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# The Association Between Gene Expression in Cumulus Oophorus Cells and Human Embryo Developmental Potential: A Systematic Review of its Prognostic Utility for Intracytoplasmic Sperm Injection Success

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## ABSTRACT

**Background**: The developmental potential of human embryos following intracytoplasmic sperm injection (ICSI) is influenced by both oocyte and follicular microenvironmental factors. Cumulus oophorus cells (COCs), which surround the oocyte and communicate bidirectionally with it through gap junctions, play a crucial regulatory role in oocyte maturation, fertilization, and early embryogenesis. The gene expression profiles of COCs have recently emerged as potential non-invasive biomarkers for assessing oocyte competence and predicting ICSI outcomes. **Objective**: This systematic review aims to evaluate the association between gene expression patterns in cumulus oophorus cells and embryo developmental potential, focusing on their prognostic utility for predicting fertilization success, embryo quality, and implantation rates in ICSI cycles. **Methods**: A comprehensive search was conducted in PubMed, Scopus, Web of Science, and Embase databases up to October 2025. Eligible studies included observational, prospective, and experimental studies investigating gene expression in COCs obtained from women undergoing ICSI. Data extraction included sample size, patient characteristics, ovarian stimulation protocols, analyzed genes, detection methods (e.g., RT-qPCR, microarray, RNA sequencing), and reported outcomes. Risk of bias was assessed using the Newcastle–Ottawa Scale for observational studies and the SYRCLE tool for animal models. **Results**: A total of 32 studies were included, encompassing over 2,800 oocyte–cumulus complexes. Several genes were consistently correlated with oocyte and embryo quality. Elevated expression of HAS2, GREM1, PTX3, CYP19A1, and AREG was associated with higher rates of fertilization and blastocyst formation. Conversely, altered expression of apoptotic and stress-related genes such as BAX, FAS, and HSP70 correlated with reduced developmental competence. Transcriptomic and microarray analyses identified distinct molecular signatures capable of discriminating between developmentally competent and incompetent oocytes. Despite promising findings, heterogeneity in study design, stimulation regimens, and normalization methods limited cross-study comparability. **Conclusion**: Gene expression profiling of cumulus oophorus cells provides a valuable, minimally invasive tool for predicting oocyte quality and embryonic developmental potential in ICSI cycles. However, the current evidence is limited by methodological variability and small sample sizes. Standardized molecular targets and validation across diverse populations are needed before clinical application. Future research integrating transcriptomic, proteomic, and metabolomic data may enhance the accuracy of non-invasive biomarkers for assisted reproductive technology (ART) outcomes.

**KEYWORDS**: Cumulus oophorus cells, gene expression, oocyte competence, embryo quality, intracytoplasmic sperm injection (ICSI), biomarkers, reproductive genetics.

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## INTRODUCTION

Infertility affects approximately 10–15% of couples of reproductive age worldwide and represents a growing public health concern with significant social and emotional implications. Assisted reproductive technologies (ART), particularly *in vitro fertilization* (IVF) and *intracytoplasmic sperm injection* (ICSI), have revolutionized the management of infertility by enabling fertilization and embryo development outside the human body. Among these, ICSI has become the preferred method for treating male and unexplained infertility because it bypasses many natural barriers to fertilization by injecting a single sperm directly into the oocyte cytoplasm. Despite remarkable technological advancements, the success rate of ICSI—measured by fertilization,

implantation, and live birth outcomes—remains suboptimal, often not exceeding 30–40% per cycle (Liu et al., 2020; Ortega et al., 2022).

A major determinant of ICSI success lies in the *developmental competence* of the oocyte, which refers to its intrinsic ability to resume meiosis, undergo fertilization, and support subsequent embryo development. However, traditional morphological evaluation of oocytes and embryos—based on cumulus expansion, zona pellucida thickness, and pronuclear morphology—provides only limited predictive value for developmental potential. This limitation has prompted researchers to investigate molecular and cellular biomarkers that can better reflect oocyte quality and improve embryo selection for transfer (Assidi et al., 2020).

One of the most promising non-invasive approaches for assessing oocyte competence is the analysis of gene expression in the *cumulus oophorus cells (COCs)*. These cells form the outer layer of the *cumulus-oocyte complex (COC)* and play an essential role in oocyte maturation through constant bidirectional communication mediated by paracrine signals, gap junctions, and extracellular vesicles (Gilchrist et al., 2008). Cumulus cells support the oocyte during folliculogenesis, mediate the transfer of ions, metabolites, and signaling molecules, and respond dynamically to hormonal cues such as the luteinizing hormone (LH) surge. Therefore, the transcriptional profile of cumulus cells is considered a mirror of the oocyte's functional status and its potential to develop into a viable embryo (Assou et al., 2021).

Advances in molecular biology and high-throughput transcriptomics have enabled the identification of numerous genes in COCs that correlate with oocyte maturity, fertilization rate, and embryo quality. Genes such as *Hyaluronan Synthase 2 (HAS2)*, *Gremlin 1 (GREM1)*, *Pentaxin 3 (PTX3)*, *Amphiregulin (AREG)*, and *Prostaglandin-Endoperoxide Synthase 2 (PTGS2)* have been repeatedly implicated in promoting cumulus expansion and enhancing oocyte competence (Feuerstein et al., 2007; McKenzie et al., 2004). Conversely, increased expression of apoptotic markers such as *BAX*, *FAS*, and *CASP3* or oxidative stress–related genes such as *HSP70* and *SOD2* has been linked with compromised oocyte quality and lower fertilization potential (Assidi et al., 2011; Li et al., 2020). These findings highlight the potential of cumulus cell transcriptomic profiling as a *surrogate marker* for embryo viability, offering a non-invasive alternative to invasive oocyte or embryo biopsy techniques.

Furthermore, with the shift toward single-embryo transfer in ART practices to reduce multiple pregnancy rates, the need for reliable molecular indicators that can predict implantation and live birth outcomes has intensified (Sfontouris et al., 2021).

By identifying cumulus cell gene expression signatures associated with high-quality oocytes and successful ICSI outcomes, clinicians can improve embryo selection accuracy and optimize personalized ovarian stimulation protocols. Such biomarkers could also contribute to refining laboratory culture conditions, minimizing embryo wastage, and reducing the emotional and financial burden on couples undergoing fertility treatment **(Ossman et al., 2023)**

Despite these promising prospects, the literature remains heterogeneous, with variability in study design, patient selection criteria, ovarian stimulation regimens, timing of cumulus cell retrieval, and molecular techniques used (qPCR, microarray, RNA-Seq). These discrepancies have hindered the establishment of standardized gene panels or validated cut-off thresholds for clinical application. Some studies report inconsistent findings, where genes considered predictive in one cohort show no significant association in another. Moreover, the mechanistic pathways linking cumulus cell transcriptional changes to oocyte competence and embryo development are still being elucidated.

Given these challenges, a systematic synthesis of the available evidence is warranted to clarify the prognostic utility of cumulus cell gene expression in predicting embryo developmental potential and ICSI success. Understanding these molecular relationships will not only advance the field of reproductive genomics but also bridge the gap between bench research and clinical embryology. **Therefore, this systematic review aims to** comprehensively evaluate the association between gene expression profiles in cumulus oophorus cells and human embryo developmental outcomes, focusing on their potential use as prognostic biomarkers for ICSI success.

**Rationale:**
Despite advances in assisted reproductive technologies (ART), predicting embryo developmental potential and ICSI success remains challenging. Conventional morphological assessment of oocytes and embryos offers limited accuracy and cannot reliably indicate developmental competence. Cumulus oophorus cells (COCs), which surround and communicate with the oocyte, reflect its physiological and molecular status through synchronized gene expression. Therefore, profiling COC gene expression provides a promising, minimally invasive tool to assess oocyte quality prior to fertilization.

Previous studies have identified several genes—such as *HAS2*, *PTX3*, *GREM1*, *AREG*, *CYP19A1*, and *BAX*—associated with fertilization rate, blastocyst formation, and pregnancy outcomes. However, findings remain inconsistent due to variations in methodologies and patient populations. A systematic synthesis of this evidence is needed to clarify which gene expression markers most accurately predict embryo developmental competence and ICSI outcomes.

**Hypothesis**
Specific gene expression profiles in cumulus oophorus cells are significantly associated with oocyte developmental competence and can serve as prognostic biomarkers for predicting fertilization success and embryo quality in intracytoplasmic sperm injection (ICSI) cycles.

## LITERATURE REVIEW

The developmental potential of the human oocyte is one of the most critical determinants of success in assisted reproductive technologies (ART), particularly intracytoplasmic sperm injection (ICSI). Despite advances in micromanipulation and embryo culture techniques, the prediction of oocyte and embryo competence remains a major challenge in reproductive medicine. Traditionally, morphological assessment of oocytes and embryos—based on cumulus expansion, cytoplasmic appearance, pronuclear morphology, and cleavage rate—has been widely used for selection prior to transfer. However, such morphological criteria are subjective and offer limited predictive power regarding fertilization potential or implantation success (Assou et al., 2021; Ortega et al., 2022). This limitation has prompted the search for more objective, molecular-based biomarkers that reflect oocyte health and developmental competence.

Cumulus oophorus cells (COCs), the somatic cells that envelop the oocyte, form an integral part of the cumulus–oocyte complex (COC) and play an essential role in the processes of folliculogenesis, oocyte maturation, and ovulation. These cells are metabolically and functionally coupled with the oocyte through transzonal projections and gap junctions, facilitating the bidirectional exchange of metabolites, ions, and signaling molecules (Gilchrist et al., 2008).

Such communication ensures synchronization between nuclear and cytoplasmic maturation, modulates meiotic arrest, and enhances cytoplasmic competence. Consequently, the transcriptomic profile of cumulus cells serves as a non-invasive proxy for assessing the oocyte's developmental status and has emerged as a promising tool for predicting outcomes in ART (Assidi et al., 2011; Feuerstein et al., 2007).

Multiple studies have demonstrated that specific genes expressed in cumulus cells are associated with oocyte maturity, fertilization rate, and embryo quality. One of the earliest identified markers, *Hyaluronan Synthase 2 (HAS2)*, encodes an enzyme critical for the synthesis of hyaluronic acid, which contributes to the viscoelastic properties of the cumulus extracellular matrix. Increased expression of *HAS2* has been strongly correlated with higher fertilization rates, better embryo morphology, and improved blastocyst development (Feuerstein et al., 2007). Similarly, *Gremlin 1 (GREM1)*, a bone morphogenetic protein (BMP) antagonist, promotes cumulus expansion and follicular differentiation. High *GREM1* expression in cumulus cells has been linked with successful oocyte fertilization and embryo progression to the blastocyst stage (McKenzie et al., 2004). Another key molecule, *Pentaxin 3 (PTX3)*, plays an essential role in stabilizing the cumulus extracellular matrix; its overexpression in cumulus cells has been consistently associated with oocyte competence and favorable ICSI outcomes (Assidi et al., 2011).

In addition to genes related to cumulus expansion, several signaling and metabolic genes have been implicated in oocyte developmental potential. For instance, *Amphiregulin (AREG)*, *Epiregulin (EREG)*, and *Prostaglandin-Endoperoxide Synthase 2 (PTGS2)* are induced by the luteinizing hormone (LH) surge and facilitate the maturation of both the oocyte and its surrounding cumulus cells **(Ali et al., 2018).**

The upregulation of these genes promotes oocyte nuclear maturation, expansion of the cumulus matrix, and improved fertilization potential (Assou et al., 2021). Similarly, genes regulating steroidogenesis—such as *CYP19A1* (aromatase) and *STAR* (steroidogenic acute regulatory protein)—have been shown to influence local estrogen production within the follicle, supporting oocyte growth and developmental competence. Elevated *CYP19A1* expression in cumulus cells correlates with higher rates of mature oocytes and increased blastocyst formation (Li et al., 2020).

Conversely, several studies have highlighted the detrimental impact of oxidative stress and apoptosis-related gene expression on oocyte quality. The presence of excessive reactive oxygen species (ROS) within the follicular environment induces the activation of apoptotic genes such as *BAX*, *CASP3*, and *FAS*, which impair oocyte developmental potential and reduce embryo viability (Li et al., 2020). On the other hand, enhanced expression of antioxidative defense genes like *SOD2* (superoxide dismutase 2) and *HSP70* (heat shock protein 70) has been associated with better embryo development and implantation potential. Thus, the balance between apoptotic and survival-related transcriptional activity in cumulus cells serves as a molecular indicator of oocyte competence **(Mahmoud et al., 2021).**

The advent of high-throughput transcriptomic technologies—such as microarray analysis and next-generation RNA sequencing—has significantly expanded the understanding of cumulus cell biology. Global gene expression profiling has revealed hundreds of genes that differentiate between developmentally competent and incompetent oocytes. Feuerstein et al. (2007) demonstrated that distinct expression patterns of *HAS2*, *PTX3*, and *GREM1* could effectively discriminate between mature metaphase II oocytes that resulted in top-quality embryos and those that failed to fertilize. Subsequent research confirmed that cumulus gene expression signatures could be used as diagnostic markers for oocyte selection prior to fertilization (Assidi et al., 2011). More recently, Li et al. (2020) integrated transcriptomic data with clinical ICSI outcomes, showing that oxidative stress and apoptosis-related gene expression directly correlated with blastocyst formation and implantation success.

Despite these advances, significant variability persists across studies regarding gene selection, ovarian stimulation regimens, timing of cumulus sampling, and normalization protocols. Differences in patient populations, age, infertility etiology, and gonadotropin exposure can also influence transcriptional profiles, limiting reproducibility and external validation (Sfontouris et al., 2021). Furthermore, while several candidate genes have demonstrated potential as biomarkers, few have been validated in large-scale, multicenter clinical trials. The lack of standardized thresholds for gene expression quantification and the absence of unified analytical methods continue to hinder clinical translation **(Iwes et al., 2023).**

Beyond mRNA analysis, recent research has expanded to include microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and proteomic analyses in cumulus cells. These molecular regulators may provide additional layers of information, as they modulate gene expression post-transcriptionally and influence follicular cell signaling. Integration of multi-omics approaches—combining transcriptomic, proteomic, and metabolomic data—has the potential to yield a comprehensive molecular signature of oocyte quality. Moreover, the application of artificial intelligence and machine learning to these large datasets could enhance the predictive accuracy of non-invasive biomarkers for embryo selection (Ortega et al., 2022).

Clinically, the ability to assess oocyte developmental potential using cumulus cell gene expression would represent a major advancement in ART practice. By identifying reliable, non-invasive molecular markers, embryologists could select the most viable oocytes for fertilization or the most competent embryos for transfer, reducing the need for invasive embryo biopsies and improving the efficiency of single-embryo transfer protocols. This molecular-based selection could decrease the emotional, physical, and financial burden on couples undergoing fertility treatment while maintaining high success rates (Sfontouris et al., 2021).

In summary, existing evidence strongly supports a biological link between cumulus cell gene expression and oocyte developmental competence. Genes involved in cumulus expansion, extracellular matrix regulation, oxidative stress response, and apoptosis appear to be critical determinants of embryo quality and ICSI success. However, inconsistencies in methodological design and insufficient clinical validation currently limit the application of these molecular findings in everyday ART practice. There remains a pressing need for systematic synthesis of available data to identify the most reproducible and clinically useful gene markers, establish standardized testing protocols, and guide future translational research in reproductive genomics.

# METHODS

### Study Design

This systematic review was designed to identify, evaluate, and synthesize published evidence on the association between gene expression in cumulus oophorus cells and human embryo developmental potential, focusing on its prognostic utility for intracytoplasmic sperm injection (ICSI) outcomes. The review followed a structured and transparent approach to ensure comprehensive coverage and methodological rigor in accordance with standard systematic review principles.

### Search Strategy

A comprehensive and systematic search was performed to capture all relevant literature addressing the relationship between cumulus cell gene expression and oocyte or embryo quality. Searches were conducted across multiple biomedical databases, including PubMed, Scopus, Web of Science, and EMBASE, covering all publications available up to October 2025. Manual searches were also carried out through reference lists of retrieved articles, relevant reviews, and conference proceedings. The search strategy utilized Boolean operators and combinations of key terms such as “cumulus oophorus cells,” “gene expression,” “oocyte competence,” “embryo development,” “fertilization,” and “ICSI.”

### Eligibility Criteria

Studies were included if they met the following criteria: (1) original human research articles published in peer-reviewed journals, (2) analysis of gene expression or transcriptional profiling in cumulus oophorus cells retrieved during IVF or ICSI procedures, and (3) reported association between gene expression levels and measurable embryological or clinical outcomes such as oocyte maturity, fertilization rate, embryo quality, blastocyst formation, implantation rate, or pregnancy success. Exclusion criteria comprised: (1) animal or in vitro model studies not involving human oocytes, (2) review articles, letters, or editorials without original data, (3) conference abstracts lacking sufficient details, (4) studies that did not assess embryo developmental potential, and (5) publications not written in English.

### Study Selection Process

Two independent reviewers conducted an initial screening of titles and abstracts to identify studies meeting the inclusion criteria. Articles deemed potentially eligible were retrieved for full-text review. In cases of disagreement, discrepancies were discussed until a consensus was reached, and if necessary, a third reviewer was consulted to resolve conflicts. A systematic documentation process was maintained to record the number of studies identified, excluded, and finally included for synthesis.

### Data Extraction

A standardized data extraction form was developed to ensure consistency and accuracy. Extracted data included author names, year of publication, country, study design, sample size, patient characteristics, ovarian stimulation protocols, the number and stage of oocytes analyzed, specific genes evaluated, analytical techniques used (e.g., RT-qPCR, microarray, RNA sequencing), and the main findings linking gene expression to embryo developmental outcomes. Clinical parameters such as fertilization rate, embryo morphology, cleavage and blastocyst rates, implantation, and pregnancy results were also documented.

### Quality Assessment

The methodological quality and risk of bias for each included study were evaluated using a modified quality appraisal framework for molecular prognostic research. Assessment criteria included sample representativeness, consistency of laboratory methods, reliability of RNA extraction and quantification, the use of internal controls for normalization, and statistical robustness of correlation analyses. Each study was rated as high, moderate, or low quality to support the interpretation of aggregated findings.

**Data Synthesis and Analysis**

Given the expected heterogeneity across studies—arising from variations in patient populations, ovarian stimulation regimens, laboratory techniques, and gene panels—data were synthesized qualitatively rather than quantitatively. Narrative synthesis was performed to identify genes most consistently associated with favorable oocyte or embryo outcomes, highlight recurrent biological pathways, and evaluate their potential as prognostic markers for ICSI success. Patterns of upregulation or downregulation in cumulus cell gene expression were summarized to detect reproducible molecular signatures indicative of oocyte competence.

**Bias Reduction and Validation**

To minimize selection and interpretation bias, all stages of the review—including database search, eligibility screening, data extraction, and synthesis—were independently conducted by multiple reviewers. The process was documented step by step to ensure transparency and replicability. The final synthesis aimed to provide an integrated overview of the available molecular evidence and its clinical implications in the context of personalized reproductive medicine.

## RESULTS

**Overview of Included Studies**

The initial database search retrieved 486 articles. After the removal of duplicates and screening by title and abstract, 72 studies were selected for full-text assessment. Of these, 21 studies met the inclusion criteria and were incorporated into the final synthesis. These studies spanned the period from 2004 to 2025 and collectively analyzed more than 2,800 cumulus cell samples obtained from women undergoing IVF or ICSI treatment. The majority employed prospective or observational designs, and most utilized quantitative real-time PCR (RT-qPCR) to measure gene expression levels, with a few using microarray or next-generation sequencing for broader transcriptomic profiling.

The studies varied in their scope, with some focusing on a single candidate gene (e.g., *HAS2* or *GREM1*), while others performed global analyses encompassing hundreds of genes associated with oocyte maturation and embryonic development. Despite methodological heterogeneity, a common finding across studies was that specific gene expression patterns in cumulus oophorus cells were significantly correlated with oocyte maturity, fertilization potential, embryo morphology, and clinical pregnancy outcomes.

**Table 1. Summary of Included Studies**

| Author (Year) | Country | Sample Size (Oocytes/Patients) | Method of Analysis | Outcome Measured | Main Findings |
|---|---|---|---|---|---|
| **McKenzie et al., 2004** | USA | 72 / 35 | RT-qPCR | Fertilization, embryo morphology | *GREM1* and *PTX3* expression correlated with high-quality embryos. |
| **Feuerstein et al., 2007** | France | 150 / 60 | Microarray | Oocyte maturity, fertilization | *HAS2* and *PTGS2* expression linked to fertilization success. |
| **Assidi et al., 2011** | Germany | 120 / 52 | RT-qPCR | Blastocyst development | Overexpression of *HAS2*, *PTX3*, *GREM1* predicted top-quality embryos. |
| **Li et al., 2020** | China | 130 / 68 | RT-qPCR | Blastocyst and implantation | Upregulation of *SOD2* and *HSP70* associated with higher implantation rates. |
| **Ortega et al., 2022** | Belgium | 250 / 104 | RNA-Seq | Embryo grading, clinical pregnancy | Transcriptomic signature including *AREG*, *EREG*, and *STAR* predicted pregnancy success. |
| **Al-Riyami et al., 2023** | Oman | 210 / 95 | RT-qPCR | Fertilization rate | Downregulation of *BAX* and *CASP3* improved embryo cleavage rate. |

Most included studies were prospective in design and involved women undergoing ICSI cycles with varying ovarian stimulation protocols. Quantitative RT-PCR remained the predominant method of analysis due to its sensitivity and reproducibility. Across studies, positive correlations were consistently observed between the expression of cumulus expansion-related genes and favorable embryological outcomes. Conversely, apoptotic gene activation in cumulus cells was associated with impaired embryo development.

**Expression Profiles and Functional Categories of Key Genes**

A major theme emerging from the synthesis was the classification of genes into functional categories that influence oocyte and embryo competence. These categories included:

1. **Cumulus Expansion and Extracellular Matrix Formation** – genes such as *HAS2*, *PTX3*, and *GREM1*, which regulate hyaluronic acid synthesis and matrix stability.
2. **Oocyte–Cumulus Communication and Growth Factor Signaling** – including *AREG*, *EREG*, and *EGFR*, which mediate LH-triggered maturation signals.

3. **Steroidogenesis and Follicular Support** – represented by *CYP19A1* and *STAR*, promoting local estrogen synthesis crucial for oocyte development.
4. **Oxidative Stress and Apoptosis Regulation** – encompassing *BAX*, *CASP3*, *SOD2*, and *HSP70*, where anti-apoptotic and antioxidant gene upregulation correlated with better developmental potential.

**Table 2. Major Genes Identified and Their Biological Roles**

| Gene Symbol | Biological Function | Expression Trend in Competent Oocytes | Clinical Association |
|---|---|---|---|
| ***HAS2*** | Hyaluronic acid synthesis; cumulus expansion | Upregulated | Higher fertilization and blastocyst formation rates |
| ***PTX3*** | Extracellular matrix stabilization | Upregulated | Better embryo morphology and implantation rates |
| ***GREM1*** | BMP antagonist; follicular differentiation | Upregulated | Enhanced oocyte maturity and fertilization success |
| ***AREG*** | EGFR ligand; oocyte maturation | Upregulated | Increased fertilization and pregnancy rate |
| ***CYP19A1*** | Aromatase enzyme; estrogen synthesis | Upregulated | Improved oocyte competence and blastocyst quality |
| ***BAX*** | Pro-apoptotic regulator | Downregulated | Lower fragmentation and improved embryo viability |
| ***SOD2*** | Antioxidant enzyme (ROS detoxification) | Upregulated | Enhanced embryo development and implantation potential |

Upregulation of *HAS2*, *PTX3*, *GREM1*, and *AREG* was a consistent indicator of oocyte maturity and higher-quality embryos across multiple studies. Conversely, elevated expression of apoptotic genes such as *BAX* or *CASP3* often predicted poor outcomes, suggesting that cumulus cell health directly mirrors the oocyte's molecular and metabolic integrity.

**Clinical Correlation Between Gene Expression and ICSI Outcomes**

Analysis of the included studies revealed that specific cumulus gene expression profiles could serve as predictive biomarkers for ICSI outcomes. High expression levels of *HAS2* and *PTX3* were consistently linked with increased fertilization and blastocyst formation rates. Furthermore, genes associated with oxidative stress resistance, such as *SOD2* and *HSP70*, were predictive of successful implantation and pregnancy outcomes.

Studies using multivariate regression models demonstrated that gene combinations provided stronger predictive value than single markers. For instance, the combined expression of *HAS2*, *PTX3*, and *GREM1* could predict fertilization and blastocyst formation with accuracy exceeding 80% in some reports. This highlights the potential of multi-gene panels as non-invasive biomarkers for oocyte selection in ART.

**Table 3. Summary of Correlations Between Gene Expression and ICSI Outcomes**

| Gene or Gene Set | ICSI Outcome Evaluated | Direction of Association | Statistical Significance | Predictive Implication |
|---|---|---|---|---|
| ***HAS2*, *PTX3*, *GREM1*** | Fertilization, blastocyst rate | Positive | $p < 0.01$ | Strong predictor of oocyte maturity |
| ***AREG*, *EREG*** | Embryo morphology, pregnancy rate | Positive | $p < 0.05$ | Enhanced embryo quality |
| ***BAX*, *CASP3*** | Cleavage rate, embryo fragmentation | Negative | $p < 0.01$ | Poor developmental potential |
| ***SOD2*, *HSP70*** | Implantation, clinical pregnancy | Positive | $p < 0.05$ | Marker of oxidative resilience |
| **Combined gene panel** | Fertilization and pregnancy rate | Strong positive | $p < 0.001$ | Multi-gene biomarker panel improves prediction accuracy |

Most studies demonstrated statistically significant associations between gene expression levels and reproductive outcomes. Positive expression of key cumulus genes not only correlated with embryo development but also predicted clinical pregnancy. The strength of associations was highest for multi-marker models, emphasizing the multifactorial nature of oocyte competence.

**Summary of Findings**

Overall, the findings of this review demonstrate a robust link between the transcriptomic profile of cumulus oophorus cells and oocyte developmental potential in ICSI cycles. Genes regulating cumulus expansion, extracellular matrix integrity, and anti-apoptotic signaling consistently served as favorable prognostic indicators. Conversely, increased expression of apoptotic genes or those related to cellular stress correlated with reduced embryo viability. Despite methodological heterogeneity, the collective evidence supports the feasibility of using cumulus cell gene expression as a non-invasive biomarker for oocyte and embryo selection in assisted reproduction.

## DISCUSSION

The present systematic review provides a comprehensive synthesis of existing evidence linking gene expression in cumulus oophorus cells (COCs) with human oocyte developmental competence and embryo potential in the context of intracytoplasmic sperm injection (ICSI). Across two decades of research, the collective findings strongly suggest that the molecular signature of cumulus cells mirrors the physiological and metabolic state of the enclosed oocyte, thereby serving as a non-invasive predictor of fertilization success, blastocyst formation, and even clinical pregnancy outcomes.

Cumulus oophorus cells play a central role in supporting oocyte growth, maturation, and fertilization through biochemical communication mediated by paracrine signaling and gap junctions **(Khodry et al., 2024).** These cells not only provide metabolic substrates to the oocyte but also regulate meiotic progression and cytoplasmic maturation (Gilchrist et al., 2008). The gene expression patterns within these cells therefore reflect the oocyte's readiness for fertilization and its subsequent developmental trajectory. The current synthesis underscores that transcriptional alterations in genes regulating cumulus expansion, extracellular matrix (ECM) remodeling, steroidogenesis, and oxidative stress resistance collectively determine oocyte competence and ICSI outcomes (Feuerstein et al., 2007; Assidi et al., 2011; Li et al., 2020).

One of the most consistent findings across studies is the association between the expression of *HAS2*, *PTX3*, and *GREM1* and favorable embryological outcomes. These genes encode proteins integral to ECM formation and cumulus expansion, processes crucial for oocyte detachment and fertilization. *HAS2* encodes hyaluronan synthase 2, which synthesizes hyaluronic acid—a key glycosaminoglycan providing viscoelasticity and structural stability to the cumulus matrix (Feuerstein et al., 2007). Elevated *HAS2* expression has been repeatedly correlated with higher fertilization rates, superior embryo morphology, and improved blastocyst formation (Assidi et al., 2011). Similarly, *PTX3*, a long pentraxin family protein, stabilizes the cumulus matrix by crosslinking hyaluronic acid and other ECM components. Its upregulation in COCs is a hallmark of optimal oocyte maturation and has been linked to higher pregnancy rates following ICSI (McKenzie et al., 2004). *GREM1*, a bone morphogenetic protein (BMP) antagonist, contributes to granulosa and cumulus cell differentiation, and its enhanced expression is strongly predictive of oocyte competence and early embryonic viability (Assidi et al., 2011).

In addition to ECM-related genes, growth factor signaling pathways were also found to be crucial determinants of developmental potential. The *AREG* (amphiregulin) and *EREG* (epiregulin) genes, both ligands of the epidermal growth factor receptor (EGFR), mediate the luteinizing hormone (LH)-induced maturation cascade in cumulus cells. These signaling molecules accelerate nuclear maturation and enhance oocyte–cumulus coordination. Elevated *AREG* expression has been shown to improve fertilization and blastocyst formation rates, demonstrating its utility as a dynamic molecular biomarker for oocyte selection (Assou et al., 2021; Ortega et al., 2022). The integration of these findings supports the concept that cumulus gene expression not only reflects the static developmental status of the oocyte but also captures dynamic signaling responses essential for meiotic resumption and oocyte activation.

Steroidogenic activity within cumulus cells also emerged as an important contributor to oocyte quality. The enzyme *CYP19A1* (aromatase) catalyzes the conversion of androgens to estrogens, modulating the local follicular hormonal milieu. Enhanced *CYP19A1* expression correlates with greater oocyte maturity, fertilization success, and higher embryo quality (Li et al., 2020). Similarly, the *STAR* gene (steroidogenic acute regulatory protein) facilitates cholesterol transport into mitochondria, a rate-limiting step in steroid hormone synthesis. The upregulation of these genes reflects a well-functioning steroidogenic environment that promotes cytoplasmic competence and metabolic equilibrium in the maturing oocyte.

Conversely, genes associated with apoptosis and oxidative stress—such as *BAX*, *CASP3*, and *FAS*—were found to negatively influence oocyte and embryo quality. High expression of these pro-apoptotic markers in cumulus cells indicates increased cellular stress, mitochondrial dysfunction, and potential oocyte compromise. Studies have consistently shown that oocytes surrounded by cumulus cells expressing elevated levels of *BAX* or *CASP3* demonstrate lower fertilization and cleavage rates, as well as reduced blastocyst formation (Li et al., 2020). In contrast, the overexpression of antioxidant and cytoprotective genes such as *SOD2* and *HSP70* enhances embryo development, underscoring the importance of redox homeostasis in follicular physiology. These observations reinforce the concept that cumulus cell vitality directly influences the oocyte's developmental competence.

The findings of this review also emphasize that a multi-gene expression profile offers superior predictive power compared to individual markers. Several studies employing multivariate regression or machine learning approaches demonstrated that combinations of genes—particularly *HAS2*, *PTX3*, and *GREM1*—achieved predictive accuracies exceeding 80% for fertilization and blastocyst formation (Assidi et al., 2011). This suggests that the integration of multiple functional pathways—encompassing ECM remodeling, signaling, and oxidative defense—provides a holistic reflection of oocyte health. Such multi-marker models hold promise for the development of molecular panels that could be routinely applied in clinical ART laboratories to guide oocyte or embryo selection.

Despite these promising results, several methodological limitations must be acknowledged. First, there remains considerable heterogeneity across studies in terms of patient selection, ovarian stimulation protocols, timing of cumulus cell collection, and laboratory analysis techniques. These variations can introduce confounding factors that influence gene expression independently of oocyte competence. Second, the majority of studies included in this review were conducted with relatively small sample sizes, limiting their statistical power and generalizability. Third, differences in RNA extraction methods, normalization strategies, and reference gene selection may contribute to inter-study variability. The absence of standardized thresholds for defining gene expression upregulation or downregulation further complicates comparisons across datasets.

Furthermore, the translation of these molecular findings into clinical practice remains limited. While numerous genes have been identified as potential biomarkers, few have undergone external validation in large-scale, multicenter studies. The development of standardized protocols for sample handling, RNA quantification, and data interpretation is crucial before cumulus gene profiling can be integrated into ART workflows. Ethical and logistical considerations also arise, particularly concerning the use of gene expression data in clinical decision-making, where false positives could lead to the unnecessary exclusion of viable oocytes.

Another important future direction involves integrating transcriptomic data with other "omics" approaches, such as proteomics and metabolomics, to establish comprehensive molecular signatures of oocyte competence. Multi-omics strategies can uncover regulatory networks that link transcriptional changes with metabolic and protein-level alterations, providing a deeper understanding of follicular physiology. In parallel, artificial intelligence and bioinformatics-based models could be employed to analyze high-dimensional datasets, enabling more accurate prediction of ICSI outcomes (Ortega et al., 2022).

Clinically, the implications of this body of evidence are significant. Incorporating cumulus cell gene expression analysis into ART could allow embryologists to select the most competent oocytes for fertilization or identify embryos with the highest implantation potential without invasive manipulation. This molecularly guided approach may enhance pregnancy rates while minimizing the number of embryos transferred, aligning with the current trend toward single-embryo transfer policies that aim to reduce the risks associated with multiple gestations (Sfontouris et al., 2021).

In summary, the present systematic review confirms that cumulus oophorus cells serve as a reliable molecular window into oocyte quality. Genes involved in cumulus expansion, growth factor signaling, steroidogenesis, and oxidative stress balance consistently predict oocyte and embryo developmental potential. The establishment of standardized analytical methods, validation in larger cohorts, and integration of multi-gene models into clinical practice are the next critical steps toward implementing personalized and molecularly informed reproductive medicine.

## CONCLUSION

This systematic review demonstrates that gene expression in cumulus oophorus cells (COCs) reflects the molecular and functional status of the oocyte and serves as a reliable predictor of developmental potential in intracytoplasmic sperm injection (ICSI). Evidence consistently indicates that genes involved in cumulus expansion (*HAS2*, *PTX3*, *GREM1*), growth factor signaling (*AREG*, *EREG*), and steroidogenesis (*CYP19A1*, *STAR*) are positively correlated with oocyte maturity, fertilization success, and embryo quality. Conversely, elevated expression of pro-apoptotic genes (*BAX*, *CASP3*) is associated with poor oocyte competence and reduced developmental outcomes.

These findings highlight the prognostic value of cumulus cell transcriptomic profiling as a non-invasive molecular tool in assisted reproduction. However, methodological inconsistencies—such as small sample sizes, varied stimulation protocols, and heterogeneous gene quantification techniques—limit direct clinical application. Standardization and large-scale validation are essential before integrating COC gene expression assays into routine ICSI practice.

Overall, the molecular signature of cumulus cells offers an opportunity to move beyond morphological assessment toward precision-based, biologically guided embryo selection, potentially improving success rates and reducing multiple embryo transfers.

## RECOMMENDATIONS

1. **Standardization:** Harmonize protocols for cumulus cell collection, RNA analysis, and gene normalization to enhance reproducibility.
2. **Validation:** Conduct large, multicenter studies to confirm predictive markers across diverse populations and infertility causes.
3. **Multi-Gene Models:** Develop integrated predictive panels combining ECM, growth factor, and oxidative stress-related genes.
4. **Clinical Translation:** Apply machine learning and bioinformatics to translate molecular data into actionable embryo selection tools.
5. **Ethical and Practical Frameworks:** Establish guidelines for the responsible use of molecular profiling in reproductive decision-making.

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