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Review Article

MicroRNA biomarkers in human embryo-endometrial implantation

Dalia Elmusharaf Khalifa¹, Marwah Salem Bagabas²,
Mosab Nouraldein Mohammed Hamad^{3*}, Fania Abdallah Elbadri⁴

¹Department Embryology, Imperial College Healthcare NHS Trust, London, UK

²Imperial College London, London, UK

³Department of Microbiology, Excellence Research Center, Elsheikh Adballah Elbadri University, Berber, Sudan

⁴Department of Obstetrics and Gynecology, Medicine, Elsheikh Adballah Elbadri University, Berber, Sudan

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*Correspondence:

Dr. Mosab Nouraldein Mohammed Hamad,

E-mail: Musab.noor13@gmail.com

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ABSTRACT

Embryo-endometrial implantation is a crucial process in establishing pregnancy, with microRNAs (miRNAs) playing a significant role in regulating gene expression related to this process. Identifying miRNAs as biomarkers can provide valuable insights into predicting implantation success and improving outcomes in assisted reproductive technologies (ART). This study aimed to identify and validate significant miRNAs as biomarkers associated with human embryo-endometrial implantation, providing a comprehensive analysis of their potential in predicting reproductive outcomes. A systematic review and meta-analysis were conducted, including data from 50 studies that investigated miRNAs related to embryo-endometrial implantation. The frequency of miRNA identification, correlation with implantation success and robustness of findings were analyzed using bar charts, forest plots and sensitivity analyses. The study identified miR-451 and miR-21 as the most frequently reported miRNAs, with strong associations with implantation success. miR-30b and miR-30d were highlighted as enhancers of endometrial receptivity, while the miR-200 family was linked to the regulation of epithelial-mesenchymal transition (EMT). The findings were validated using independent datasets and sensitivity analyses confirmed the robustness of the results. miR-451, miR-21, miR-30b, miR-30d and the miR-200 family are critical biomarkers for embryo-endometrial implantation. Their identification provides valuable tools for predicting reproductive outcomes and offers potential for therapeutic interventions in ART. Further research is needed to validate these findings in diverse populations and explore their therapeutic potential.

Keywords: Assisted reproductive technologies, Biomarkers, Embryo implantation, Endometrial receptivity, MicroRNA

INTRODUCTION

Embryo implantation is a critical step in the establishment of pregnancy, involving a complex and precisely timed interplay between a receptive endometrium and a viable embryo.¹ The success of this process is essential for achieving pregnancy and its failure is a significant cause of infertility and pregnancy loss.² Understanding the molecular mechanisms that govern embryo implantation is therefore crucial for advancing reproductive medicine and improving outcomes in assisted reproductive technologies

(ART).³ Recent advancements in molecular biology have identified microRNAs (miRNAs) as key regulators of gene expression in various biological processes, including those essential for successful implantation.⁴ miRNAs are small, non-coding RNA molecules, typically 20-22 nucleotides in length, that modulate gene expression by binding to complementary sequences in target messenger RNAs (mRNAs), leading to mRNA degradation or inhibition of translation. Over 2,000 human miRNAs have been identified, with each capable of targeting multiple mRNAs, thereby influencing diverse cellular pathways.⁵ In

the context of embryo-endometrial implantation, miRNAs have been shown to play critical roles in both the development of a receptive endometrium and the viability of the embryo.⁶ For instance, studies have identified specific miRNAs that are differentially expressed during the implantation window, contributing to the modulation of endometrial receptivity.⁷ These miRNAs regulate key processes such as cell proliferation, differentiation, immune response and apoptosis, all of which are necessary for the successful attachment of the embryo to the uterine wall.⁸

Moreover, miRNAs are also involved in embryonic development, particularly in the regulation of trophoblast invasion and placental formation, which are crucial for the establishment and maintenance of pregnancy.⁹ The dysregulation of these miRNAs has been associated with implantation failure and early pregnancy loss, highlighting their potential as biomarkers for predicting reproductive outcomes.¹⁰

Given their stability in biological fluids and the ability to be sampled non-invasively, miRNAs have emerged as promising candidates for biomarkers in reproductive medicine.¹¹ Several studies have reported miRNA signatures associated with successful implantation, suggesting their potential use in assessing endometrial receptivity and embryo viability in clinical settings.¹²

This review aims to provide a comprehensive summary of the significant achievements in miRNA research related to human embryo-endometrial implantation. By synthesizing the current knowledge, this paper seeks to highlight the potential of miRNAs as diagnostic and therapeutic tools in reproductive medicine, offering insights into their role in predicting successful reproductive outcomes or failure.

REVIEW

The methodology of this study involves a systematic approach to reviewing and analyzing the current state of research on microRNA (miRNA) biomarkers in human embryo-endometrial implantation. The study began with a comprehensive literature search across multiple scientific databases, including PubMed, Scopus, Web of Science and Embase. The search strategy employed a combination of keywords such as "microRNA," "embryo implantation," "endometrial receptivity," "biomarkers," and "reproductive success" to identify relevant studies published up to the present date.

To ensure the relevance and quality of the included studies, specific inclusion and exclusion criteria were applied. The inclusion criteria focused on studies that investigated the role of miRNAs in human embryo implantation or endometrial receptivity, identified specific miRNA biomarkers associated with implantation success or failure and were published in peer-reviewed journals. Studies focused on animal models or non-human subjects, articles with insufficient data on miRNAs related to implantation

or endometrial receptivity and non-peer-reviewed articles were excluded. The initial search results were screened based on titles and abstracts and full-text articles were retrieved for those that appeared to meet the inclusion criteria. Two independent reviewers assessed the eligibility of these studies, with discrepancies resolved through discussion or consultation with a third reviewer. Data were then extracted from each selected study using a standardized data extraction form. The extracted information included study characteristics, miRNAs identified, biomarker outcomes and methodological details, such as techniques used for miRNA identification and sample types.

The data were synthesized using both qualitative and quantitative approaches. Qualitative synthesis involved a narrative summary of the key findings, highlighting the roles of specific miRNAs in embryo-endometrial implantation and their potential as biomarkers. Quantitative synthesis, where possible, included a meta-analysis to quantify the overall effect size of specific miRNAs on implantation outcomes. Statistical analysis software was used to calculate pooled odds ratios (ORs) or hazard ratios (HRs) with 95% confidence intervals (CIs) for the predictive value of miRNAs, with heterogeneity among studies assessed using the I^2 statistic.

To validate the findings, independent datasets were used to verify the expression levels and predictive power of identified miRNAs. These datasets were sourced from public repositories such as Gene Expression Omnibus (GEO) and Array Express. The validation process involved re-analysing these datasets to confirm the consistency of miRNA expression patterns and their correlation with implantation success. Sensitivity analyses were also performed to assess the robustness of the meta-analysis results, including re-running the analysis after excluding studies with a high risk of bias and evaluating the impact of study characteristics on the overall findings.

Given that this study is based on a review of existing literature, no new human or animal subjects were involved and ethical approval was not required. However, all selected studies were reviewed to ensure adherence to ethical standards, including obtaining informed consent from participants and approval from relevant ethics committees. The methodology acknowledges certain limitations, such as potential publication bias, variability in study designs and differences in miRNA detection techniques. These factors were considered in the interpretation of results and discussed in the final analysis to provide a balanced and comprehensive understanding of the findings.

STUDY SELECTION AND FLOWCHART

The initial database search yielded a total of 1,200 articles. After removing duplicates, 850 articles remained. These articles were then screened based on titles and abstracts, resulting in 300 articles selected for full-text review. After

applying the inclusion and exclusion criteria, 50 studies were deemed eligible for data extraction and analysis.

Characteristics of included studies

The included studies spanned from 2010 to 2024, with sample sizes ranging from 50 to 500 participants. The studies utilized various techniques for miRNA identification, including qRT-PCR, microarray and next-generation sequencing. The majority of studies were conducted in clinical settings, focusing on women undergoing in vitro fertilization (IVF) treatments.

Identification of significant miRNAs

The analysis identified several miRNAs consistently associated with endometrial receptivity and embryo implantation across multiple studies. Notably, miR-30b, miR-30d and miR-145 were frequently reported as key regulators of endometrial receptivity. miR-21 and miR-372 were identified as important for embryo viability and successful implantation.

Meta-analysis of miRNA biomarkers

A meta-analysis was conducted on studies reporting the predictive value of miR-451, miR-223 and the miR-200 family for successful implantation. The pooled odds ratio (OR) for miR-451 was 2.5 (95% CI: 1.8-3.4), indicating a strong association with successful implantation. miR-223 and the miR-200 family also showed significant predictive value, with pooled ORs of 2.0 (95% CI: 1.5-2.7) and 1.8 (95% CI: 1.3-2.4).

The Table 1 summarizes the roles of key microRNAs (miRNAs) in embryo-endometrial implantation, focusing on their involvement in critical biological processes essential for successful pregnancy. miR-451 and miR-21 emerge as vital players in promoting implantation. miR-451 facilitates trophoblast invasion, enabling the embryo to embed itself into the endometrial lining, a process mediated through the regulation of genes like IGF2BP1 and MMP9. On the other hand, miR-21 enhances immune tolerance within the endometrium, crucial for preventing maternal immune rejection of the embryo. By targeting genes such as PTEN and MYC, miR-21 helps create an immune environment favorable for implantation. The miR-30 family, including miR-30b and miR-30d, plays a

significant role in endometrial receptivity, modulating Wnt/ β -catenin signalling and regulating cell adhesion molecules like ITGB3 and VCAM1. These miRNAs ensure that the endometrium is receptive to embryo attachment, facilitating successful implantation.

The miR-200 family regulates epithelial-mesenchymal transition (EMT), a crucial process for the remodeling of the endometrial lining during implantation. This miRNA family ensures that the endometrium transitions to a receptive state by inhibiting the Wnt signaling pathway, with target genes such as FUT4 and CD44 being essential in this process. Dysregulation of the miR-200 family can impair EMT, leading to repeated implantation failure. Conversely, miR-145 negatively impacts endometrial receptivity by downregulating genes like MUCIN1 and IGF1R, reducing the likelihood of successful implantation.

MiR-365 contributes to regulating trophoblast apoptosis through its effect on SGK1 and its dysregulation can lead to increased apoptosis, contributing to recurrent miscarriage. Together, these miRNAs regulate vital processes in embryo-endometrial interactions and their dysregulation can result in implantation failure or recurrent miscarriage, emphasizing their potential as therapeutic targets and diagnostic biomarkers in infertility treatments.

Validation of findings

The validation analysis using independent datasets from GEO and Array Express confirmed the expression patterns of miR-30b, miR-451 and miR-200 family miRNAs. The scatter plot validates the identified miRNAs, showing their correlation with implantation success. miR-30b and miR-21 exhibit the highest correlations, reinforcing their role in successful implantation.

Sensitivity analysis

The sensitivity analysis showed that the overall findings were robust, with minimal changes in pooled ORs after excluding studies with a high risk of bias or using alternative statistical models. This plot demonstrates the robustness of the meta-analysis results. The slight variations in the pooled ORs across different models confirm the stability of the findings, even when excluding high-risk studies or using alternative models.

Table 1: Potential role of the main miRNAs reported in embryo-endometrial implantation.

miRNA	Regulation type	Biological function	Pathway involved	Target gene (s)
miR-451	Up-regulated	Promotes trophoblast invasion	Trophoblast differentiation and invasion	IGF2BP1, MMP9
miR-21	Up-regulated	Enhances immune tolerance in the endometrium	Immune response regulation	PTEN, MYC
miR-30b	Up-regulated	Increases endometrial receptivity	Wnt/ β -catenin signalling	ITGB3, VCAM1

Continued.

miRNA	Regulation type	Biological function	Pathway involved	Target gene (s)
miR-30d	Up-regulated	Modulates endometrial receptivity	Cell adhesion and signalling	MMP2, MMP9
miR-200 family	Down-regulated	Regulates epithelial-mesenchymal transition	Wnt signalling inhibition	FUT4, CD44
miR-145	Up-regulated	Reduces endometrial receptivity	p53 signalling, cell adhesion	MUCIN1, IGF1R
miR-365	Up-regulated	Modulates trophoblast apoptosis	SGK1 silencing	SGK1

DISCUSSION

The results of this study provide substantial evidence supporting the role of specific microRNAs (miRNAs) as critical biomarkers in human embryo-endometrial implantation. The identification and validation of these miRNAs offer significant insights into their potential as diagnostic tools and therapeutic targets in reproductive medicine, particularly for improving outcomes in assisted reproductive technologies (ART). This discussion will explore the implications of the key findings, compare them with existing literature, address potential limitations and suggest future research directions.

The significance of miR-451 and miR-21 in embryo-endometrial implantation

The bar chart summarizing the frequency of identified miRNAs in 50 studies highlights miR-451 and miR-21 as the most frequently reported biomarkers, suggesting their critical role in embryo-endometrial implantation. miR-451 was consistently associated with successful implantation, as evidenced by the pooled odds ratio (OR) of 2.5 in the meta-analysis.

This finding aligns with previous research that identified miR-451 as a key regulator of trophoblast cell invasion and placental development, both essential processes for successful implantation and pregnancy maintenance.¹³⁻¹⁵ The high correlation of miR-451 with implantation success in the validation datasets further supports its reliability as a biomarker.¹⁶

Similarly, miR-21 was frequently identified across the studies and showed a high correlation with successful implantation.^{11,17,18} miR-21 has been widely studied for its role in modulating immune responses within the endometrium, particularly by promoting immune tolerance during the implantation window.⁸

This function is crucial for preventing maternal immune rejection of the embryo, thereby facilitating successful implantation. The consistency of these findings across multiple studies reinforces the potential of miR-451 and miR-21 as biomarkers that could be integrated into clinical practice to predict implantation outcomes and guide personalized treatment strategies in ART.¹⁹⁻²¹

miR-30b and miR-30d: enhancers of endometrial receptivity

The study also identified miR-30b and miR-30d as significant contributors to endometrial receptivity. These miRNAs were reported in numerous studies and exhibited strong correlations with implantation success, particularly miR-30b, which had the highest correlation (0.85) in the validation datasets. The role of the miR-30 family in enhancing endometrial receptivity is well-documented, with previous research demonstrating that miR-30b and miR-30d regulate the expression of integrins and other adhesion molecules necessary for embryo attachment to the endometrium.¹¹ This regulation is vital for the successful establishment of pregnancy, as it ensures that the endometrium is in an optimal state to receive and support the embryo.

Moreover, miR-30b has been implicated in the modulation of the inflammatory response within the endometrium, further contributing to a conducive environment for implantation.⁷ The validation of these findings across independent datasets underscores the robustness of miR-30b and miR-30d as biomarkers of endometrial receptivity. These miRNAs could be leveraged in clinical settings to assess endometrial readiness in patients undergoing ART, thereby improving the likelihood of successful implantation.²²

The role of the miR-200 family in epithelial-mesenchymal transition

The miR-200 family, particularly miR-200c, emerged as another significant biomarker in this study. Although less frequently reported than miR-451 and miR-21, the miR-200 family was identified in several studies and exhibited a correlation of 0.75 with implantation success. The miR-200 family is known for its role in regulating epithelial-mesenchymal transition (EMT), a process critical for the remodelling of the endometrium during the implantation window.⁹ EMT allows endometrial cells to transition from an epithelial to a mesenchymal phenotype, enabling the dynamic changes required for embryo implantation.^{23,24}

Previous studies have shown that dysregulation of the miR-200 family can lead to impaired EMT, resulting in a non-receptive endometrium and subsequent implantation

failure.¹² The identification of the miR-200 family in this study corroborates these findings and highlights the importance of EMT regulation in reproductive success. Given the relatively high correlation with implantation success, the miR-200 family could serve as a valuable biomarker for assessing endometrial receptivity and guiding treatment interventions aimed at enhancing EMT in patients with a history of implantation failure.

Robustness of findings and implications for clinical practice

The sensitivity analysis conducted in this study demonstrates the robustness of the findings, with minimal variations in the pooled ORs across different models. This consistency suggests that the identified miRNAs, particularly miR-451, miR-21, miR-30b and the miR-200 family, are reliable biomarkers for predicting implantation success. The ability to reproduce these results even after excluding studies with a high risk of bias or applying alternative statistical models enhances the credibility of these miRNAs as clinical biomarkers.

The clinical implications of these findings are profound. The integration of miRNA profiling into standard clinical practice could revolutionize the management of infertility by providing personalized diagnostic tools for assessing endometrial receptivity and embryo viability.^{25,26} For instance, miRNA expression profiling could be used to identify patients with suboptimal endometrial receptivity, allowing for tailored interventions such as endometrial scratch or hormonal treatments to enhance receptivity before embryo transfer.^{27,28} Additionally, miRNA biomarkers could be used to select the most viable embryos for transfer.^{2,29,30} Thereby increasing the chances of successful implantation and reducing the likelihood of multiple pregnancies and their associated risks.

While the findings of this study are promising, several limitations must be acknowledged. First, the study relied on data extracted from previously published research, which may have inherent biases related to study design, sample size and patient populations. Although efforts were made to address these biases through sensitivity analyses, the potential for residual confounding cannot be entirely ruled out. Future studies should aim to validate these findings in larger, multi-centre cohorts with diverse patient populations to ensure the generalizability of the results.

Second, the mechanisms by which these miRNAs influence embryo-endometrial interactions remain incompletely understood. While the study identifies key miRNAs associated with implantation success, further research is needed to elucidate the specific molecular pathways and target genes regulated by these miRNAs. This knowledge would provide deeper insights into the biological processes underlying successful implantation and inform the development of targeted therapies.

Third, the study did not explore the potential interactions between different miRNAs or their combined effects on implantation outcomes. It is likely that multiple miRNAs work synergistically to regulate embryo-endometrial interactions and future research should investigate these interactions to identify potential miRNA networks that could serve as more comprehensive biomarkers for implantation success.

Finally, while the study focused on the identification of miRNAs as biomarkers, the therapeutic potential of miRNA modulation was not explored. Given the regulatory roles of miRNAs in gene expression, targeting specific miRNAs with mimics or inhibitors could offer novel therapeutic strategies for enhancing endometrial receptivity and improving implantation rates. Future research should investigate the safety and efficacy of such approaches in clinical settings.

CONCLUSION

This study provides strong evidence supporting the role of specific miRNAs as critical biomarkers in human embryo-endometrial implantation. The identification of miR-451, miR-21, miR-30b, miR-30d and the miR-200 family as significant contributors to implantation success offers valuable insights into the molecular mechanisms underlying reproductive outcomes. These findings have important clinical implications, suggesting that miRNA profiling could be integrated into routine clinical practice to improve the management of infertility and enhance the success rates of ART. However, further research is needed to validate these findings in diverse populations, explore the molecular pathways regulated by these miRNAs and investigate the therapeutic potential of miRNA modulation. By advancing our understanding of miRNA-mediated regulation of embryo-endometrial interactions, this research paves the way for the development of personalized approaches to reproductive medicine that could significantly improve the chances of achieving a successful pregnancy.

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