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Gene Expression Profiling of Cumulus-oocyte Complex in Infertile Women: A Systematic Review and Integrated Bioinformatic Analysis

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Abstract— The cumulus-oocyte complex (COC) is an important complex for oocyte maturation. Gene expression in the cumulus cells surrounding the oocyte is crucial for regulating maturation. Abnormal gene expression in the cumulus-oocyte complex may inhibit oocyte maturation and subsequently lead to infertility. A thorough understanding of impacted gene expression profiles and molecular pathways in infertile women is necessary. However, the gene expression profiles vary between studies. Thus, a comprehensive literature search across EBSCOhost, PubMed, ScienceDirect, and Scopus databases was conducted to robustly identify gene expression profiles of cumulus-oocyte complexes and the molecular pathways involved in infertility development. Terms including (“Infertile women” OR Sterility OR Subfertility OR Infertility OR “Sub-fertility”) AND (“Gene expression” OR Profiling OR Transcriptome) AND (“In-vitro fertilization” OR “In-vitro fertilisation” OR IVF) were used as keywords in all fields. 2090 studies were retrieved, and all were screened against the inclusion and exclusion criteria. After the screening, five studies were selected, and 897 differentially expressed genes (DEGs) were identified across them. The integrated bioinformatic analysis using DAVID, STRING, and Cytoscape revealed a protein-protein interaction (PPI) network comprising 30 high-intermolecular-interaction clusters. Based on Gene Ontology (GO) annotations, most DEGs are expressed in the extracellular space, cytosol, and mitochondria. Correspondingly, the DEGs were identified as modulating communication with the extracellular environment via signalling pathways and regulating proliferation via DNA transcription and the cell cycle. This systematic review further elucidates the genes and molecular mechanisms within the cumulus-oocyte complex involved in the development of infertility.

Keywords—cumulus cells; gene expression; infertility, in vitro fertilization; oocytes

I. INTRODUCTION

Infertility nowadays has become a concerning medical condition affecting about 17.5% of the adult population or 1 among 6 individuals worldwide [1]. The intricacy of infertility is a result of the complicated interactions between several factors, including lifestyle decisions, underlying medical issues, environmental effects, and genetic predispositions [2].

The cumulus-oocyte complex (COC) is a crucial component of oogenesis, one of the complicated processes regulating fertility [3]. The cumulus cells that surround the oocyte in the ovarian follicle are responsible for providing crucial support and nutrition, which in turn controls the oocyte's development and maturity [4]. Significantly, the cumulus cell's gene expression profile functions as a

molecular hallmark indicative of the oocyte's capacity for development and general state of reproductive health.

The discovery of biomarkers linked to infertility has attracted considerable interest in the scientific community lately. Biomarkers have enormous potential to predict reproductive outcomes and guide individualised treatment plans. In cumulus cells, genes such as Gremlin 1 (GREM1), Prostaglandin-Endoperoxide Synthase 2 (PTGS2), Bone morphogenetic protein 15 (BMP15), and Hyaluronan Synthase 2 (HAS2) have been identified as key regulators of oocyte quality and cumulus cell activity. However, these studies were performed in a single cohort. The samples came from distinct populations that represent only a small fraction of the whole population, and researchers used different methods to identify DEGs. Therefore, these individual results cannot support a strong conclusion about the pathogenesis of infertility, given the various biases involved.

Thus, a systematic review is crucial to provide robust insight into the gene expression mechanisms among infertile individuals in order to comprehend the disruption of biochemical pathways in infertile women. Integrating bioinformatic approaches enables the systematic analysis of large-scale genomic datasets, identifying dysregulated pathways and molecular signatures associated with infertility.

While our study focuses on a cohort of patients with clinical infertility, we clarify that our objective is not to compare infertile and fertile populations but rather to identify molecular biomarkers associated with oocyte competence in the IVF context. Thus, this systematic review aimed to determine gene expression patterns associated with infertility, their protein-protein interactions and annotations, and the biological pathways involved in cumulus cells across populations through integrated bioinformatic analysis. By illuminating the molecular reasons behind infertility, this systematic review aims to pave the way for customised therapies tailored to the specific needs of individuals with reproductive challenges.

II. MATERIALS AND METHODS

This review has been officially registered in PROSPERO (No.CRD42024527475)

A. Search strategy

This systematic review followed the PRISMA guidelines. A thorough literature search was conducted in June/2024 using ScienceDirect, EBSCOhost, Scopus, and PubMed to identify relevant studies published up to that date. Additional keywords were generated using Medical Subject Headings (MeSH) terminology, including Oocytes, Infertility, and "Fertilization in Vitro". The following keyword sets were combined with ("AND"): (1) "Infertile women" OR Sterility OR Subfertility OR Infertility OR "Sub-fertility"; (2) "Gene expression" OR Profiling OR Transcriptome; (3) "In-vitro fertilization" OR "In-vitro fertilisation" OR IVF. The bibliographies of the retrieved papers provide further references.

B. Inclusion criteria

All studies, including case-control, cross-sectional, prospective, and retrospective, that reported abstracts examining DEGs in infertile IVF patients were included. To ensure data homogeneity, we included only clinical studies reporting differentially expressed genes (DEGs) in cumulus cells and oocytes from IVF patients.

C. Exclusion criteria

This systematic review excluded case reports and in silico, in vitro, and in vivo studies. Studies that involved interventions were excluded. Studies that collected blood samples or any samples other than cumulus cells and oocytes were excluded. Studies that included chronic diseases such as diabetes, hypertension, and cancer patients were excluded.

D. Screening of Articles for Eligibility

Screening of all articles pooled from all resources was conducted through three phases. In the first phase, duplicate papers were removed, and articles with irrelevant titles were excluded. Next, the abstracts were reviewed, and articles that did not fulfil the inclusion criteria were excluded. Lastly, screening was conducted by thoroughly reviewing the full texts of the remaining articles, and articles that did not meet the inclusion criteria were excluded in this third phase. All authors participated in selection, screening, and data extraction. The PRISMA flow diagram (Figure 1) summarises the article selection process and the reasons for excluding articles.

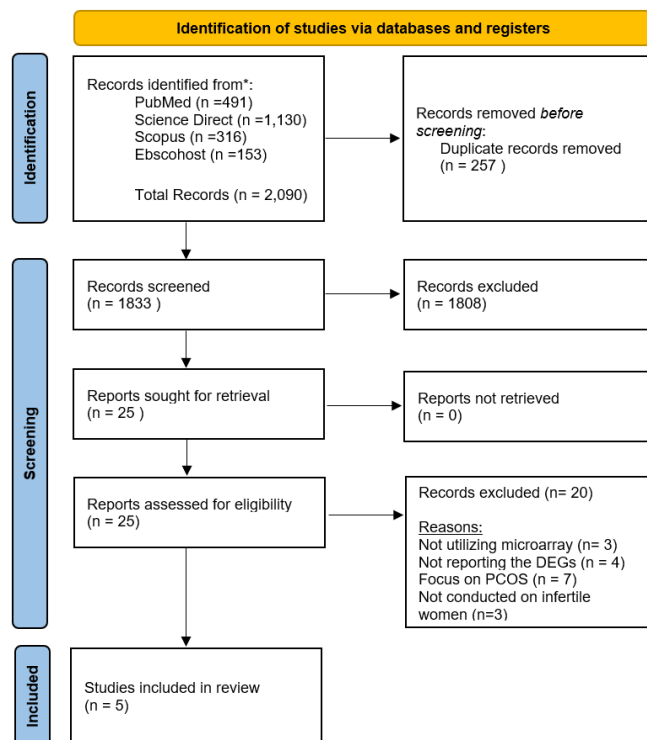


Figure 1. PRISMA flow diagram

E. Data extraction

Data were extracted from all studies that met the inclusion criteria. All the authors participated in data extraction. A data collection form was utilised to standardise the data-gathering process, and each data extraction was conducted separately. The authors resolved disagreements by majority vote. Reasons for exclusion were recorded. The following information was gathered: (1) name of the author; (2) year of publication; (3) paper title; (4) country; (5) study design; (6) sample type; (7) sample size; (8) mean age; (9) gene-expression technique; (10) list of DEGs; (11) list of upregulated DEGs; and (12) list of downregulated DEGs.

To harmonise DEG lists from heterogeneous profiling platforms, all reported gene identifiers were standardised to official HGNC Gene Symbols, ensuring consistent nomenclature and facilitating deduplication. Although studies in this review used different significance thresholds (0.05 and 0.001), heterogeneity was addressed by retaining DEGs deemed significant and expert-verified by the original authors.

F. Study Quality

Using the Joanna Briggs Institute critical assessment methods, MFA and NFMM independently assessed each paper's study quality. MHE and NAH verified the study's quality findings. Studies that received a total score of less than 50% were categorised as low quality and had a high risk of bias; studies that received a total score between 50 and 69% were categorised as moderate quality and had a moderate risk of bias; and studies that received a score of more than 69% were categorised as high quality and had a low risk of bias.

G. Protein-Protein Interaction (PPI) Network, clustering, and visualisation

All retrieved DEGs were pooled and analysed using PPI functional enrichment analysis via STRING software (<https://string-db.org/>) to identify the PPI network [5]. To combine the gene expression patterns of the DEGs and visualise the molecular interaction networks, the STRING results were imported into Cytoscape (<http://www.cytoscape.org>) [6]. Target network module analysis and protein clustering were conducted using the Cytoscape MCODE plug-in. Max depth = 100, K-score = 2, degree cut-off = 2, node score cut-off = 0.2, and node density cut-off = 0.1 were the criteria used to choose the modules.

H. Gene ontology (GO) and pathway enrichment analysis

All genes in each cluster were analysed using the Database for Annotation, Visualisation, and Integrated Discovery (DAVID) to identify gene ontology and functional annotation enrichment associated with infertility [7-12]. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway was used to assess gene involvement in the infertility-related pathway.

III. RESULTS

A total of 2090 potentially relevant studies were found using the keywords across the four databases. After title

screening, 257 duplicates were eliminated, and the abstracts of the remaining 1833 were screened further. After the abstracts were evaluated, 1808 papers were excluded. The full paper of the other 25 papers was then retrieved. After a thorough review of the full text, 20 papers that did not meet the inclusion and exclusion criteria were excluded. Finally, five studies were selected for this systematic review (Tanriverdi et al., 2013; Wathlet et al., 2012; Xu et al., 2015; Zhang et al., 2005). These five studies were published between 2005 and 2015. Sampling bias was prevented through homogeneous sampling by strictly adhering to the specific inclusion and exclusion criteria. Only studies that reported gene expression were included, and the sample size ranged from 3 to 47. Table 1 summarises the included studies.

A. Patient recruitment and sample collection details.

All studies described sample collection. All studies were conducted on infertile women undergoing IVF treatments stimulated with gonadotropin-releasing hormone agonist (GnRH-a). The ovarian response was monitored by ultrasound assessment of follicular development prior to oocyte retrieval. All studies collected oocyte samples under ultrasound guidance 34-36 hours after hCG stimulation. All studies reported on cumulus gene expression. Gasca et al. (2007) reported on gene expression in both cumulus cells and oocytes. However, only the gene expression results from cumulus cells were retrieved from the Gasca et al. (2007) study.

B. Study quality.

Supplementary Table 1 contains a comprehensive quality assessment of the included studies. This systematic review included four high-quality (low-risk) and one moderate-quality (moderate-risk) study.

C. Identification of DEGs in the cumulus-oocyte complex of infertile women

DEGs were pooled from all the included studies. After removing duplicates, 897 DEGs were retrieved for further integrated bioinformatic analysis.

D. Identification of key candidate genes and pathways via Protein-Protein Interaction (PPI) Network and Modular Analysis.

A total of 897 proteins from the selected DEGs were filtered into a PPI network complex containing 878 nodes and 5634 edges with a PPI enrichment p-value of $< 1.0e-16$ and an average local clustering coefficient of 0.359. The molecular interaction networks were visualised by importing the network data from STRING into Cytoscape. The Molecular Complex Detection (MCODE) algorithm was employed, and nine significant modules from the PPI network complex were identified. Figure 2 shows the nine significant clusters created from the PPI network complex generated from the DEGs in the cumulus-oocyte complex of infertile women. The functional-annotation clustering showed that cluster 1 comprises 47 nodes and 246 edges (score = 10.696), cluster 2 consists of 45 nodes and 218 edges (score = 9.909), cluster 5 consists of 22 nodes and 42 edges (score=4), cluster 6 consists

of 4 nodes and 6 edges (score=4), cluster 8 consists of 4 nodes and 5 edges (score=3.333), cluster 12 consists of 23 nodes and 34 edges (score=3.091), cluster 13 consists of 3 nodes and 3 edges (score =3), cluster 14 consists of 3 nodes and 3 edges (score =3), and cluster 21 consists of 3 nodes and 3 edges (score =3). Gene Ontology (GO) and pathway enrichment analysis of the identified clusters

The GO and pathway enrichment analysis identified nine clusters; the most prominent clusters (1, 2, 5, 6, 8, 12, 13, 14, and 21) were involved in the microenvironment, cell signalling, and cellular metabolism. The DEGs in cluster 1 were primarily located at the membrane (GO:0016020) and the cytoplasm (GO:0005737). These DEGs are involved in the immune response and microenvironment regulation, with a primary molecular function in chemokine activity. In contrast, the DEGs in cluster 2 were primarily localised to the extracellular region (GO:0005576), involved in angiogenesis and neutrophil chemotaxis, and had a notable molecular function of CXCR chemokine receptor binding. Cluster 5 comprised DEGs primarily localised to the membrane (GO:0016020) and involved in cholesterol biosynthesis (KW-0152) and lipid metabolism (KW-0443). The DEGs in Cluster 6 are located in the cytosol (GO:0005829) and are major participants in exocytosis (GO:0006887), intracellular protein transport (GO:0006886), and the positive regulation of cell migration (GO:0030335). Table 2 summarises the functional annotation of all the prominent clusters. Cluster 8 included DEGs localised to the mitochondrial matrix (GO:0005759) and was strongly involved in fatty acid metabolism (KW-0276), oxidation (GO:0006635), and lipid metabolism (KW-0443). The DEGs in Cluster 12 were mostly found in the lipid droplet (KW-0551) and are involved in lipid degradation (KW-0442) and metabolism (KW-0443). Cluster 13 consisted of DEGs involved in ribosome structure (GO:0003735), found in the mitochondrion (GO:0005739), and involved in mitochondrial translation (GO:0032543). In contrast, Cluster 14 included DEGs localised to the collagen-containing extracellular matrix (GO:0062023) and involved in extracellular matrix structural support conferring compression resistance (GO:0030021). The DEGs in Cluster 21 are involved in the galactose metabolic process (GO:0006012), amino sugar and nucleotide sugar metabolism (hsa00520), and carbohydrate metabolic process (GO:0005975).

E. Selection of candidate genes for infertility

Based on the PPI network and functional annotation clustering (Table 3), we selected 11 candidate genes for their involvement in relevant GO terms and their interactions with other proteins in the PPI network via undirected first-neighbour nodes (Figure 2). Candidate genes were filtered based on their biological significance in pathways critical to oocyte competence, follicular development, and implantation via Gene Ontology and KEGG pathway analyses. The candidate genes include ITGB1, CXCL1, CXCL8, CXCL12, FDPS, EXOC5, ACAA1, RAB7A, MRPL18, VCAN, and GALK2. Genes from Cluster 1 interacted closely with those from Cluster 14, whereas Clusters 21, 12, and 13 did not. Genes from Clusters 5, 8, 12, 13, and 21 are involved in energy production, while genes from Clusters 1, 2, 6, and 14 are involved in microenvironmental mechanisms.

IV. DISCUSSION

Based on the functional annotation clustering analysis, which yielded 30 clusters of DEGs, the major molecular mechanisms in the cumulus cells of infertile women can be divided into cell signalling, inflammatory response, and energy metabolism.

A. Microenvironment and Inflammatory Responses

DEGs in cluster 1 are primarily located at the membrane and the external side of the membrane. These DEGs are associated with cells' microenvironmental signalling and inflammatory response, offering insights into the interplay between these mechanisms within the immune microenvironment (IME). The homeostasis of IME is greatly influenced by M1 and M2 macrophages polarisation [13], which perturbation ultimately leads to the loss in ovarian function [14], folliculogenesis impairment [15], and recurrent implantation failure (RIF) [16]. From the DEGs in cluster 1, ITGB1 is found to be upregulated in infertile women. ITGB1 expression is associated with the IME by infiltrating CD8+ T cells, dendritic cells, macrophages, and neutrophils [17]. Thus, the dysregulation of ITGB1 contributes to an unfavourable microenvironment for viable pregnancies to occur [18]. Another candidate gene discovered is CXCL12, located in the extracellular exosome (GO:0070062), which is also a ligand for the CXCR4 receptor involved in oocyte maturation via cytokine-cytokine receptor interaction, CXCL12-CXCR4 signalling (hsa04060). In this review, CXCL12 is upregulated, validating its role in the expansion of cumulus cells through a positive regulation of monocyte chemotaxis (GO:0090026) via the activation of the SH2 domain, leading to the MAPK pathway [19-21]. CXCL12 in the cumulus cells has also demonstrated a sperm-attractant property towards the cumulus-oocyte complex during IVF via its interaction with the CXCR4 receptor localised on the capacitated spermatozoa [19]. However, although CXCL12 plays a role in cumulus expansion, the expression of CXCL12 is still upregulated in infertile women. Thus, indicating that CXCL12 is involved not only in cumulus expansion. Instead, CXCL12 is related to the overproduction of matrix metalloprotease (MMP) 9 and MMP2, which have recently been discovered to cause abnormal implantation [22]. A candidate gene obtained from Cluster 14 is versican (VCAN), found in the extracellular region (GO:0005576) to stabilise hyaluronic acid on the surface of cumulus cells by its interaction with integrins and other extracellular matrix components containing collagens (GO:0062023) [23]. VCAN expression, which accelerates 20- to 50-fold due to the LH surge, is found to be indicative of oocyte competence, implantation rates, and the pregnancy outcome due to its ability to modulate cumulus expansion, thus allowing the required cell-to-cell signalling process to occur [4, 24]. In infertile women, VCAN becomes upregulated, which we postulate as an attempt to remodel the extracellular matrix in response to the deterioration of ovarian reserve [25].

B. Cell Signalling

Another candidate gene highlighted in this study is CXCL1, whose expression might be linked with the activation of Toll-like receptor 4 (TLR4) on immune cells, aiding in the

B-cell extravasation during inflammatory response (KW-0395) [26]. The upregulation of CXCL1, as observed in this review, is postulated to be involved in the neutrophil chemotaxis (GO:0030593) in infertile women with chronic endometritis to mediate the antimicrobial humoral immune response (GO:0061844) [27]. Another candidate gene was CXCL8 or Interleukin-8 (IL8), which is found in the extracellular region (GO:0005576) and acts as a ligand for both CXCL1 and CXCR2 receptors in the induction of chemotaxis during angiogenesis (GO:0001525) [28]. CXCL8 is discovered to be downregulated in infertile women, indicating that the failure to conceive could be due to an inconducive angiogenic environment due to the decreasing phosphorylation of ERK1/2, hence the alteration in the microenvironment [29]. CXCL8's downregulation affects the migration of neutrophils (GO:0030593) and the production of reactive oxygen species (ROS) via the regulation of G protein-coupled receptor signaling pathway (GO:0045744) [30]. This overproduction of ROS due to the disrupted negative regulation of GO:0045744 is implicated in decreased female fertility, as its accumulation disrupts redox equilibrium, hence damaging the oocyte [31, 32]. Another highlighted candidate gene is exocyst complex component 5 (EXOC5) or Sec10 from Cluster 6. EXOC5 is found in the cytosol (GO:0005829), which constitutes a multiprotein complex involved in exocytosis (GO:0006887) where the fusion of secretory vesicles with the plasma membrane during cell-to-cell signalling takes place. A functioning exocyst complex is required for the exocytosis process after sperm fertilisation to prevent polyspermy by reconstructing the impermeability of the zona pellucida through the translocation of cortical granules [33, 34]. In this review, EXOC5 is found to be downregulated in infertile women, postulating its implication on the cystogenesis process of germ cells to form germline cysts during gametogenesis [35, 36]. Apart from that, the underexpression of EXOC5 may be associated with the disruption in the folliculogenesis as previously reported in a study where the deposition of collagen abundant in the extracellular matrix in age-related fibrosis was detected in cKO [37, 38]. We also identified a candidate gene, Ras-related protein (RAB7A), found in the cytosol (GO:0005829) that is predominantly involved in lipid droplet degradation (KW-0442) via autophagy when cells are under nutrient stress. Its downregulation in infertile women disrupts the degradation of cargo contents and the release of macromolecules into the cytosol [39, 40]. The underexpression of RAB7A leads to the failure in autophagy, which is usually necessary during nutrient deprivation, in which triglycerides stored in the lipid droplets will be hydrolysed into free fatty acids and ATP to fulfill cellular demands for viable pregnancies to occur [41].

C. Energy Metabolism

In Cluster 5, a candidate gene discovered is farnesyl diphosphate synthase (FDPS), localised in the membrane (GO:0016020), involved in the synthesis of geranyl pyrophosphate and farnesyl pyrophosphate (FPP), both substrates of GTPase Rac1 during cholesterol synthesis (KW-0152) [42]. The downregulation of FDPS in infertile women contributes to disrupted embryogenesis, for FDPS acts to fulfill the demand of cholesterol in the oocytes, where

cholesterol cannot be synthesized de novo due to the lack of lipoprotein receptors [43, 44]. Dysregulated lipid metabolism (KW-0443), including cholesterol biosynthesis by FDPS, is therefore unable to provide a follicular environment rich with the precursor for steroid hormones such as estradiol and progesterone necessary for oocyte development to be synthesised [45, 46]. Next is Acetyl-CoA acyltransferase 1 (ACAA1) from Cluster 8, which plays a major role in fatty acid metabolism (KW-0276) by catalysing the formation of acetyl-CoA, which is a substrate for ATP production through the citric acid cycle, TCA [47]. The ATP production driven by ACAA1 could generate approximately 106 ATP molecules compared to glucose metabolism, implying its notable potential in promoting oocyte maturation [48]. Therefore, the downregulation of ACAA1 in infertile women may interfere with the transition of the 8-cell embryo to the blastocyst due to the lack of energy demand for rapid proliferation [49]. The reduction in oocyte maturation rate was also observed apart from delayed meiosis resumption when fatty acid oxidation was inhibited in mice [50]. Another candidate gene discovered in Cluster 13 is mitochondrial ribosomal protein L18 (MRPL18), which is one of the structural constituents of the ribosome (GO:0003735), localised in the mitochondrion (GO:0005739). MRPL18 is alternatively translated, producing a cytoplasmic isoform to integrate with the 80s ribosome complex under stress conditions, highlighting its role during ATP production in cell survival mode [51]. MRPL18's upregulation is associated with its role in oocyte maturation, which requires a large amount of ATP to cater to the continuous cellular transcription and translation process [52]. Moreover, it is previously reported that along with other MRP proteins, MRPL18 knockout will impact the gastrulation initiation by day 7.5, as well as a significant reduction in ATP production [53]. However, the upregulation of MRPL18 in infertile women is not indicative of viable pregnancies, as the increased expression of MRPL18 may be a compensatory response to the declining mitochondrial function in infertile women [54]. Another candidate gene revealed from Cluster 21 is GALK2, which is involved in the galactose metabolic process (GO:0006012). In its deficiency, galactosemia type 2 may occur, where, interestingly, primary ovarian insufficiency was detected as one of the long-term complications of this condition among 80% of females [55]. In infertile women, GALK2 is found to be upregulated, which could be explained as a compensatory mechanism to ingest galactose into galactose-1-phosphate to prevent galactose toxicity in the Leloir pathway of galactosemia sufferers [56].

D. Limitations of the Study

Several limitations must be considered in this systematic review, primarily the absence of fertile populations as a control group for comparing gene expression among already infertile IVF populations. Thus, the molecular biomarkers identified are predictive of oocyte competence, implantation rate, and IVF success, rather than underlying etiological drivers of clinical infertility. Because these findings reflect only biomarkers influencing oocyte developmental potential, more studies should compare biomarkers between infertile and fertile populations to distinguish which genes are standard

in reproductive health and which are unique and clinically proven to affect fertility status.

Another limitation is the methodological heterogeneity in defining the statistical significance of the DEGs. This review includes studies using different thresholds ($p < 0.05$ and $p < 0.001$), based on the primary authors' established high-stringency cut-offs. Given that this is a systematic review on reported DEGs, we adopted an approach to preserve all DEGs deemed significant by the rigorous statistical thresholds set by the original authors, rather than standardising to only one, and a potentially biased cut-off, ensuring that our analysis is based upon high-confidence and validated data points from the primary literature.

Additionally, this review comprises literature published between 2005 and 2015. While these studies established the foundational molecular characterisation of cumulus cells in infertile populations, the lack of more recent literature may reflect a notable shift in research toward newer omics technologies and single-cell transcriptomics. Therefore, future research should validate these findings and incorporate new datasets to determine whether this molecular characterisation persists as clinical and bioinformatic standards evolve.

V. CONCLUSION

The molecular interactions in the cumulus cells of infertile women require in-depth studies to identify comprehensive molecular mechanisms that influence oocyte competence and IVF outcomes, providing better insights into biomarker panels for predicting embryo potential. The integrated bioinformatic analysis from this systematic review reveals three major molecular mechanisms involved in infertility: microenvironment and inflammatory responses, cell signalling, and energy metabolism. ITGB1, CXCL1, CXCL8, CXCL12, FDPS, EXOC5, ACAA1, RAB7A, MRPL18, VCAN, and GALK2 were identified as key genes that regulate oocyte competency and development. These genes can be utilised to develop a biomarker panel to screen for oocyte incompetence and to identify targeted genes for developing better treatment strategies.

AUTHORS' CONTRIBUTIONS

M.H.E: Conceptualisation, Methodology; N.Z: Writing-Original Draft, Reviewing and Editing; N.F.M.M., N.A.H., Z.S & M.F.A: Reviewing and Editing; All authors: Review, Improvement, and Approval of the Manuscript. Overall, this manuscript is entirely collaborative work, and all authors met the journal's contribution requirements.

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CONFLICT OF INTEREST

The authors have no competing interests relevant to this article.

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DECLARATION OF AI-ASSISTED TOOLS USAGE

AI-assisted tools were used solely for language editing to improve grammar, clarity, and readability. An AI-detection assessment reported a score below 15%. The authors have critically reviewed and verified all revisions and accept full responsibility for the accuracy, originality, integrity, and entire content of the manuscript.

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