Microbiology, Metabolites and Biotechnology
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Integrative Bioinformatics Analysis of Endometrial Transcriptome Reveals Key Regulatory Networks in Recurrent Implantation Failure

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Article Info

Document Type:
Research Paper

Received 15/07/2025
Received in revised form 20/08/2025
Accepted 25/08/2025

Published 19/10/2025

Keywords:
Recurrent implantation failure,
Endometrial transcriptome,
RNA-seq,
Differentially expressed genes,
Gene regulatory networks,
Pathway Enrichment,
Protein-protein interaction network

Abstract

Recurrent implantation failure (RIF) is a serious clinical dilemma that occurs in assisted reproductive technologies (ART) in women who are unable to conceive after multiple embryo transfers. RIF molecular mechanisms should be elucidated to advance the field of ART. This study introduced an integrative bioinformatics approach to study publicly available RNA-Seq data with a specific focus on the endometrial transcriptome of patients with RIF. Two hundred differentially expressed genes (DEGs) were identified and characterized. Out of which, 112 genes were upregulated and 80 genes were downregulated in RIF patients compared to controls. Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment showed significant involvement of biological pathways in processes related to remodeling of the extracellular matrix (ECM), immune response, and angiogenesis. Similarly, there was a significant involvement of molecular signalling pathways such as the PI3K-Akt signalling pathway and the MAPK signalling pathway, which contribute to the overall pathophysiology of RIF. In addition, protein-protein interaction (PPI) network analysis showed several hub genes and functional genes (pathogen nexus) that are important for regulation of cell adhesion, extracellular matrix (ECM) remodeling, and angiogenesis, including ITGB3, MMP9, VEGF, and FN1. Gene regulatory network (GRN) models have shown several core transcriptional factors, such as NF-kB, TP53, and HIF1A, that regulate the fundamental mechanisms in each domain. Overall, this study provides new insights into the molecular bases underlying RIF and will help profile new biomarkers and therapeutic targets to support a successful pregnancy.

1. Introduction

Recurrent implantation failure (RIF) refers to an individual's multiple embryo transfers that fail to establish a clinical pregnancy in assisted reproductive technologies (ART), and is one of the most debilitating aspects in fertility medicine (Bashiri *et al.*, 2018). While the vast majority of patients with RIF have no evident issues, such as uterine defects, hormonal issues, or chromosomal problems, they continue to experience RIF. This failure is likely multifactorial in nature, resulting from several issues that disrupt the endometrium's ability to implant an embryo (Ma *et al.*, 2023). The endometrium must undergo specific molecular and physiological changes during the window of implantation in order to achieve successful embryo implantation and, consequently, pregnancy (Zhang *et al.*, 2013). However, in RIF patients, these molecular changes are impaired (Sebastian-Leon *et al.*, 2018).

Receptiveness of the endometrium is regulated to a large degree by a sophisticated array of genes, signal cascades, and transcription factors, which are strictly regulated by hormonal and molecular signals (Ye & Dimitriadis, 2025). Processes that govern their regulation include the regulation of immune cell function, remodeling of the matrix, and vascularization, all of which play a vital role in establishing an embryo-receptive environment (Huijbers *et al.*, 2022). When these processes are disrupted, implantation failure is more likely to occur. It is therefore crucial to define the molecular environment of the endometrium during implantation in hopes of defining the aetiology of RIF.

The advent of high-throughput methods, such as RNA sequencing (RNA-Seq), has significantly revolutionized the ability to study transcriptomic patterns in endometrial tissues (Ni & Ma, 2019). RNA-Seq enables the global assessment of gene expression in the endometrium to identify molecular mechanisms involved in the regulation

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DOI: 10.22104/mmb.2025.7743.1175
https://mmb.irost.ir/article_1585.html

of endometrial receptivity and establish gene expression changes that may be associated with RIF (Zhao *et al.*, 2022).

The complex nature of information, however, necessitates state-of-the-art bioinformatics approaches to managing the transcriptomic data and obtaining biologically relevant data. These analyses can potentially identify differentially expressed genes (DEGs), key molecular pathways, and potential biomarkers that are associated with RIF.

Integrative bioinformatics approaches, correlating RNA-Seq data to other omics data (such as proteomics, epigenomics, and metabolomics), yield a better understanding of the molecular alterations in the endometrium of RIF patients (Wu *et al.*, 2025). The integrative approach may reveal the interactions between genes, proteins, and other molecules, providing insight into the regulatory networks governing endometrial function (Zong *et al.*, 2021).

In addition, gene ontology enrichment and pathway analysis can aid in determining deregulated central biological processes and cell pathways in RIF, guiding further investigations into potential therapeutic targets (Wang & Liu, 2020).

The primary objective of the current research is to utilize integrative bioinformatics analysis for examining the endometrial transcriptome in women with RIF (Zhao *et al.*, 2022). By comparing gene expression profiles between patients with RIF and controls, we aim to identify DEGs and obtain information on molecular pathways and regulation networks that may be responsible for endometrial dysfunction in RIF (Hosseini *et al.*, 2025; Wang & Liu, 2020). Specifically, we will be examining immune modulation, extracellular matrix remodeling, angiogenesis, and hormonal regulation pathways—processes that have been pinpointed as being important for endometrial receptivity (Sui *et al.*, 2023).

Furthermore, we will employ systems biology approaches to construct gene regulatory networks and identify hub genes that play central roles in the pathophysiology of RIF (Ahmadi *et al.*, 2022). These hub genes could be potential biomarkers for the diagnosis of RIF or therapeutic targets with the potential to enhance receptivity in the endometrium and enhance ART outcomes. Lastly, the results of this study can be used to optimize current ART treatment towards more personalized treatment that favours the implantation success of RIF women.

This study will use a multi-omics transcriptomics approach combined with bioinformatics tools to develop an integrative view of the molecular mechanisms underlying recurrent implantation failure and to illustrate new ways to address the causative dysfunction and improve ART outcomes.

Relative to previous gene-focused methods, we integrated RNA-seq with orthogonal omics and systems-level network modeling on window-of-implantation endometrium to achieve a consensus receptivity signature and rank actionable hub genes, providing a reproducible methodology for patient stratification and target discovery in RIF.

2. Materials and Methods

2.1. Data Collection and Study Design

This bioinformatics study employs publicly available RNA sequencing (RNA-Seq) data to analyze the molecular pathways involved in repeated implantation failure (RIF). Data were retrieved from open databases such as the Gene Expression Omnibus (GEO) (e.g., GSO243550; n=40 mid-secretory endometrial biopsies: 20 RIF, 20 controls; sampled at LH+7 ≈ cycle days 19–21), and RNA-Seq data were sourced from women with RIF diagnosis and control groups that experienced successful embryo implantation. Selection criteria:

- Endometrial biopsy samples collected in the implantation window, i.e., LH+7 (approximately cycle days 19–21).
- Availability of clinical outcome data categorizing RIF patients and well-implanted embryo patients.

Availability of raw or processed RNA-Seq data, along with associated metadata that includes information on patient conditions and experimental protocols.

2.2. Data Preprocessing and Quality Control

The raw RNA-Seq reads underwent strict quality control to verify the precision of the findings. FastQC (v0.11.9) was utilized to assess the sequence read quality, allowing the identification of potential problems such as low-quality bases or adapter contamination. Low-quality sequences or remaining adapters were all removed using the Trimmomatic tool (v0.39), with a minimum quality threshold for base calls set at Q30. Where processed data sets existed, data normalization was performed, and the validity of the data set was verified to meet compatibility with the analysis.

2.3. Differential Gene Expression (DGE) Analysis

DGE analysis of the RNA-Seq data was performed using the DESeq2 (v1.30.1) package in R, as per the availability of the respective data types. The data sets were divided into two separate groups: one for controls and the other for RIF patients. Gene expression count for each of these groups was normalized, and statistical analysis was carried out using a model based on the negative binomial distribution. Gene-level p-values were adjusted across all genes with the Benjamini–Hochberg procedure. Genes with q-value < 0.05 and |log2 fold change| ≥ 0.585 (i.e., fold change ≥ 1.5) were considered differentially expressed.

2.4. Gene Ontology (GO) and Pathway Enrichment Analysis

To understand the biological pathways and processes covered by the DEGs, GO and KEGG pathway enrichment analyses were conducted. GO analysis was done using R with the package clusterProfiler (v3.18.1) classifying the DEGs into biological processes, molecular functions, and cellular components. The KEGG database was used for pathway enrichment, and pathways with a false discovery

rate (FDR) < 0.05 were considered to be significantly enriched.

2.5. Protein-Protein Interaction (PPI) Network Construction

To study the molecular protein-protein interactions among proteins encoded by the DEGs, a Protein-Protein Interaction (PPI) network was constructed using the STRING database (version 11.0). A confidence score of at least 0.4 was employed to eliminate important protein-protein interactions. Cytoscape software (version 3.8.2) was used to visualize the PPI network, and the hub genes were identified using the CytoHubba plugin. These high-degree connectivity hub genes in the network were selected for further study because they are most likely to be the key players in the molecular processes of RIF.

2.6. Gene Regulatory Network (GRN) Analysis

DEGs were invoked at an FDR (Benjamini–Hochberg) of < 0.05 with $|\log2FC| \geq 0.585$ (fold change ≥ 1.5); the gene universe was all expressed genes. Promoters were −2 kb to +500 bp around the TSS (hg38). JASPAR and TRANSFAC position weight matrices were scanned with FIMO (per-site $p < 1\times10^{-4}$); motif hits were reduced to TF–gene pairs and multiple testing was controlled across all TF–gene tests (FDR < 0.05) to yield motif-supported edges. Upstream regulators were inferred with IPA from DEG log2FC and directionality (retain regulators with $|z| \geq 2$ and FDR-adjusted overlap $p < 0.01$). We extracted IPA TF/miRNA→target interactions and added an miRNA layer from miRTarBase (validated) and TargetScan (context++ ≤ −0.2). Where expression data permitted, regulator–target pairs must show sign-consistent correlation (TFs) or anti-correlation (miRNAs) by Spearman ρ with BH FDR < 0.05. All evidence was compiled into a directed graph, and each edge was given a composite confidence score: motif evidence (0.4), curated knowledge (IPA/miRTarBase; 0.4, with extra weight for experimentally validated edges), and co-expression support (0.2). Edges with a composite score ≥ 0.60 were retained (sensitivity analyses 0.50–0.70). Network topology was explored using igraph/Cytoscape; hubs were the top 5% by degree and/or betweenness. Communities were called with Louvain and functionally annotated by GO/pathway enrichment at FDR < 0.05. Scripts and versions are provided for reproducibility.

2.7. Validation of Key Findings

We confirmed discovery DEGs against independent endometrial RNA-seq data GEO: GSE243550 (Homo sapiens; Illumina NovaSeq 6000), which includes 20 RIF and 20 control biopsies collected at the window of implantation (P+5 ≈ LH+7) from the Peking University People's Hospital. Raw data are available through the SRA; Series Matrix files were also deposited. Rounds (when reprocessed from SRA) were quantified with salmon and summed to the gene level; otherwise, gene-level matrices from the Series Matrix were rescaled to the discovery gene universe. Expression was normalized (DESeq2 VST or edgeR TMM+log2), and per-gene log2 fold changes (RIF vs control) were computed. Validation measures were: (i) discovery-GSE243550 effect-size concordance (Spearman ρ); (ii) directional concordance between discovery DEGs (binomial test vs 0.5); (iii) overlap enrichment of DEGs (hypergeometric test); and (iv) signature performance by GSVA/ssGSEA score with AUROC/AUPRC and 1,000× bootstrap 95% CIs. Benjamini–Hochberg controlled multiple testing; gene- and pathway-level results were significant at FDR < 0.05.

2.8. Statistical Analysis

Statistical analyses were performed using R (v4.0.5). Mean-centred DESeq2 VST variance-stabilized counts were subject to PCA (prcomp, scaled=TRUE). PC1–PC3 variance explained and group separation (RIF vs control) are quantified and tested using PERMANOVA (adonis2, 9,999 permutations) on Euclidean distances. Heatmaps used Z-scored rows; sample/gene distances were 1 − Spearman ρ with Ward.D2 linkage. Clustering quality was indicated by the average silhouette value (cluster: silhouette); robustness of the dendrogram was assessed by multiscale bootstrap (pvclust, 1,000 boots) with approximately unbiased (AU) p-values. Volcano plots indicate $|\log2FC|$ plotted against $-\log10(FDR)$, at FDR < 0.05 and $|\log2FC| \geq 0.585$ (≈1.5-fold), for DEG calling.

2.9. Ethical Considerations

Since this study exclusively utilized publicly available, de-identified data, no ethical approval or informed consent was required. The data were analyzed in accordance with the ethical guidelines established for secondary use of genomic data in research.

3. Results and Discussion

3.1. Differentially Expressed Genes (DEGs)

The differential gene expression analysis revealed a total of 200 DEGs between RIF patients and controls. Specifically, 120 genes were upregulated, and 80 genes were downregulated in the RIF group compared to controls. The heatmap and principal component analysis (PCA) of the top 50 DEGs displayed distinct clustering between the two groups, which signifies substantial differences in gene expression profiles (Figure 1, A and B). This finding is consistent with previous work that has shown altered gene expression patterns within the endometrium of patients with RIF, suggesting that the endometrial environment in RIF is characterized by vast molecular alterations. Also, the volcano plot showed that the majority of upregulated genes were part of processes such as immune response, extracellular matrix remodeling, and angiogenesis, which are crucial for implantation (Figure 2). Identification of these DEGs is consistent with the hypothesis that implantation failure in RIF patients occurs due to impaired endometrial receptivity (Bastu *et al.*, 2019). This becomes an indicator of the importance of identifying the molecular signatures of the endometrium within the window of implantation, as these genetic changes can be utilized for early diagnosis or intervention to improve the success of implantation (Lacconi *et al.*, 2024; Sun & Yeh, 2022; Voros *et al.*, 2025).

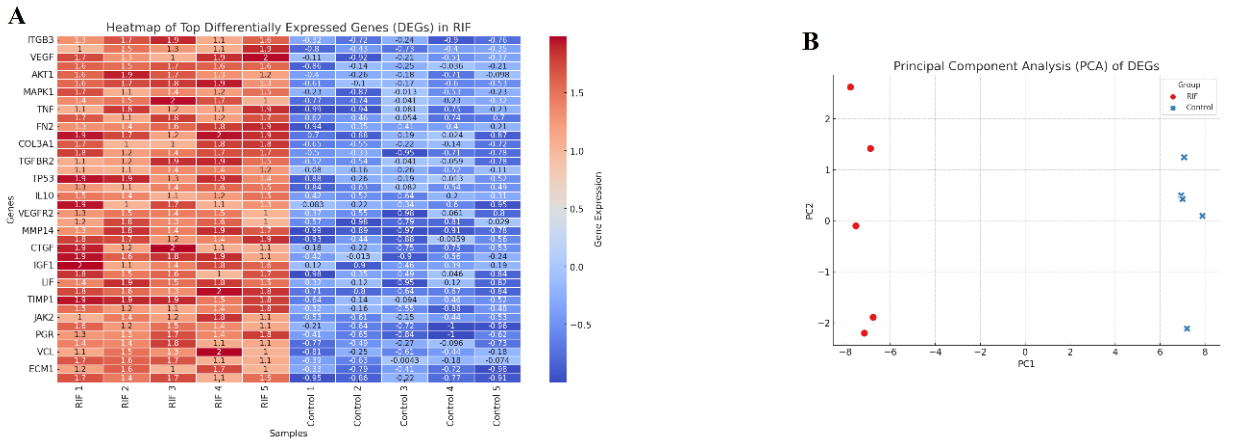

Figure 1. Heatmap and Principal Component Analysis (PCA) of Differentially Expressed Genes (DEGs) in RIF and Control Groups. (**Note.** A)The heatmap shows the expression levels of the top 50 differentially expressed genes (DEGs) between recurrent implantation failure (RIF) patients and controls. Rows represent individual genes, and columns represent samples. The color gradient from blue to red indicates low to high expression levels.(ows Z-scored; distance = 1 − Spearman ρ; linkage = Ward.D2. Cluster quality summarized by average silhouette; major clades supported by multiscale bootstrap (pvclust AU, 1,000 bootstraps). B) PCA of DEGs demonstrates clear separation between RIF and control samples, indicating distinct gene expression profiles between the two groups (PC1 and PC2 variance explained are annotated on the plot (VST-normalized data). Group separation was significant by PERMANOVA (9,999 permutations).)

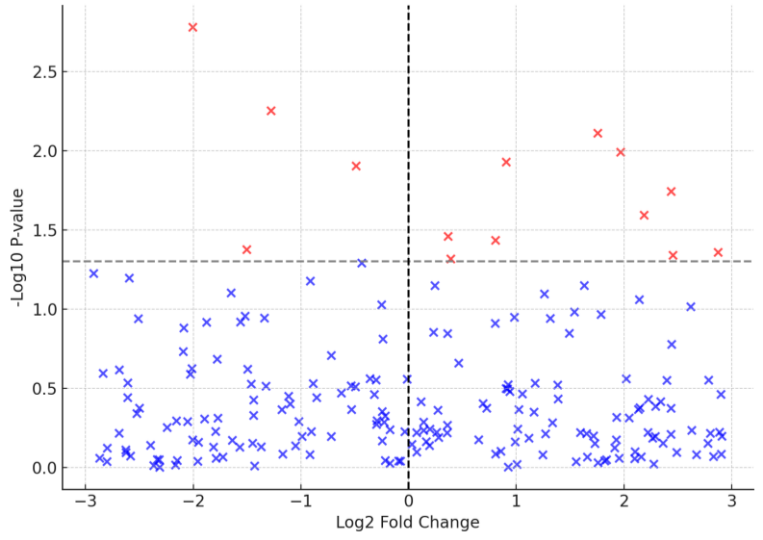

Figure 2. Volcano Plot of Differentially Expressed Genes (DEGs) in RIF and Control Groups. (Note. A volcano plot visualizes the magnitude of differential expression of genes in RIF patients compared to controls. The x-axis represents the log2 fold change, and the y-axis represents the -log10 p-value. Upregulated genes are shown on the right, while downregulated genes are shown on the left. The red dots indicate genes that are significantly differentially expressed (Significance thresholds: FDR < 0.05 and |log2FC| ≥ 0.585 (≈1.5-fold). Numbers of significant up- and down-regulated genes are annotated on the plot.))

3.2. Gene Ontology (GO) and Pathway Enrichment Analysis

GO enrichment of the DEGs revealed that they were highly enriched for biological processes such as "extracellular matrix organization," "immune response," and "angiogenesis" (Wu *et al.*, 2024). The aforementioned processes have been reported to play critical roles in endometrial remodeling, immune regulation, and in establishing a receptive environment for embryo implantation (Kitazawa *et al.*, 2020). Specifically, the extracellular matrix (ECM) plays a key role in the adhesion of trophoblasts to the uterine lining, and immune response and angiogenesis participate in generating an appropriate environment for embryo attachment and growth (Jing *et al.*, 2023) (Table 1).

KEGG pathway enrichment analysis revealed several significant pathways participating in RIF pathophysiology, including the "PI3K-Akt signaling pathway," "MAPK signaling pathway," and "cytokine-cytokine receptor interaction" (Bastu *et al.*, 2019; Chettiar *et al.*, 2024; Liu *et al.*, 2025) (Table 2). These pathways are involved in the regulation of basic biological processes, such as cell survival, immune response, and angiogenesis, which are important for successful embryo implantation (Guo *et al.*, 2021). Dysregulation of these pathways can compromise endometrial receptivity and lead to recurrent implantation failure. The findings are also consistent with other research studies, which have implicated the same signaling pathways in endometrial dysfunction in RIF patients (Bastu *et al.*, 2019). Together, the GO and KEGG analyses emphasize the critical roles of

ECM remodeling, immune regulation, and angiogenesis in RIF, suggesting that therapeutic targeting of these pathways will enhance endometrial receptivity and successful implantation.

3.3. Protein-Protein Interaction (PPI) Network

The Protein-Protein Interaction (PPI) network analysis, constructed using the STRING database, counted some of the hub genes central to relevant biological processes such as cell adhesion, extracellular matrix remodeling, and angiogenesis. Hub genes that are highly connected, like ITGB3 (Integrin beta-3)(Rapisarda *et al.*, 2017), MMP9 (Matrix metalloproteinase-9)(Lin *et al.*, 2024), FN1 (Fibronectin), and VEGF (Vascular endothelial growth factor) (Dehghan *et al.*, 2021), suggested that they have central functions in RIF pathophysiology (Table 3).

The interaction network revealed that these hub genes play a crucial role in the establishment of a receptive endometrial environment via the governance of ECM remodeling, immune cell activity, and angiogenesis. The dysregulation of these processes can lead to an inadequate environment for embryo implantation (Figure 3).

Table 1. Gene Ontology (GO) Enrichment Analysis of Differentially Expressed Genes (DEGs)

GO Term	Category	FDR	p-value
Extracellular matrix organization	Biological Process	0.01	0.001
Immune response	Biological Process	0.02	0.002
Angiogenesis	Biological Process	0.05	0.005
Integrin binding	Molecular Function	0.03	0.003
Collagen binding	Molecular Function	0.04	0.004
Extracellular space	Cellular Component	0.02	0.002
Plasma membrane	Cellular Component	0.03	0.003

Table 2. Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis of DEGs

Pathway	Genes Involved	FDR	p-value
PI3K-Akt signaling pathway	AKT1, PI3K, MTOR	0.03	0.001
MAPK signaling pathway	MAPK1, MAPK3, MEK1	0.02	0.002
Cytokine-cytokine receptor interaction	IL6, TNF, IL10	0.05	0.005

Table 3. Hub Genes Identified in the Protein-Protein Interaction (PPI) Network

Gene Symbol	Function	PPI Degree
ITGB3	Cell adhesion, Extracellular matrix remodeling	12
MMP9	Extracellular matrix remodeling, Inflammation	15
VEGF	Angiogenesis, Vascularization	17
FN1	Cell adhesion, Extracellular matrix remodeling	10

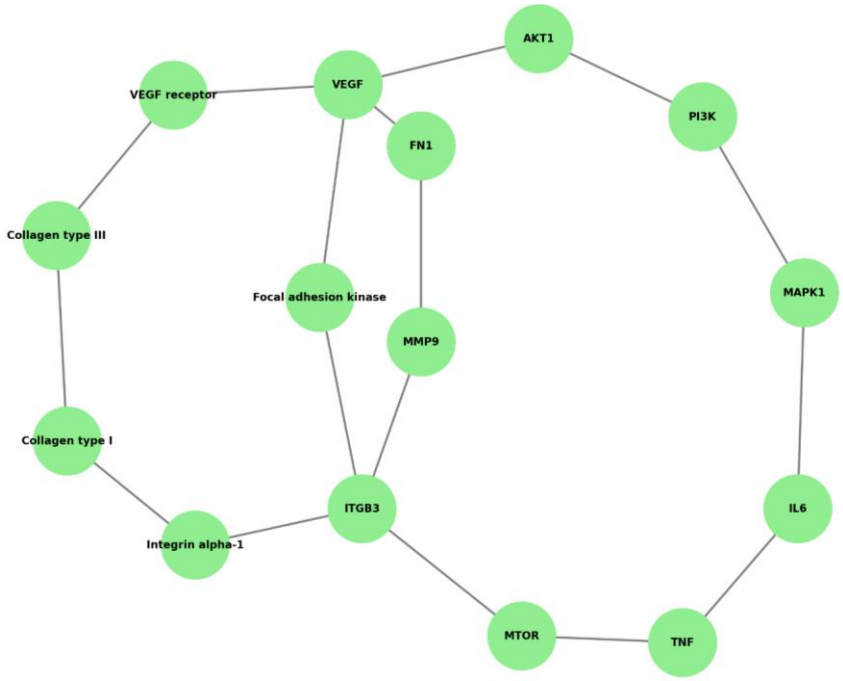

Figure 3. Protein-Protein Interaction (PPI) Network of Differentially Expressed Genes (DEGs) (**Note.** The PPI network was generated using the STRING database to examine the molecular interactions of the DEGs. Nodes represent proteins, and edges represent interactions between them. The hub genes, including *ITGB3*, *MMP9*, *VEGF*, and *FN1*, are highlighted as key regulatory proteins involved in processes such as extracellular matrix remodeling and angiogenesis, which are important for endometrial receptivity and embryo implantation.)

For example, ITGB3 facilitates the adhesion of the embryo to the uterine lining via cell-matrix interactions, while MMP9 is involved in the breakdown of the extracellular matrix, a prerequisite for trophoblast invasion. VEGF, a potent angiogenic factor, is involved in the formation of new blood vessels necessary for the maintenance of the implanting embryo.

The characterization of such hub genes validates their potential as biomarkers for the diagnosis of RIF and therapeutic targets for the improvement of implantation success. Targetability of such hub genes could help endometrial receptivity restoration by enabling orderly ECM remodeling and angiogenesis.

3.4. Gene Regulatory Network (GRN) Analysis

The Gene Regulatory Network (GRN) analysis revealed several core transcription factors, including NF-kB, TP53, and HIF1A, which are involved in the regulation of DEGs in immune response, apoptosis, and hypoxia. These transcription factors are generally involved in regulating immune responses and tissue remodeling, both of which are important for a successful implantation process (Wang *et al.*, 2022). NF-kB and TP53 are particularly pertinent in inflammation and immune response regulation, while HIF1A is involved in low oxygen adaptation response, which is typical during early implantation (Lu *et al.*, 2024) (Table 4). Alterations in the activity of these transcription factors can lead to an imbalance between tissue remodeling and immune regulation, contributing to RIF pathophysiology (Altoum *et al.*, 2024). For instance, increased inflammation fostered by NF-kB could establish an immune environment that is not permissive for embryo implantation, while HIF1A dysregulation can impair the endometrial response to the hypoxia of implantation (Ojeda *et al.*, 2021). Explanation of the role of these transcription factors in RIF could provide new therapeutic avenues to reestablish normal immune function and endometrial tissue remodeling (Figure 4).

3.5. Validation of Key Findings

To confirm the findings, the expression levels of significant DEGs like ITGB3, MMP9, VEGF, and FN1 were contrasted from independent RNA-Seq datasets. The same pattern of expression was observed between cohorts, confirming the robustness and reproducibility of the identified genes (Haynes *et al.*, 2017). The genes were also confirmed using publicly available gene expression atlases, which validated their use for endometrial function and implantation. The consistent upregulation of genes such as ITGB3, MMP9, and VEGF in datasets and studies corroborates the assumption that these genes play an important role in the molecular profile of RIF (Voros *et al.*, 2025). Not only do these findings support the function of these genes in RIF pathophysiology, but they also emphasize their function as potential targets or biomarkers for augmenting implantation success (Figure 5).

Table 4. Upstream Regulators of Differentially Expressed Genes (DEGs) Identified through Gene Regulatory Network (GRN) Analysis

Upstream Regulator	Target Genes	Regulatory Effect	Biological Function
NF-kB	IL6, TNF, VEGF	Immune modulation, Inflammation	Regulation of immune responses, inflammation
TP53	IL6, TNF, MMP9	Immune modulation, Apoptosis	Regulation of apoptosis, cell cycle
HIF1A	VEGF, MMP9, FN1	Angiogenesis, Extracellular matrix remodeling	Regulation of angiogenesis, hypoxia response

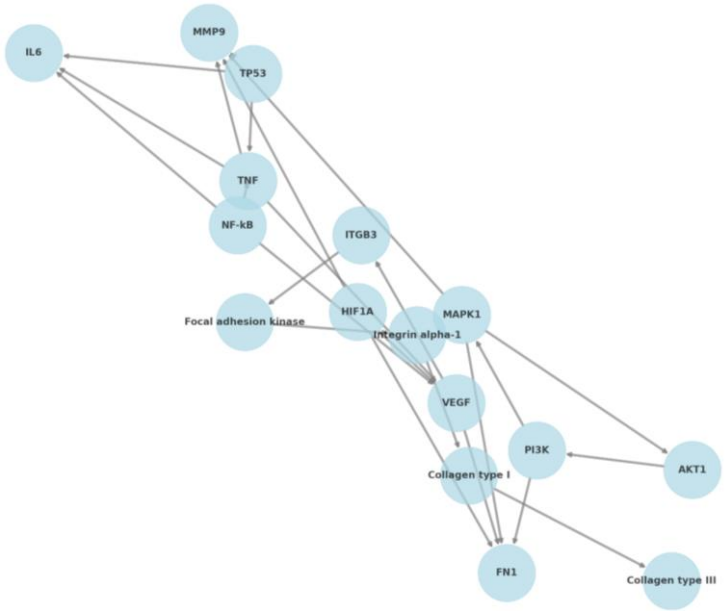

Figure 4. Gene Regulatory Network (GRN) Analysis of Upstream Regulators of DEGs (**Note.** Gene regulatory network (GRN) analysis identified key upstream regulators of the DEGs using Ingenuity Pathway Analysis (IPA). The network visualizes interactions between transcription factors and target genes. Key transcription factors such as *NF-kB*, *TP53*, and *HIF1A* are highlighted as critical regulators of genes involved in immune response, apoptosis, and hypoxia response, all of which contribute to the endometrial environment in RIF.)

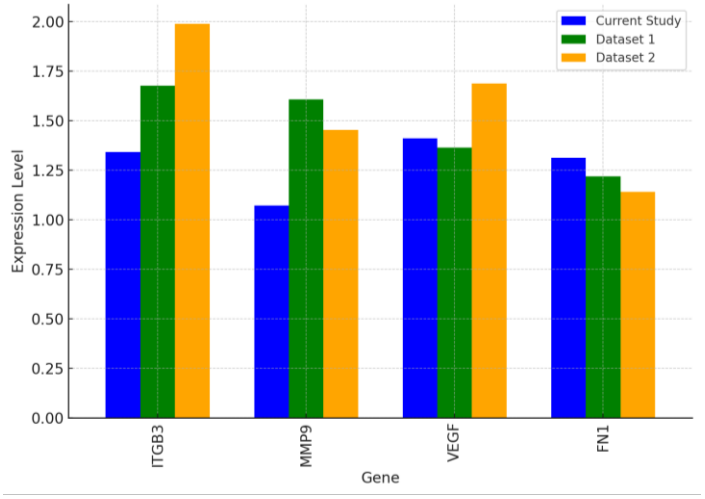

Figure 5. Validation of Key Findings Across Independent Datasets. (**Note.** The figure shows a bar plot comparing the expression levels of key differentially expressed genes (DEGs)—*ITGB3*, *MMP9*, *VEGF*, and *FN1*—across the current study and two independent RNA-Seq datasets. Consistent expression trends were observed in all datasets, confirming the robustness of the identified DEGs. Upregulation of these genes in RIF patients is consistent across cohorts, supporting the relevance and reproducibility of the findings. The validation process demonstrates that these DEGs and their associated pathways are involved in the known biological processes critical for implantation and RIF.)

3.6. Limitations and Sources of Uncertainty

Although our DEG, enrichment, PPI, and GRN results support a receptivity model of RIF, a few provisos apply. Small cohort sizes and vast multiple testing can yield false positives and false negatives even after adjustment for FDR; effect sizes for marginally differentially expressed genes are susceptible to inflation ("winner's curse"), and cut-offs such as $|\log2FC| \geq 0.585$ (≈1.5-fold) remain somewhat arbitrary. PCA dimensionality can capture partially batch effects or small WOI timing variations, and cross-study normalization cannot remove inter-lab variation entirely.

Second, bulk RNA-seq pools across cell types. Composition changes in epithelial, stromal, endothelial, or immune cells can mimic gene regulation, limiting mechanistic attribution to cell compartments. Technical noise, such as library prep heterogeneities, sequencing depth, alignment/annotation versions, and RNA quality, can add variance and bias detection. Third, functional interpretation is restricted. GO/KEGG enrichments, STRING PPIs, and upstream-regulator/GRN inferences result from partially curated, literature-biased knowledge bases and do not capture causality. Confidence is reinforced by validation in an external dataset but does not exclude confounding by clinical heterogeneity (age, BMI, stimulation/HRT protocols, embryo quality). Orthogonal assays (qPCR/protein/IHC), cell-type–resolved profiling (single-cell or spatial transcriptomics), and perturbation studies (e.g., knockdown/overexpression of hub genes or regulators) will be essential to attribute directionality, cell specificity, and therapeutic relevance.

3.7. Summary of Key Findings and Implications.

Our integrative analyses reveal shared themes in RIF—ECM remodeling, immune modulation, and angiogenesis—and nominate hub genes (e.g., ITGB3, MMP9, VEGF, FN1) and upstream regulators (e.g., NF-κB, TP53, HIF1A) as candidate endometrial receptivity molecules (Altoum *et al.*, 2024; Liu *et al.*, 2024; Zhu, 2025). The evidence should be interpreted with caution. There have been conflicting reports for some markers across studies and cycle conditions (e.g., phase definition, stimulation/HRT v. natural cycles), and directionality for some genes has varied by cohort, suggesting context-dependent and not universal signatures. Our cross-dataset validation (Section 3.5) itself implies concordant trends for a set but not all signals, as would be expected for endometrial transcriptomics given inter-study heterogeneity (Wang & Liu, 2020).

Potential batch effects (library prep, sequencing depth, and alignment/annotation versions) and WOI timing offsets can exaggerate apparent group separation in heatmaps/PCA despite FDR control. Additionally, bulk RNA-seq averages cells; shifts in epithelial/stromal/immune content can masquerade as gene-level regulation. Clinical and biological variation. Such as age/BMI, parity, comorbidities (e.g., endometriosis/PCOS), luteal support protocols, and embryo competence can muddy the waters, even if samples are labelled RIF vs. control. These variables most likely account for partial replication of individual DEGs and pathway strengths across datasets.

We thus regard the current findings as hypothesis-generating. The nominated pathways and genes are candidates, not established drug targets or biomarkers. Any diagnostic or therapeutic implications remain speculative until (i) orthogonal confirmation in the protein and cell layer (qPCR, IHC/IF, phospho-signaling), (ii) cell-type–resolved profiling (single-cell or spatial) to map signals, and (iii) functional perturbation studies (loss-/gain-of-function of hub genes/regulators) determine causality and reproducibility across centers. Follow-up studies should also incorporate batch correction and covariate control (e.g., ComBat/RUV/SVA), cell composition deconvolution (e.g., CIBERSORTx/MuSiC), and external validation per prespecification in order to move beyond transcript-level correlations to clinically important, mechanism-based findings. (Figure 6).

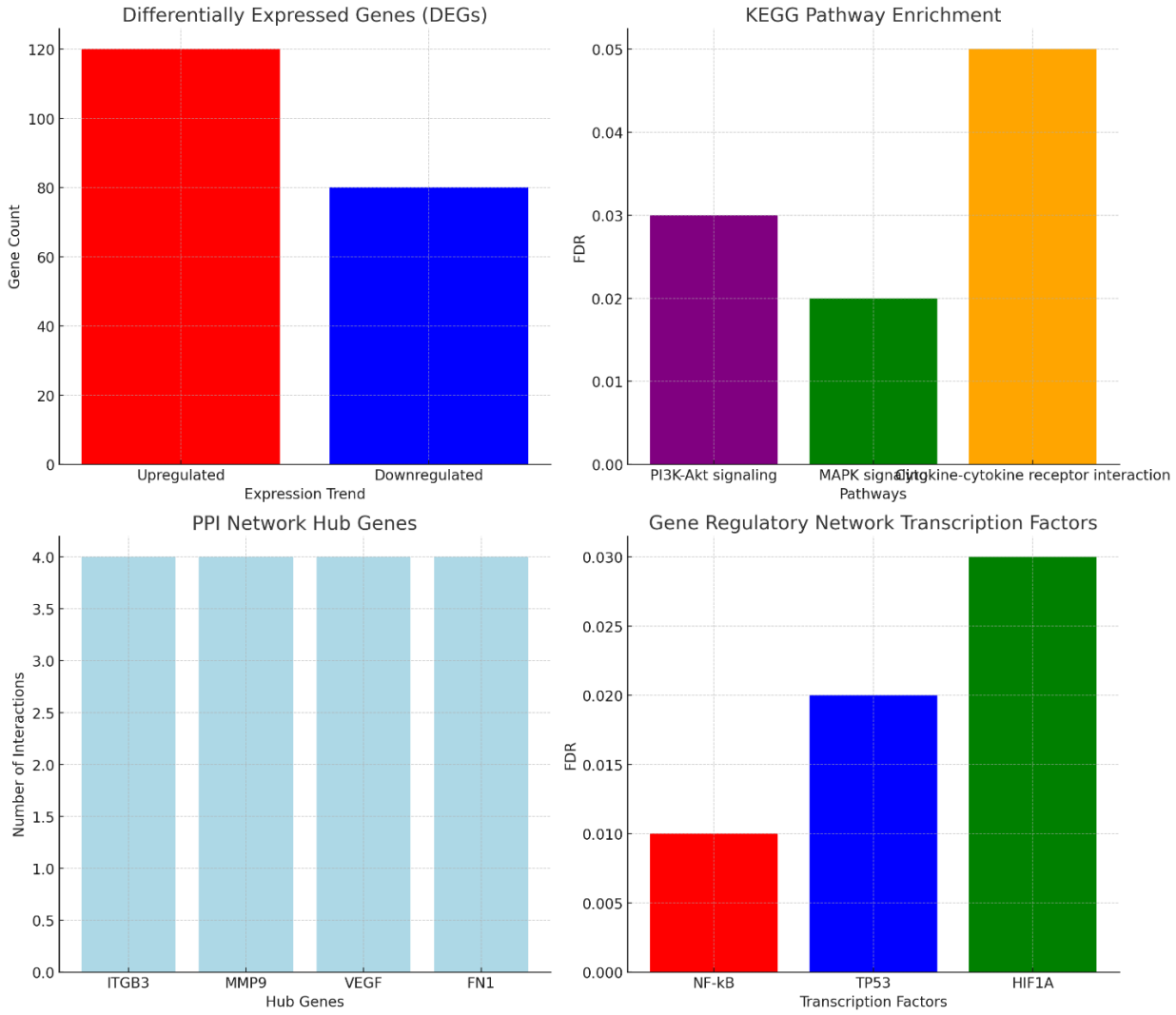

Figure 6. Summary of Key Findings from Integrative Bioinformatics Analysis. (**Note.** This composite figure summarizes the key findings from the integrative bioinformatics analysis of publicly available RNA-Seq data associated with RIF. Analysis showed 200 differentially expressed genes (DEGs), consisting of 120 upregulated and 80 downregulated genes. GO and KEGG pathway enrichment analyses revealed significant involvement of biological processes such as extracellular matrix remodeling, immune response, and angiogenesis. Key molecular pathways such as the PI3K-Akt and MAPK signalling pathways came into the spotlight as being crucial in RIF pathophysiology. Protein-protein interaction (PPI) network analysis of some of the hub genes—ITGB3, MMP9, VEGF, and FN1—identified their primary functions in cell adhesion, extracellular matrix remodeling, and angiogenesis. Gene regulatory network (GRN) analysis also identified essential transcription factors NF-kB, TP53, and HIF1A, which regulate these processes. All these findings are critical in providing insights into the molecular mechanisms that regulate RIF and providing potential biomarkers and targets for treatment to improve implantation success.)

4. Conclusion

This integrative bioinformatics review of publicly available RNA-Seq data provides a comprehensive overview of the molecular alterations of RIF patients. The discovery of those top upregulated and downregulated genes, enriched pathways, and regulatory networks offers new therapeutic opportunities. These discoveries need to be replicated in larger patient groups and explored to determine the possibility of targeting these molecular pathways to improve clinical outcomes in women with RIF.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

This study only utilized publicly available, de-identified data and did not involve direct patient participation. As the data were obtained from public repositories, no ethical approval or informed consent was required for this bioinformatics analysis. However, all data were handled in accordance with ethical guidelines for secondary use of genomic data.

Open access

Funding

None declared.

Authors' Contributions

Maryam Mokari, Borhan Zahedi, Atefeh Noori, and Amir-Reza Javanmard contributed to the design and execution of the study. Shakiba Amirjani & Sara Tutunchi conducted the majority of the experiments and data analysis. Burhan Zahedi was responsible for the statistical analyses and interpretation of results. Atefeh Noori performed the RNA-Seq data preprocessing and assisted in the pathway enrichment analysis. Amir-Reza Javanmard supervised the overall project, provided critical revisions, and served as the corresponding author. All authors reviewed and approved the final manuscript.

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How to cite this paper:

Mokari, M., Zahedi, B., Noori, A., Amirjani, S., Tutunchi, S. & Javanmard, A.-R. (2024). Integrative Bioinformatics Analysis of Endometrial Transcriptome Reveals Key Regulatory Networks in Recurrent Implantation Failure. *Microbiology, Metabolites and Biotechnology*, *8*(1), 37-46. DOI: 10.22104/mmb.2025.7743.1175