre-eclampsia or its utility in differentiating between the two conditions. We acknowledge this as a limitation of the study and have now included it in the Discussion section. Future studies will aim to investigate sFLT-1's potential in pre-eclampsia and its diagnostic distinction from ectopic-pregnancy.

4. In fact, there are many published miRNAs that can be used for screening and diagnosis of ectopic pregnancy. The authors have chosen to focus on eight specific miRNAs in this study. It would be beneficial for the manuscript if the authors could elaborate on the rationale and criteria behind the selection of these particular miRNAs.

This study focuses on eight specific miRNAs associated with ectopic pregnancy. The selection process involved a comprehensive review of existing literature, focusing on miRNAs that have demonstrated significant changes in expression levels in the context of ectopic pregnancy compared to normal pregnancy and non-pregnant controls. These miRNAs were chosen based on their potential role in key biological processes such as implantation, inflammation, and trophoblast function.

The selection criteria included biological relevance, diagnostic potential, technical feasibility, novelty, and innovation. The selected miRNAs were chosen based on their availability of robust and validated detection methods that could be integrated into the diagnostic Approach.

The study employed well-established miRNA target prediction programs to identify potential gene targets of the differentially expressed miRNAs. The tools used included miRBase, a database providing comprehensive information about miRNA sequences and their known targets, and DIANA Tools – mirPath, which performed pathway enrichment analysis to identify biological pathways significantly associated with the target genes of the differentially expressed miRNAs. The results of the gene target predictions and pathway analyses are summarized in Table 2 of the manuscript, providing insights into the potential biological functions and implications of the miRNAs in the context of ectopic pregnancy.

5. The manuscript provides valuable insights into the use of miR-519d as a diagnostic marker for ectopic pregnancy. However, to enhance the depth and applicability of the findings, it would be beneficial if the authors could include results from combination tests involving other miRNAs beyond miR-519d.

We appreciate your suggestion to include results from combination tests involving other miRNAs. However, we did not perform combination analyses with additional miRNAs because our preliminary investigations indicated that these miRNAs do not significantly contribute to the diagnostic accuracy for ectopic pregnancy in conjunction with miR-519d. Including these analyses would not provide meaningful insights or improve the clinical applicability of our findings. Therefore, we focused solely on miR-519d, which we identified as the most relevant and useful marker in this context.

6. The authors present an intriguing analysis of miRNA roles in diagnosing ectopic pregnancies. However, to provide a more complete scientific context and enhance the manuscript's utility, it would be beneficial if the authors could include a comprehensive list of miRNA target genes in the supplementary materials.

We have included a detailed list of miRNA target genes in the supplementary materials. This supplementary document provides an extensive overview of the genes targeted by the miRNAs discussed in our study, offering additional context and supporting the findings presented in the manuscript.

7. Please highlight the functions of different miRNAs in Table 2, especially the potential downstream signalling and biological functions mediated by miR-519d.

We would like to point out that these functions, including the downstream signaling pathways and biological roles of miR-519d, are thoroughly discussed in the manuscript's discussion section. Additionally, we have included detailed descriptions and analyses of these functions in the supplementary document.

VERSION 2 – REVIEW

REVIEWER NAME	***Dr. Jiasong Cao***
REVIEWER AFFILIATION	
REVIEWER CONFLICT OF INTEREST	No competing interests.
DATE REVIEW RETURNED	01-Sep-2024

GENERAL COMMENTS	I have reviewed the revised manuscript on the novel diagnostic approach for ectopic pregnancy. You have comprehensively addressed my comments and improved the manuscript significantly. The manuscript now adequately addresses my concerns and is suitable for publication. Thank you for considering my suggestions.

VERSION 2 – AUTHOR RESPONSE

Dear Editor,

Thank you for providing the opportunity to revise our manuscript entitled “Integrating MicroRNA Profiling and Protein Markers: A Novel Diagnostic Approach for Ectopic Pregnancy” (manuscript ID

gocm-2024-000034.R2). We have carefully addressed all the comments from the reviewer and the editor. Please find our detailed responses below:

Reviewer 1: Dr. Jiasong Cao

Comment:

I have reviewed the revised manuscript on the novel diagnostic approach for ectopic pregnancy. You have comprehensively addressed my comments and improved the manuscript significantly. The manuscript now adequately addresses my concerns and is suitable for publication. Thank you for considering my suggestions.

Response:

Thank you for your positive feedback and for acknowledging the improvements made to the manuscript. We appreciate your valuable suggestions which have significantly enhanced the quality of our work. We are pleased to hear that the revised manuscript now adequately addresses your concerns and is deemed suitable for publication.

Associate Editor

Comment 1:

Please see the comments in the attachment, and make sure that each one is replied and modified.

Response 1:

We have carefully reviewed the comments in the attachment and have addressed each one as follows:

Office:

• Comment: What this study adds? More detailed.

Response: We have expanded the section "What this study adds" to provide a more detailed explanation of the novel contributions and implications of our research.

• Comment: In Figure 2, the authors highlight and color some of the results but without notes.

Response: We have converted Figure 2 into Table 1 as suggested. The table does not include any highlighted or colored results.

• Comment: The data within the table are exported directly from the software without any uniformation of the number of digits or standardization.

Response: Thank you for pointing out the lack of uniformity in the number of digits and standardization in the table data. We have reviewed the data and standardized the number of decimal places to ensure consistency across all entries. Specifically, we have formatted all numerical values to six decimal places in column 2 (p-value). The updated table now reflects this standardization. We believe this improves the clarity and readability of the data presented.

Mingzhu Li:

• Comment: Does the font need to be bold?

Response: yes, we have reviewed the manuscript and ensured that the font needs to be bold to highlights the results.

• Comment: Please change to the format of refs as (23) and (24).

Response: We have updated the references to the requested format, using (23) and (24) as specified.

• Comment: Please mark the a., b., c. in the top left corner of the picture, and move the content: a) β-HCG, b) progesterone... to the legend.

Response: We have marked the a., b., c. in the top left corner of the picture and moved the content (a) β-HCG, b) progesterone...) to the legend as requested.

• Comment: Please also use standard presentation for AUC.

Response: We have revised the presentation of AUC to follow the standard format.

• Comment: It is recommended that Figure 2 be presented in a standard tabular format.

Response: We have converted Figure 2 into Table 1 as suggested, presenting the data in a standard tabular format.

Holly Snow:

Comment: There's no mention about the supplementary of "MiR gene targets and Pathway analysis" in the content, and the title of this table (S Table 1) should be more specific, such as "from …".

Response: We have included a mention of the supplementary file "MiR gene targets and Pathway analysis" in the main content. Additionally, we have updated the title of S Table 1 to be more specific, such as "from MiR gene targets and Pathway analysis".

Comment 2:

Please upload the revised file within 3 days.

Response 2:

We will ensure that the revised manuscript is uploaded within the specified timeframe.

Editor Comments

Comment 1:

Please provide a point-by-point response to the Editor's comments and reviewer's comments.

Response 1:

We have provided a detailed point-by-point response to all comments from both the reviewer and the editor in this document.

Comment 2:

One of the co-authors in the submission system is different from the main document.

System: Jothi Malar Ramalingam,

Main Document: Jothimalar Ramalingam

Response 2:

We apologize for the discrepancy. The correct name is Jothi Malar Ramalingam. We have updated the main document to reflect this.

Comment 3:

Citation for table 1 is missing in the main text. Please recheck and amend accordingly.

Response 3:

Thank you for your comment. The data presented in Table 1 was generated by the authors based on our original research and analysis. Therefore, it does not require an external citation. We have mentioned this in the main document-marked copy

Comment 4:

Kindly label the provided supplementary file.

Response 4:

We have labelled the supplementary file in Main Document as requested.

Additional Information:

We have ensured that all ORCID IDs have been added to the co-authors' ScholarOne accounts as required.

Please let us know if there are any further modifications needed. Thank you for your time and consideration.

Kind regards,

Dr Sathya Selvarajan

07.09.2024

Dear Editor,

Thank you for your email and for the opportunity to revise our manuscript entitled “Integrating MicroRNA Profiling and Protein Markers: A Novel Diagnostic Approach for Ectopic Pregnancy” (manuscript ID gocm-2024-000034.R2). We have addressed the issues raised and made the necessary corrections as outlined below:

1. Author Affiliation Mismatch:

o We have corrected the author affiliation for Selvarajan, Sathya to ensure consistency between the submission system and the main document.

2. Table Citations Missing:

o The in-text citations for ‘Tables 1a and 1b’ have been added. We have also reviewed and ensured that all table citations are in ascending order throughout the manuscript.

Additionally, we have ensured that all co-authors have linked their ORCID IDs to their ScholarOne accounts as requested.

We appreciate your guidance and look forward to your Positive feedback.

Regards,

Dr. Sathya Selvarajan

Date 10.09.2024

Dear Editor,

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Kind regards,

Dr Sathya Selvarajan