

ORIGINAL RESEARCH ARTICLE

Transcriptomic analysis reveals inflammatory and metabolic dysregulation in unexplained female infertility

Ritika Patial¹, Sonalika Ray², Kashmir Singh³, and Ranbir C. Sobti^{3*}

¹Centre for Systems Biology and Bioinformatics, Faculty of Science, Panjab University, Chandigarh, Chandigarh, India

²Division of Biotechnology and Molecular Medicine, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana, United States of America

³Department of Biotechnology, Faculty of Science, Panjab University, Chandigarh, Chandigarh, India

Abstract

Infertility affects the majority of women, and many cases have no clear cause. Endometrial abnormalities are suspected contributors, but the molecular changes behind them remain poorly mapped. We analyzed endometrial tissue transcriptomes from the NCBI GEO dataset GSE92324 to identify differences between women with implantation failure and fertile controls. We performed principal component analysis to assess sample variance and performed differential gene expression analysis with DESeq2. The analysis highlighted 168 significant genes (adjusted $p < 0.05$, $|\log_2FC| > 2$), of which 47 were upregulated and 121 were downregulated. Eleven of these were reproduced in an independent cohort (GSE111974) with fully concordant direction (overlap above chance, hypergeometric $p = 1.3e-16$): *CAPN6*, *CHST4*, *CLDN22*, *DUOXA1*, *ENPP3*, *PKHD1L1*, *SCGB1D4*, *SLC39A14*, *SLC7A4*, *TRPM6*, and *WNK4*. These reproducible genes mapped to epithelial ion transport (*WNK4*, *TRPM6*, *SLC7A4*, *SLC39A14*, *ENPP3*), tight junction and barrier function (*CLDN22*, *SCGB1D4*), and mitochondrial dysfunction (*CAPN6*), the same pathways that emerged as the most strongly inhibited across the analysis. Functional enrichment pointed to immune-inflammatory pathways, lipid metabolism, and steroid biosynthesis. Ingenuity Pathway Analysis showed increased innate immune responses, altered lipid metabolism, and inhibited mitochondrial function. Upstream regulator analysis identified *PTEN*, *PRKAA1*, *HDAC4*, *IL10RA*, and *RAD51*, which regulate metabolic and anti-inflammatory signaling. Weighted gene co-expression network analysis identified a turquoise module with a strong negative correlation with the trait of interest (correlation = -0.84 , $p < 0.0001$), pointing to *C7orf50*, a cholesterol-metabolism gene not previously tied to infertility, as a candidate worth following up. Five further genes, *C1orf106* (*INAVA*), *C15orf59*, *LINC00461*, *C15orf48* (*NMES1*), and *C10orf99* (*GPR15LG*), had no prior reported link to infertility. Together, these findings map the genes, co-expression modules, and pathways disrupted in unexplained female infertility and nominate a reproducible set of ion-transport and barrier genes as candidate markers.

Keywords: Unexplained female infertility; Endometrial transcriptomics; Weighted gene co-expression network analysis; Ingenuity Pathway Analysis; Ion transport

*Corresponding author:

Ranbir C. Sobti
(rcsobti@pu.ac.in)

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1. Introduction

Infertility affects a substantial proportion of the reproductive-age population worldwide, with a global prevalence estimated at 12.87%.¹ According to recent data, one in every six people of reproductive age will experience infertility at some point in their lifetime, translating to approximately 110 million women and 55 million men living with the condition globally as of 2021.² The prevalence of infertility has shown a consistent upward trend over the past decade, with an average annual increase of 0.68% between 1990 and 2021.² This rise is driven by a combination of demographic shifts, delayed childbearing, and changing lifestyle patterns that together impair reproductive function.³ Infertility carries not only significant medical consequences but also profound psychological, social, and economic burdens for affected individuals and their families.³ Understanding its underlying causes has therefore become a priority in reproductive medicine.

Female infertility arises from hormonal, structural, genetic, and lifestyle-related causes. Hormonal imbalances, particularly disruption of the hypothalamic–pituitary–ovarian (HPO) axis, impair follicle development, ovulation, and luteal function.⁴ Structural problems such as tubal blockage, fibroids, and endometriosis obstruct implantation,⁵ and genetic factors such as chromosomal disorders and single-gene mutations affect ovarian development;⁶ the *FMRI* premutation, for example, confers approximately a 20% risk of premature ovarian insufficiency.⁷ Lifestyle factors are increasingly recognized as contributors: abnormal body weight disrupts ovulation through altered estrogen and insulin levels;⁸ diets high in trans fats increase ovulatory infertility risk while antioxidant-rich diets improve outcomes;⁸ smoking and alcohol damage oocyte quality;⁹ and circadian disruption from poor sleep dysregulates the HPO axis and impairs receptivity.¹⁰ Despite this understanding, a significant proportion of cases remain unexplained even after thorough clinical investigation.

Unexplained infertility in females refers to cases where standard diagnostics, including ovarian reserve testing and tubal patency assessment, yield normal results, yet implantation consistently fails.⁵ This category represents a major clinical challenge because the absence of an identifiable cause limits treatment options and leaves patients without a clear therapeutic pathway. Emerging evidence suggests that subtle molecular abnormalities in the endometrium, particularly during the implantation window, may underlie many of these unexplained cases.^{11,12} The endometrium undergoes dynamic molecular reprogramming during the window of implantation,

and any disruption in this process, whether in immune signaling, metabolic activity, or gene expression, can render it non-receptive to an embryo.¹¹ These molecular-level changes are often undetectable by conventional histological or hormonal assessment, making transcriptomic profiling of endometrial tissue a particularly valuable strategy for uncovering the hidden biology of unexplained infertility.¹² Infertility is now increasingly recognized as a condition shaped not only by reproductive organ abnormalities but also by systemic metabolic and inflammatory states, many of which originate from modifiable lifestyle factors.^{8,13} This systemic perspective calls for an integrated molecular analysis that goes beyond immune profiling to capture the full landscape of endometrial dysfunction.

Transcriptomic approaches have begun to clarify the molecular basis of infertility, but existing studies are limited in scope. Dong *et al.*¹⁴ profiled granulosa cells in metabolic syndrome-associated infertility but not endometrial tissue, and Da Luz *et al.*¹⁵ examined cumulus cells in endometriosis without extending to the implantation window. Vargas *et al.*¹¹ and Bui *et al.*¹² profiled the receptive-phase endometrium and confirmed immune and receptivity-related pathways, but did not integrate upstream regulator or co-expression network analyses. Heydarian *et al.*¹³ addressed lifestyle determinants but lacked molecular resolution at the endometrial level. A common limitation across these studies is their narrow focus on immune and cytokine responses, with little attention to metabolic dysregulation, lipid and steroid biosynthesis, mitochondrial function, upstream regulators, network-level organization, reproducibility across cohorts, and under-investigated genes.

To address this gap, we analyzed publicly available transcriptomic data from endometrial tissue of women with unexplained infertility using the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) dataset GSE92324, which includes samples collected during the implantation window under controlled ovarian stimulation conditions.¹⁶ We performed differential gene expression analysis (DGE) using DESeq2 to identify genes with significant expression changes between infertile cases and fertile controls.¹⁷ We conducted functional enrichment analysis using Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology frameworks to characterize the biological processes, molecular functions, and cellular components associated with the differentially expressed genes (DEGs).^{18,19} We applied weighted gene co-expression network analysis (WGCNA) to identify co-expression modules significantly associated with the infertility trait and to uncover hub genes driving these modules.²⁰ We used Ingenuity

Pathway Analysis (IPA) to identify significantly activated and inhibited canonical pathways, upstream regulators, and disease and biofunction annotations relevant to unexplained infertility.²¹ We further constructed a protein–protein interaction (PPI) network using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database to map the functional relationships among the DEGs and identify key interaction hubs.²² This integrative multi-layered approach extends beyond single-method transcriptomic studies and provides a detailed molecular portrait of endometrial dysfunction in unexplained infertility, with the potential to identify novel biomarkers for infertility and inform targeted therapeutic strategies.

2. Materials and methods

2.1. Dataset extraction

Gene expression data were obtained from the NCBI GEO dataset GSE92324,¹⁶ which comprises transcriptomic profiles of endometrial tissue collected during the implantation window. The dataset has two groups: women with implantation failure and unexplained infertility, and proven fertile oocyte donors as controls. Eighteen samples were included: 10 implantation failure cases and 8 controls, all collected under ovarian stimulation during an *in vitro* fertilization (IVF) cycle. Raw counts were screened before analysis. Duplicate gene symbols, genes with zero or very low expression, and genes with no measurable variance across samples were removed, leaving only genes with usable signal for subsequent analyses.

2.2. Data normalization

The filtered expression matrix was log-transformed. Log transformation stabilizes variance, pulls in extreme expression values, and brings the data closer to a normal distribution.²³ This step is important for differential expression and principal component analysis (PCA), where a few highly expressed genes can otherwise dominate.²⁴ Normalizing first helps reduce technical noise that can mask real biological differences and makes subsequent statistics more reliable.

2.3. Exploratory data analysis

Principal component analysis of the log-transformed matrix was used to assess overall variability and identify outliers. PCA reduces dimensionality, making sample-to-sample variation easier to identify.²⁵ Using log-transformed values kept highly expressed genes from skewing the principal components.

2.4. Differential gene expression analysis

Differential expression between cases and controls was

investigated using DESeq2 in R version 4.2 (R Foundation for Statistical Computing, Austria).¹⁷ A gene was considered significantly expressed at $p < 0.05$ and adjusted $p < 0.05$. Adjusted p -values were computed using the Benjamini–Hochberg procedure, which controls the false discovery rate across the full set of tested genes.²⁶ A second filter, $\log_2$ fold change (FC) ≥ 2 or ≤ -2 , was applied to focus on genes with larger expression shifts. Results were displayed as a volcano plot using the EnhancedVolcano package with significance against FC.

2.5. Cross-referencing with Comparative Toxicogenomics Database and literature

The DEGs were cross-referenced with the Comparative Toxicogenomics Database (CTD) to identify those with curated associations with female infertility. Each gene was assessed for curated therapeutic (T) or marker/mechanism (M) annotations linking it to the disease, and genes carrying only inferred associations were recorded separately. To identify genes with no prior reported link to infertility, a targeted literature search was run in PubMed using each gene symbol combined with infertility-related terms and their synonyms. Searches were restricted to human studies in female populations and queried Title/Abstract fields together with the corresponding Medical Subject Headings (MeSH) terms. Genes returning no relevant records were treated as previously unreported candidates for follow-up.

2.6. Functional enrichment

Differentially expressed genes were annotated with the clusterProfiler package²⁷ across the three Gene Ontology domains: biological process, molecular function, and cellular component.¹⁸ KEGG pathway enrichment was performed on the same gene set to assign DEGs to known pathways.¹⁹

2.7. Ingenuity Pathway Analysis

Ingenuity Pathway Analysis was used for subsequent causal analysis.²⁸ Canonical pathways, diseases and biofunctions, and upstream regulators were considered significant at $p < 0.05$, with activation z -scores ≥ 2 indicating activated pathways and ≤ -2 indicating inhibited ones.²¹

2.8. Weighted gene co-expression network analysis

Co-expression structure was built with the WGCNA package.²⁰ Sample clustering was performed to identify outliers. A soft-threshold power beta was selected using the pickSoftThreshold function to approximate a scale-free topology, thereby avoiding an arbitrary cutoff. An adjacency matrix was built at the chosen beta, and then converted to a topological overlap measure (TOM) to score co-expression similarity between gene pairs; TOM

dampens noise and spurious links. Modules were clustered hierarchically, and modules with eigengene dissimilarity below 0.25 were merged.

2.9. Module–trait relationships

Module–trait associations were tested using Pearson's correlation coefficient between each module eigengene and the clinical trait (condition), with $p < 0.05$ as the significance threshold. Pearson correlation was used rather than a rank-based alternative because WGCNA computes correlations between module eigengenes, which are continuous summary variables representing the expression profiles of gene modules. The WGCNA framework is based on Pearson correlation to quantify the similarity between gene-expression profiles and module eigengenes. Within trait-linked modules, hub genes were identified by combining Gene Significance, the absolute correlation between a gene and the trait, with Module Membership, the correlation between a gene's expression and the module eigengene. Genes with high Gene Significance and Module Membership were treated as hub genes.

2.10. Protein–protein interaction network

To examine interactions among the DEGs ($|\log_2FC| \geq 2$, adjusted $p < 0.05$), the gene set was submitted to STRING at a confidence cutoff of 0.700 to limit false positives.²² The network was imported into Cytoscape VERSION (Cytoscape Consortium, United States of America) for visualization, and hub genes were ranked with the CytoHubba plugin.

2.11. Independent validation

To validate whether the DEGs were reproduced in an independent cohort, a separate endometrial transcriptomic dataset (GEO accession GSE111974) was analyzed with the same case-versus-control contrast. DEGs in the validation set were identified using an adjusted $p < 0.05$ and $|\log_2FC| > 2$. The identified DEGs were compared with genes reported in CTD and the PubMed literature.

3. Results

3.1. Infertile and control endometrium separate clearly along the main axis of variation

In the PCA plot (Figure 1A), control samples clustered tightly within the blue ellipse, indicating consistent expression profiles. The infertility group (red ellipse) formed a separate but looser cluster, suggesting greater heterogeneity among cases. Principal component 1 (PC1) accounted for 23.57% of the variance, and PC2 accounted for an additional 12.47%, indicating that most

of the variation aligns with implantation status. No outliers showed up in either group.

3.2. A stringent fold-change filter narrows 1,775 differentially expressed genes to 168 high-confidence genes

DESeq2 analysis between cases and controls returned 1,775 significant DEGs at adjusted $p < 0.05$. Furthermore, the $|\log_2FC| > 2$ threshold filtered this number to 168 genes (47 upregulated and 121 downregulated). The top upregulated genes were *GPX3* ($\log_2FC = 4.62$), *CXCL14* (4.40), *AOX1* (3.91), *HSFX1* (3.75), *CDA* (3.48), *PPARGC1A* (3.25), *TSPAN8* (3.24), *NNMT* (3.10), *LAMB3* (3.09), and *C3* (3.01). These genes map onto oxidative stress response (*GPX3*²⁹), inflammation and immune modulation (*CXCL14*, *C3*), metabolic reprogramming (*AOX1*, *NNMT*^{30,31}), mitochondrial biogenesis (*PPARGC1A*³²), and extracellular matrix remodeling (*TSPAN8*, *LAMB3*).

The top downregulated genes were *WNK4* ($\log_2FC = -4.20$), *GJB2* (-4.25), *TRPM6* (-4.27), *CAPN6* (-4.42), *HPCAL4* (-4.60), *OPRPN* (-4.67), *CHST4* (-4.70), *GP2* (-5.07), *NLRP5* (-5.17), and *SLC7A4* (-5.54). These genes are involved in pathways central to fertility: ion transport and signaling (*WNK4*, *TRPM6*^{33,34}), gap-junctional communication (*GJB2*³⁵), proteolysis (*CAPN6*³⁶), immune and inflammatory modulation (*OPRPN*, *GP2*, *CHST4*), maternal-effect and embryo development (*NLRP5*^{37,38}), and amino acid transport (*SLC7A4*³⁹). The volcano plot (Figure 1B) shows the distribution of upregulated and downregulated genes.

3.3. Eleven differentially expressed genes reproduced in an independent cohort

Differentially expressed gene analysis on the validation dataset GSE111974 identified 24 genes, of which 11 overlapped with the 168 DEGs from dataset GSE92324 (Figure 1C). These genes included *CAPN6*, *CHST4*, *CLDN22*, *DUOXA1*, *ENPP3*, *PKHD1L1*, *SCGB1D4*, *SLC39A14*, *SLC7A4*, *TRPM6*, and *WNK4*. All 11 genes were downregulated in both cohorts, illustrating complete directional concordance (11/11). The shared genes were clustered into functional themes, such as epithelial ion transport and solute handling (*WNK4*, *TRPM6*, *SLC7A4*, *SLC39A14*, *ENPP3*), tight-junction barrier function (*CLDN22*, *SCGB1D4*), oxidative and immune modulation (*DUOXA1*, *CHST4*), and proteolysis (*CAPN6*). *PKHD1L1*, which encodes a fibrocystin-related membrane protein, was also identified, though its role in endometrial function remains uncharacterized and warrants further study.

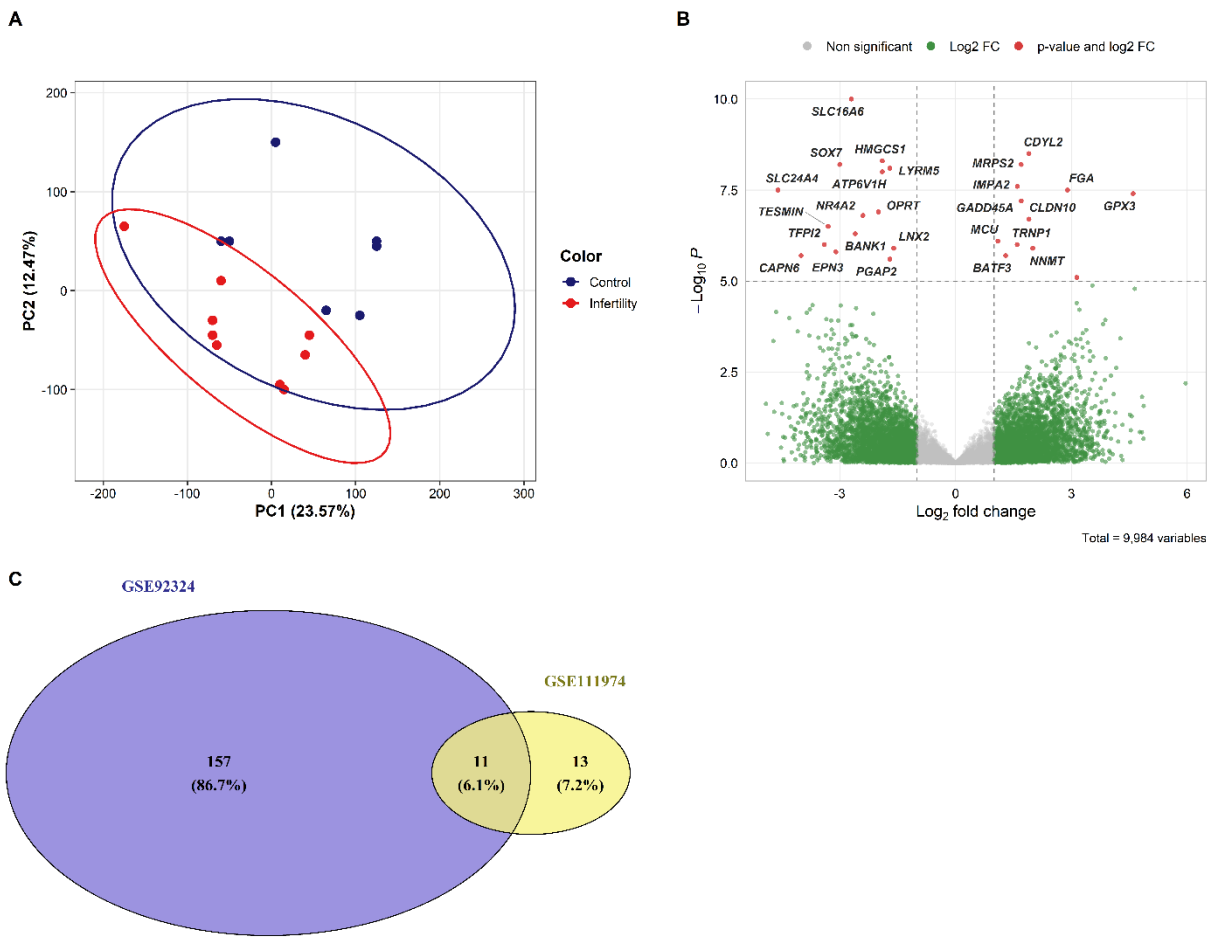


Figure 1. Principal component analysis and differential gene expression in endometrial tissue from women with implantation failure versus fertile controls. (A) Principal component analysis of the dataset. The blue ellipse represents control samples, and the red ellipse represents samples from unexplained infertility cases. (B) Volcano plot showing the differentially expressed genes (DEGs) between control and infertility cases. Genes with significant changes are highlighted. (C) Overlap between discovery (168) and validation (24) DEGs. Eleven genes are shared and are downregulated in both cohorts. Abbreviations: FC: Fold change.

3.4. Most differentially expressed genes lack curated infertility links, and five have no prior infertility evidence

Cross-referencing the 168 DEGs from dataset GSE92324 with CTD showed that none had curated therapeutic or marker/mechanism roles for female infertility, and 163 carried only inferred associations. A targeted PubMed search found four coding and one non-coding gene with no prior direct evidence linking them to infertility: *C1orf106* (*INAVA*), *C15orf59*, *LINC00461*, *C15orf48* (*NMES1*), and *C10orf99* (*GPR15LG*). These stand out as new candidates worth following up. The 11 cross-validated genes carried only inferred disease associations, with none holding a curated therapeutic or marker/mechanism annotation for female infertility. The parallel PubMed search returned no

relevant human female studies for 9 of the 11 genes, with only *ENPP3* and *SCGB1D4* yielding prior literature linking them to infertility.

3.5. A turquoise co-expression module tracks strongly with infertility and surfaces *C7orf50*

Sample clustering based on the Pearson distance produced a dendrogram with no outliers; all 18 samples were included (Figure 2B). A soft-threshold power of $\beta = 10$ yielded an approximately scale-free network (Figure 2C). Average-linkage hierarchical clustering grouped 8,245 genes into 45 modules. When correlating each module eigengene with condition, the turquoise module showed a strong negative correlation ($\text{cor} = -0.84$, $p < 0.0001$) and the purple module showed a weaker positive correlation ($\text{cor} = +0.65$,

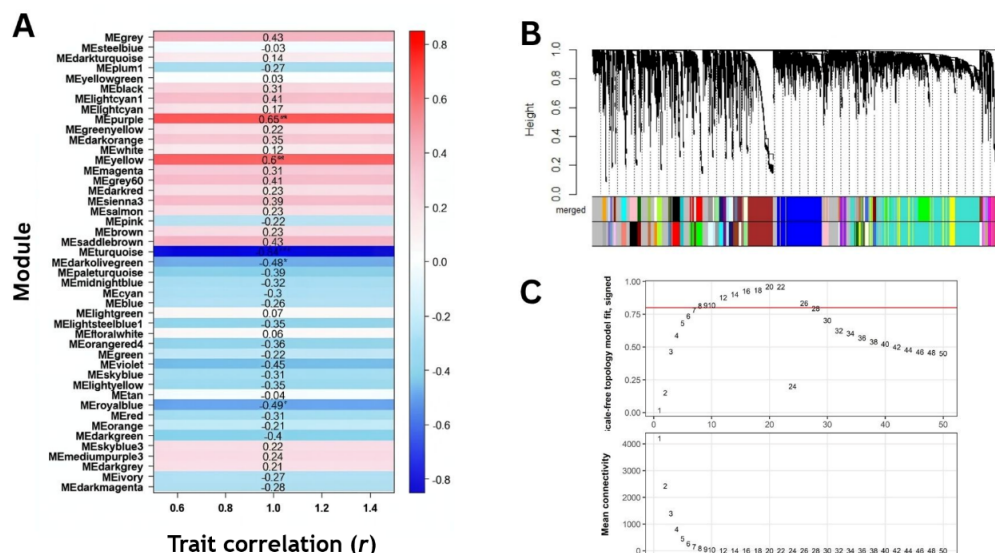


Figure 2. Weighted gene co-expression network analysis of transcriptomic data. (A) Heatmap correlating module eigengenes with traits of interest. Color represents strength of correlation (red: positive, blue: negative), with asterisks indicating statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). (B) Cluster dendrogram showing hierarchical clustering of gene expression profiles, with a dynamic tree cut used to identify modules; color bands represent merged modules. (C) Scale-free topology fit index and mean connectivity analysis to determine the optimal soft-thresholding power; power = 10 achieves high scale-free topology with acceptable mean connectivity.

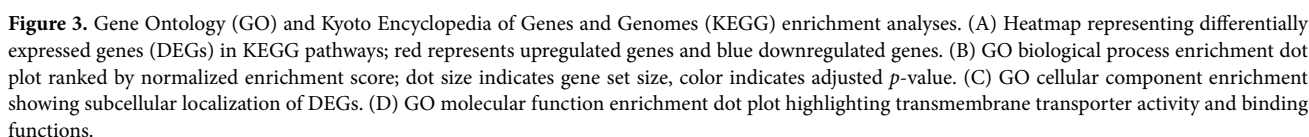
$p < 0.001$; Figure 2A). Many module genes overlapped with the DEG list, supporting their relevance to the infertile phenotype. Ten of the 11 cross-validated genes (all except *CHST4*), together with the novel candidates *C7orf50* and *C1orf106* (*INAVA*), were members of the trait-associated turquoise module, consistent with their downregulation tracking the infertile phenotype.⁴⁰ Literature review of the module identified three genes new to this context: *C7orf50*, *LOC652276*, and *CASTOR3*.

3.6. Upregulated pathways center on innate immunity; downregulated pathways on lipid and steroid metabolism

The KEGG enrichment analysis identified several upregulated immune pathways (Figure 3A): Hematopoietic cell lineage ($p = 0.0005$), natural killer (NK) cell-mediated cytotoxicity ($p = 0.0008$), and complement and coagulation cascades ($p = 0.0014$), involving genes such as *CR1*, *CSF1R*, *TNF*, *CD3E*, and *PIK3*-family members. Uterine NK cells normally support trophoblast invasion and vascular remodeling in a restrained way, so enrichment of cytotoxicity pathways suggests an overactive NK phenotype. This finding is consistent with previous infertility studies reporting that elevated NK cytotoxicity, not just NK cell number, is implicated in recurrent pregnancy loss and unexplained infertility.⁴¹⁻⁴³

The complement cascade was also upregulated, including genes *C1*, *C3*, and *C4*. Complement activity at the implantation site is normally tightly controlled.^{44,45} Persistent activation in the absence of an embryo suggests a pro-inflammatory microenvironment that could promote immune-cell recruitment and contribute to endometrial tissue or vasculature remodeling. Excessive complement activation has been associated with pregnancy complications, including preeclampsia linked to complement-regulator mutations.⁴⁶ Our results are in agreement with earlier reports of an inflammation-rich endometrium. Chemokine signaling, antigen processing and presentation, and Fc epsilon receptor I signaling further contribute to an abnormal immune environment.^{47,48}

Downregulated pathways indicated the opposite direction, toward suppressed metabolism: terpenoid backbone biosynthesis, biosynthesis of unsaturated fatty acids, butanoate and propanoate metabolism, mucin-type O-glycan biosynthesis, and steroid biosynthesis. Recurring genes included *ACAT1*, *ACAT2*, *HADHA*, *ECHS1*, polypeptide N-acetylgalactosaminyltransferase (*GALNT*)-family members, and *CYP*-family members, spanning lipid and energy metabolism, glycan modification, and steroid synthesis, all of which are needed for a receptive endometrium.⁴⁹⁻⁵¹ Because cholesterol is the precursor of estradiol and progesterone synthesis and supports



cytoplasmic vesicle lumen, secretory granule lumen, early endosome, and blood microparticle. Molecular function terms covered potassium ion transport, monocarboxylic acid binding, transmembrane transporter activity, and retinoic acid and retinoid binding.

3.8. Ingenuity Pathway Analysis shows activated acute-phase and lipid signaling and inhibited mitochondrial and ion-transport pathways

Ingenuity Pathway Analysis identified both activated and inhibited canonical pathways (Figure 4A). Activated pathways included acute-phase response signaling, consistent with systemic inflammation and innate immune activity during implantation.⁵⁵ Liver X receptor/retinoid X receptor activation was involved in cholesterol homeostasis

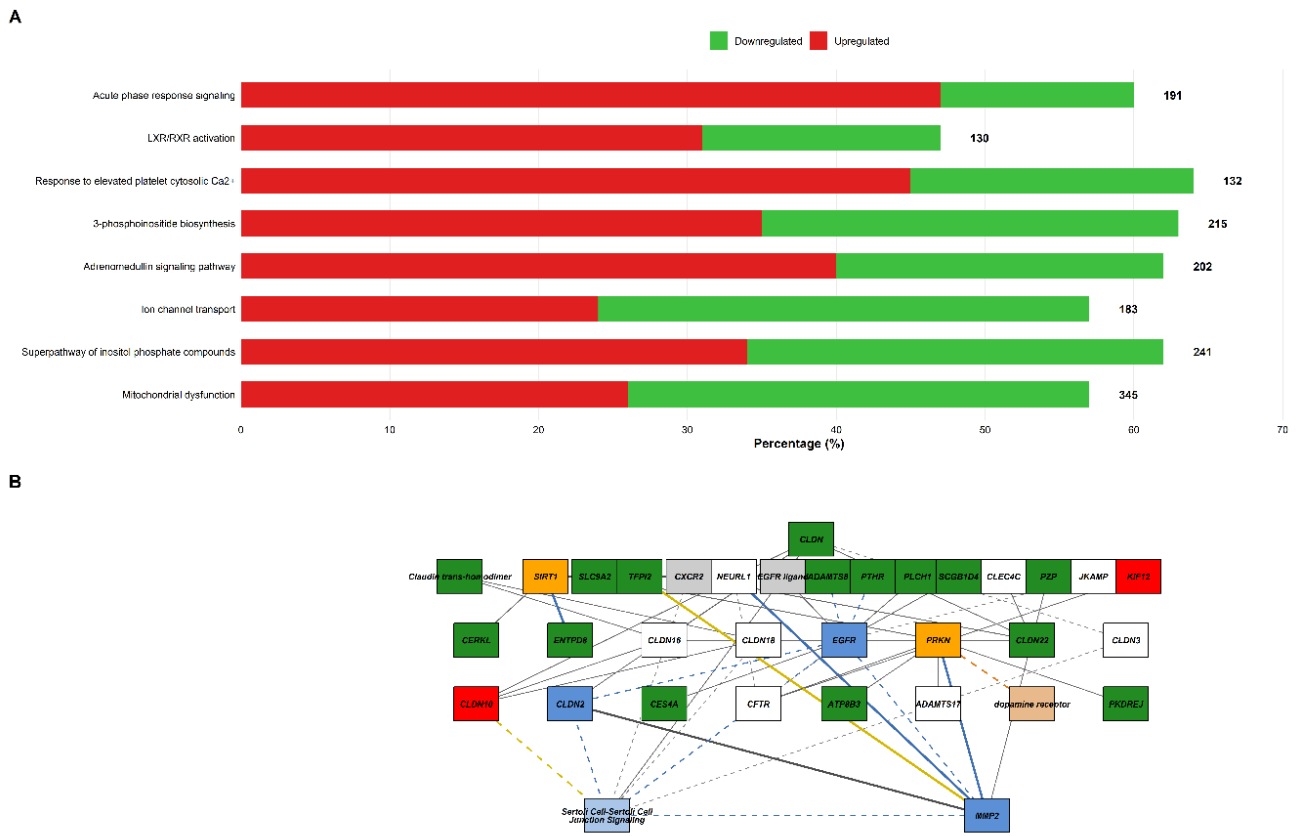


Figure 4. Ingenuity Pathway Analysis (IPA). (A) Bar plot of top canonical pathways significantly impacted in infertility. Color indicates the proportion of genes upregulated (red), downregulated (green), or unchanged (gray). (B) Network showing functional connectivity of genes. Node color indicates expression change (red: up, green: down, white: unchanged); solid lines indicate direct interactions; dashed lines indicate indirect interactions; blue and orange lines indicate predicted inhibition or activation.

and membrane remodeling during receptivity.^{56,57} Neutrophil degranulation and response to elevated platelet cytosolic Ca²⁺ were also activated, indicating neutrophil-driven inflammation and platelet-related vascular changes.⁵⁸

Several metabolic and signaling pathways were inhibited. These included the superpathway of inositol phosphate compounds and 3-phosphoinositide biosynthesis, the adrenomedullin signaling pathway,⁵⁹ and the ion channel transport pathway.³⁵ Two of the validated genes, *TRPM6* and *WNK4*, are associated with the ion channel transport pathway. The mitochondrial dysfunction pathway was the most notably inhibited and included the validated gene *CAPN6*, along with the downregulated biogenesis regulator *PPARGC1A* (PGC-1α), consistent with reduced mitochondrial function.⁶⁰

3.9. Upstream regulators converge on metabolic stress and lost anti-inflammatory control

Ingenuity Pathway Analysis of upstream regulators,

cross-checked with CTD, identified regulators with only inferred infertility associations; none listed as therapeutic targets or markers. Among activated regulators, *PRKAA1* (AMP-activated protein kinase alpha 1 [AMPKα1]) affected the expression of *PPARGC1A*, *PLA2G2A*, and *SOD2*, all of which are genes involved in metabolic and mitochondrial pathways. AMPK is activated under low-energy conditions; therefore, its activation suggests that the infertile endometrium is experiencing energy stress.⁶¹ Histone deacetylase 4 (HDAC4; encoded by *HDAC4*) acted on *IGFBP1*, *NR4A2*, *PTGS2*, and *GADD45A*, linked to apoptosis, oxidative stress, and embryonic development. Phosphatase and Tensin Homolog (PTEN; encoded by *PTEN*) targeted *C3*, *GPX3*, *CXCL14*, *PPARGC1A*, and *PTGS2*, modulating immunometabolic control of receptivity; disrupted phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) signaling, downstream of PTEN, is associated with decidualization failure, which is linked to implantation failure and recurrent loss.⁶² *BRCA1* was activated; it is associated with oxidative-stress-related and

developmental genes, including *GADD45A*, *CAMK2B*, *HSD11B2*, and *NEDD9*.

On the suppressed side, *IL10RA* and its downstream genes (*C3*, *CA2*, *PPARGC1A*, *SORBS2*) were strongly reduced. Interleukin (IL)-10 is a tolerogenic cytokine that inhibits harmful immune responses in pregnancy, and reduced IL-10 signaling is associated with pregnancy complications and immune-mediated implantation failure.⁶³ *RAD51*, central to homologous recombination DNA repair, was also reduced, potentially compromising genomic stability under oxidative stress. *MYC*, *FOXA1*, *ERG*, and *EGFR* affect developmental, angiogenic, and immune genes (*LAMB3*, *GADD45A*, *PTGS2*, *HSD11B2*, *NTRK2*), while inhibition of *RAF1* and *CASR* suppresses mitogen-activated protein kinase (MAPK) and vascular signaling. *PTGS2*, which encodes cyclooxygenase-2 (COX-2), stood out, targeted by nine regulators across inflammatory, metabolic, and vascular pathways, while *PPARGC1A* and *SOD2* were each modulated by several regulators (*PTEN*, *PRKAA1*, *MYC*, *IL10RA*), reinforcing their central role in the infertile phenotype. None of the 11 validated genes appeared as upstream regulators themselves; however, several regulators converged on the same metabolic and ion-transport pathways represented by the validated genes.

3.10. Disease and biofunction analysis reflects shared inflammatory and epithelial mechanisms

Disease and biofunction analyses identified nine activated and four inhibited terms, several of which are outside the usual infertility frame. The strongest terms were gastrointestinal: inflammation of the gastrointestinal tract, gastroenteritis, abnormality of large intestine, enteritis, and colitis. These annotations do not indicate actual gastrointestinal disease in these women; rather, they reflect inflammatory and epithelial-barrier mechanisms that the endometrium shares with intestinal tissue. Downregulated annotations included transport of ions and transport of monovalent inorganic cations, involving the validated genes *SLC15A2*, *TRPM6*, and *WNK4*, which are required for fluid balance and epithelial polarization at implantation. Suppression of sperm movement within the uterus was also observed, consistent with the ion-transport and mitochondrial suppression seen elsewhere.

3.11. Network analysis identifies claudin and parathyroid hormone-receptor isoforms as uncharacterized in fertility

Interaction-network analysis for endocrine system disorders, gastrointestinal disease, and organismal injury identified two entries, *CLDN* (which encodes claudin) and

PTHR (which encodes parathyroid hormone receptor), with no therapeutic or mechanistic roles in CTD. *CLDN1* and *CLDN4* have known links to implantation and endometrial barrier function, but the generic *CLDN* group annotated has not been associated with infertility, suggesting that isoforms such as *CLDN2*, *CLDN10*, *CLDN22*, and *CLDN18* participate in tight-junction signaling during reproductive failure. Furthermore, *PTHR* lacks an established association with infertility and requires mechanistic follow-up. In the network (Figure 5), *EGFR* and *MMP2* served as central nodes connecting *CLDN* isoforms and regulators such as *PRKN*, *SIRT1*, and *TFPI2*, suggesting disrupted tight-junction remodeling and proteolytic balance. *CES4A*, *SLC9A2*, and *SCGB1D4* were downregulated; they are associated with barrier function, transport, and immune modulation. *CLDN22* and *SCGB1D4* were also among the genes independently validated in the replication cohort (Section 3.3).

3.12. Protein-protein interaction network of the DEGs

The DEGs were submitted to STRING, and the resulting network was exported to Cytoscape for visualization. The network comprised 164 nodes and 18 edges with significant enrichment ($p = 9.11 \times 10^{-7}$, Figure 6), indicating that the interactions were non-random. Several of the validated genes, including *WNK4*, *TRPM6*, *SLC7A4*, *SLC39A14*, *ENPP3*, *CLDN22*, and *SCGB1D4*, were present among the interacting nodes. The top hub genes ranked with the CytoHubba plugin were *ENPP3*, *ATP12A*, *SLC7A1*, *SLC15A2*, *SLC1A1*, *GPR37*, *UCK2*, *PRR15*, *ENTPD8*, and *WNK4*; two of which, *ENPP3* and *WNK4*, were also among the independently validated genes. Given the limited number of edges, the PPI network is presented as supporting evidence rather than a primary result.

4. Discussion

We used differential expression analysis, KEGG, GO, IPA, WGCNA, and an independent validation cohort to identify under-investigated genes and pathways that contribute to unexplained female infertility. The most significant result was a set of 11 genes that were downregulated in a second endometrial cohort, with functions converging on epithelial ion transport and tight-junction barrier function. Around this core, the endometrium showed increased innate immune and inflammatory activity, along with reduced lipid, steroid, and mitochondrial metabolism. WGCNA identified a turquoise co-expression module that was strongly and negatively correlated with the infertile trait ($\text{cor} = -0.84$, $p < 0.0001$). This module contained most of the validated genes and identified *C7orf50* (cholesin), a cholesterol-related gene with no prior link to infertility.

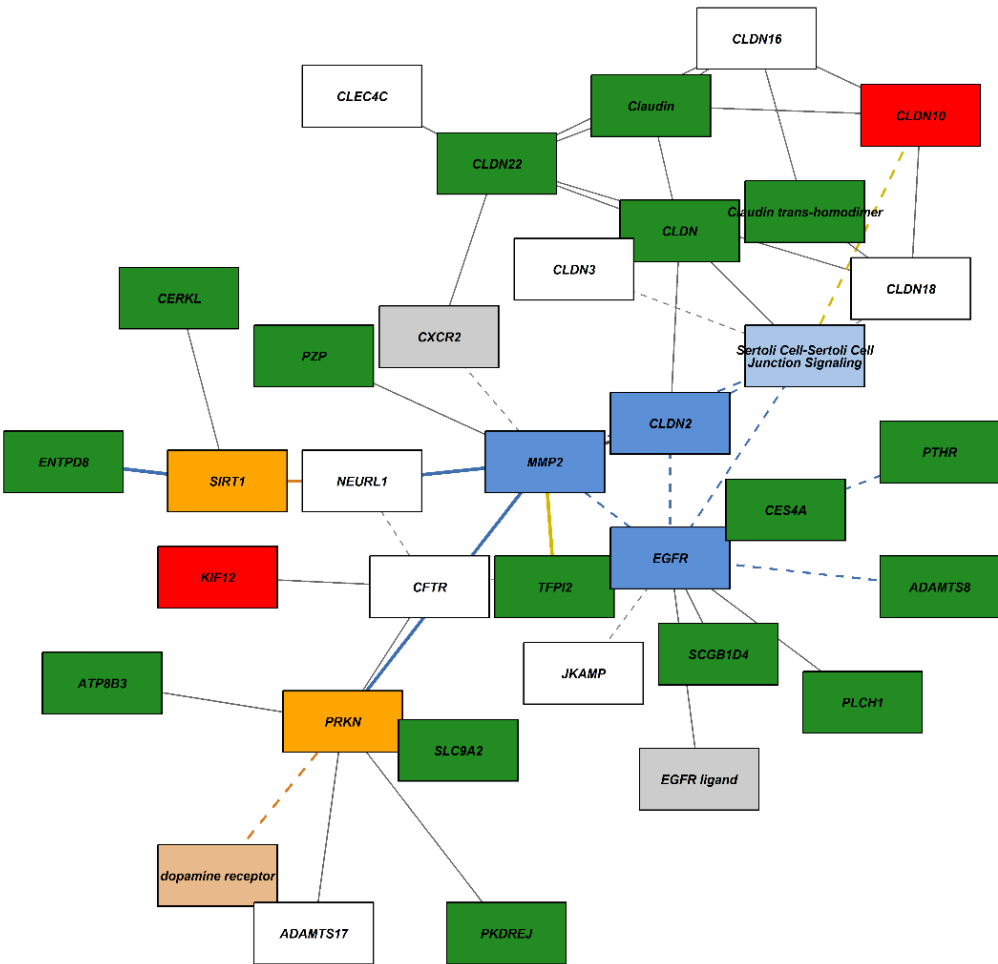


Figure 5. Ingenuity Pathway Analysis network showing isoforms of *CLDN* responsible for novel tight-junction signaling events related to reproductive failure

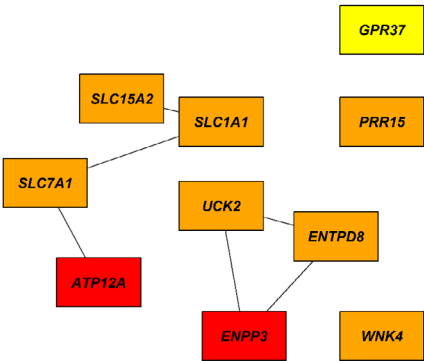


Figure 6. Search Tool for the Retrieval of Interacting Genes/Proteins (STRING)-based protein–protein interaction network (164 nodes, 18 edges; $p = 9.11 \times 10^{-7}$). Each node is a differentially expressed gene; edges indicate validated functional interactions.

Validation strengthens the evidence supporting this gene set. Starting with a single discovery dataset of 18 samples, we used reproducibility across an independent cohort as a criterion for prioritizing robust findings. Eleven genes were reproducibly identified across two separate endometrial cohorts and showed the same direction of dysregulation, with an overlap substantially greater than expected by chance (hypergeometric $p = 1.3 \times 10^{-16}$). These genes were concentrated in several functional categories, including ion transport and solute handling (*WNK4*, *TRPM6*, *SLC7A4*, *SLC39A14*, *ENPP3*) and tight-junction barrier integrity (*CLDN22*, *SCGB1D4*). Together, these findings suggest that altered luminal ion balance and epithelial barrier function are reproducible features of the endometrium in unexplained female infertility rather than dataset-specific findings.

4.1. Novel and validated genes

The validated genes are particularly compelling because they were identified consistently across complementary analytical approaches. *WNK4* and *TRPM6* were identified among the discovery DEGs and reproduced in the validation cohort, while also appearing in the inhibited ion channel transport pathway, downregulated ion-transport disease annotations, and PPI hub-gene set. *CAPN6* was reproduced in the validation set and was also associated with the inhibited mitochondrial dysfunction pathway.³⁶ *CLDN22* and *SCGB1D4* were reproduced in the validation set and were represented in the tight-junction network. Concordance across multiple independent analytical approaches provides stronger evidence for a gene as a candidate than identification by a single analysis. Of the 11 validated genes, only *ENPP3* and *SCGB1D4* had previously been implicated in infertility, leaving the other 9 reproducible genes largely unexplored in this context. Five additional DEGs, *C1orf106* (*INAVA*), *C15orf59*, *LINC00461*, *C15orf48* (*NMES1*), and *C10orf99* (*GPR15LG*), had no previously reported link to infertility. *PKHD1L1*, which encodes a fibrocystin-related membrane protein, was also reproduced across cohorts but has no established role in the endometrium. These findings identify biological territory that is poorly represented in existing knowledge bases and highlight candidates that warrant direct experimental investigations.

4.2. Biological interpretation

The reproducible genes point to two interconnected disruptions at the epithelial surface. The first involves ion transport. *WNK4* and *TRPM6* contribute to magnesium and electrolyte balance,^{33,34} whereas *SLC7A4* and *SLC39A14* mediate the transport of amino acids and metal ions.³⁹ If these transport processes are collectively impaired,

luminal fluid composition, pH, and epithelial polarity may be altered, potentially affecting the properties of the surface to which an embryo must attach.³⁵ The second involves barrier integrity. Downregulation of the claudin isoform *CLDN22* and the secretoglobin *SCGB1D4* suggests that tight-junction remodeling at the receptive surface may be disrupted. In parallel, the inhibited mitochondrial dysfunction pathway and reduced expression of the mitochondrial biogenesis regulator *PPARGC1A* are consistent with impaired cellular energy metabolism,³² whereas suppression of terpenoid and steroid biosynthesis pathways would reduce local estradiol and progesterone.⁵¹ Together, these findings suggest an endometrium with impaired epithelial homeostasis, altered barrier function, and reduced metabolic capacity at a stage when coordinated epithelial and metabolic changes are required for receptivity.

4.3. Inflammatory signaling

Our enrichment results indicate a pro-inflammatory endometrial state. NK cell-mediated cytotoxicity and complement and coagulation cascades were both increased.⁴⁸ Uterine NK cells normally contribute to implantation; however, excessive NK-cell activity or dysregulated complement activation may adversely affect the embryo or disrupt maternal-fetal immune tolerance.⁴¹⁻⁴³ Increased expression of acute-phase and neutrophil-degranulation genes is also consistent with a tissue exhibiting an injury-like inflammatory response.^{55,58} *IL10RA*, which encodes the receptor for the anti-inflammatory cytokine IL-10, was downregulated, potentially further favoring a pro-inflammatory state.⁶⁴ These findings are not novel individually. Rather, they are consistent with previous studies of infertility and therefore provide supporting evidence for the broader biological picture rather than constituting the primary finding of this study.

4.4. Metabolic and energy deficits

Several metabolic pathways were suppressed, spanning cholesterol and steroid synthesis, unsaturated fatty acid production, and short-chain fatty acid metabolism.⁴⁸⁻⁵⁰ Mitochondrial dysfunction was among the top inhibited pathways, suggesting impaired energy production and reactive oxygen species handling.^{30,31} Reduced expression of *PPARGC1A*, together with increased expression of the antioxidant genes *SOD2* and *GPX3*, is consistent with a compensatory response to oxidative stress.²⁹ The terpenoid backbone pathway, which supplies precursors for cholesterol and downstream steroid hormone biosynthesis, including estradiol and progesterone, was also suppressed. Reduced cholesterol biosynthetic activity could therefore

affect local steroid hormone signaling and membrane composition, potentially altering the epithelial properties required for embryo attachment.⁵²

4.5. Upstream regulators and DNA damage

Ingenuity Pathway Analysis upstream-regulator analysis indicated metabolic stress and weakened anti-inflammatory control. Activated *PRKAA1* (AMPK α 1) suggests that the endometrium may be responding to reduced energy availability by promoting catabolic processes.⁶¹ *PTEN* was predicted to be activated, and disruption of PI3K/AKT signaling downstream of PTEN has been linked to impaired decidualization.⁶² On the inhibited side, *IL10RA* indicates reduced anti-inflammatory signaling, whereas reduced *RAD51* suggests impaired DNA repair capacity. Chronic inflammation and oxidative stress can induce DNA damage, and reduced *RAD51* activity could permit damage to accumulate and contribute to cellular senescence. Senescent cells can, in turn, release pro-inflammatory factors, potentially reinforcing the inflammatory state observed in the enrichment analyses. None of the 11 validated genes were identified as upstream regulators; however, the predicted regulators converged on metabolic and ion-transport pathways represented by the validated genes.

4.6. Shared epithelial and inflammatory gene programs

The disease and biofunction analysis identified gastrointestinal terms such as colitis and enteritis. These findings do not indicate that the patients had gastrointestinal disease; rather, they likely reflect inflammatory and epithelial-barrier gene programs shared by the endometrium and intestinal tissues. Thus, the result reinforces the barrier-and-inflammation theme rather than identifying a distinct biological process. It may also be consistent with emerging evidence for a gut–reproductive link, in which dysbiosis and impaired barrier integrity can affect immune tolerance and hormone signaling.^{64,65} In this context, *C7orf50* could potentially link intestinal lipid signaling with local steroid availability.^{40,66}

4.7. Claudin and parathyroid hormone–receptor isoforms

The IPA interaction network identified claudin (*CLDN*) and parathyroid hormone receptor (*PTH*) entries with no curated infertility role. *CLDN1* and *CLDN4* have established associations with implantation, and claudin-3 and claudin-4 are dysregulated in the endometrium of women with impaired implantation.⁶⁷ However, the generic claudin entry identified in this analysis has not been specifically linked to infertility, leaving open the

possibility that individual isoforms, such as *CLDN2*, *CLDN10*, *CLDN18*, and *CLDN22*, may contribute to tight-junction signaling in reproductive failure. The validated barrier genes *CLDN22* and *SCGB1D4* are represented within this network, while the downregulated transporters *WNK4* and *TRPM6* further support a potential disruption of epithelial polarity and fluid homeostasis. *PTH* lacks an established role in fertility and therefore warrants direct experimental investigation.

4.8. Limitations

Several limitations should be considered when interpreting these findings. The discovery analysis was based on a single dataset of 18 samples, which limits statistical power. We addressed this limitation by evaluating an independent cohort, in which 11 DEGs were reproduced in the same direction; however, this cohort was modest in size and analyzed on a different platform. The study was computational and has no protein-level or experimental validation, so the proposed mechanisms remain inferential. Some findings, such as the shared gastrointestinal annotations and the uncharacterized claudin and parathyroid hormone–receptor isoforms, are based on functional annotations and existing literature rather than direct measurements. CTD cross-referencing identified a lack of curated evidence supporting the novelty of these candidates, but it does not establish a causal role in fertility. Pearson correlation was retained for WGCNA because the analysis was performed on module eigengenes, which are continuous, multi-gene summary measures that are generally more amenable to correlation-based analysis than individual gene-level counts. Nevertheless, a rank-based correlation analysis could be included as a sensitivity analysis given the limited sample size. We did not perform a meta-analysis pooling the two cohorts because they differed in platform and study design; pooling them could therefore introduce additional heterogeneity into the estimated effects.

4.9. Clinical implications and future directions

The reproducible genes provide a short list of candidate markers of endometrial dysfunction in unexplained infertility. *WNK4*, *TRPM6*, *CAPN6*, *CLDN22*, and *SCGB1D4*, together with *C7orf50* and other understudied candidates, could be evaluated as a focused expression panel for endometrial receptivity. These findings represent biomarker candidates only and do not support any treatment implications. Protein-level confirmation and prospective clinical validation would be required before these candidates could be considered for clinical use. Key next steps include validation in additional independent cohorts, protein-level confirmation of the leading

candidates, and direct assessment of whether *C7orf50* and the claudin isoforms vary with endometrial receptivity status.

5. Conclusion

In this study, unexplained female infertility was associated with an endometrial state characterized by increased innate immune and inflammatory activity and lowered lipid, steroid, and mitochondrial metabolism. Eleven genes were identified in an independent cohort and converged on epithelial ion transport, tight-junction barrier function, and mitochondrial dysfunction, with *WNK4*, *TRPM6*, and *CAPN6* emerging as the clearest leads. Several of these genes, together with *C7orf50* and five additional candidates, *C1orf106* (*INAVA*), *C15orf59*, *LINC00461*, *C15orf48* (*NMES1*), and *C10orf99* (*GPR15LG*), have not previously been linked to fertility. These reproducible yet under-investigated genes, along with the ion-transport and barrier pathways in which they are implicated, offer testable biomarkers for unexplained female infertility and potential targets for future experimental study.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Conceptualization: Ritika Patial

Data curation: Ritika Patial

Formal analysis: Ritika Patial, Sonalika Ray

Investigation: Ritika Patial

Methodology: Ritika Patial, Kashmir Singh

Project administration: Kashmir Singh, Ranbir C. Sobti

Supervision: Kashmir Singh, Ranbir C. Sobti

Writing–original draft: Ritika Patial

Writing–review & editing: Sonalika Ray, Kashmir Singh, Ranbir C. Sobti

Ethics approval and consent to participate

This study is based on the re-analysis of publicly available transcriptomic data and does not involve any new human or animal subjects. Therefore, ethics approval and participant consent were not required.

Consent for publication

Not applicable.

Availability of data

The gene expression datasets analyzed in this study are publicly available in the NCBI Gene Expression Omnibus (GEO) repository under accession numbers GSE92324 (discovery) and GSE111974 (validation).

Further disclosure

Artificial intelligence (AI) tools were used in the preparation of this manuscript solely for grammar checking and improving the overall structure and outline of the content. No AI tools were used for data analysis, interpretation, or generation of scientific results. All scientific content, conclusions, and interpretations are the original work of the authors. The paper has been deposited in a preprint server, BioRxiv (doi: 10.64898/2026.01.24.701467).

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