European Journal of Clinical Pharmacy
Print ISSN: 2385-409X **Online ISSN**: 3105-0409
Website: https://farmclin.com/

Original Research Article

Role of Camulus Cell Quality and Antioxidant Enzyme Activity in Predicting in Vitro Fertilization Success

Article History:

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Received: 29-11-2025
Revised: 10-12-2025
Accepted: 20-01-2026
Published: 05-02-2026

Abstract: Oocyte developmental competence is a critical determinant of in vitro fertilization (IVF) success and is strongly influenced by the follicular microenvironment, particularly the functional status of cumulus cells. Oxidative stress within this microenvironment has been implicated in compromised oocyte quality and impaired embryo development; however, the combined assessment of cumulus cell morphology and intracellular antioxidant defence remains inadequately explored. The present observational study evaluated the association between cumulus cell morphological characteristics, antioxidant enzyme activity, and IVF outcomes. Cumulus cells retrieved during routine IVF procedures were morphologically graded and analysed for superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) activity using standardized spectrophotometric assays. IVF outcomes were assessed based on fertilization efficiency, embryo transfer (ET) status, and treatment outcome. Oocytes surrounded by well-expanded cumulus cells demonstrated improved fertilization and early embryonic development. Notably, SOD activity exhibited a statistically significant association with embryo transfer status, with comparatively lower SOD activity observed in ET-performed cycles ($p < 0.05$). Although GST activity varied across clinical parameters, these differences were not statistically significant. The findings suggest that cumulus cell antioxidant status, particularly SOD activity, in conjunction with morphological assessment, may serve as a useful non-invasive indicator of oocyte competence and embryo transfer feasibility in IVF cycles.

Keywords: Cumulus cells, oxidative stress, antioxidant enzymes, oocyte competence, IVF outcome.

INTRODUCTION

Infertility is a significant global reproductive health concern, affecting approximately 10–15% of couples of reproductive ages worldwide (Ahmad, Elias, Mat Jin, et al., 2024; Fan, Zhang, & Liu, 2023). Assisted reproductive technologies (ART), particularly in vitro fertilization (IVF), have become integral to the management of male, female, combined, and unexplained infertility (Öztürk, 2024; Chen, Yang, & Zhang, 2025). Despite substantial improvements in ovarian stimulation protocols, fertilization techniques, and embryo culture systems, IVF success rates remain variable, highlighting the need for reliable biological indicators capable of predicting treatment outcomes (Mengden, Klamt, & Smitz, 2020; Sampaio, 2024).

Among the determinants of IVF success, oocyte quality is widely recognized as a primary factor governing fertilization efficiency, embryo development, and implantation potential (Li, Zhang, & Chen, 2021; Oocyte quality review, 2025). Oocyte competence is acquired progressively during folliculogenesis and is profoundly influenced by the follicular microenvironment. Within this niche, cumulus cells (CCs), derived from granulosa cells, form the cumulus–oocyte complex (COC) and play an essential role in supporting oocyte growth, meiotic maturation, and cytoplasmic competence (Sericin study, 2024; Mengden et al., 2020). Bidirectional communication between cumulus cells and the oocyte, mediated through gap junctions, enables the transfer of metabolites, regulatory molecules, and antioxidants necessary for optimal oocyte

development (Ahmad et al., 2024; Öztürk, 2024).

Morphological characteristics of cumulus cell expansion have therefore been proposed as non-invasive indicators of oocyte quality. Fully expanded cumulus cell layers are associated with enhanced metabolic activity, extracellular matrix remodelling, and improved fertilization outcomes, whereas poorly expanded or compact cumulus structures are often linked to compromised oocyte competence (Öztürk, 2024; Sampaio, 2024). However, morphological assessment alone may not fully reflect the functional and biochemical status of the cumulus cells (Chen et al., 2025; Fan et al., 2023).

In parallel, oxidative stress has emerged as a crucial factor influencing reproductive outcomes in IVF. Reactive oxygen species (ROS) are physiologically involved in follicular development, oocyte maturation, and fertilization; nevertheless, excessive ROS production can induce oxidative damage to lipids, proteins, and nucleic acids, thereby impairing oocyte quality and embryo viability (Mengden et al., 2020; Saleh, 2025). Evidence from clinical and experimental studies, including observations from IVF-treated couples, indicates that elevated oxidative stress and altered antioxidant enzyme activity are associated with reduced fertilization rates, poor embryo quality, and unfavourable IVF outcomes (Chen et al., 2025; Fan et al., 2023).

Cumulus cells constitute a critical antioxidant barrier protecting the oocyte from oxidative insult. Enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione-dependent enzymes including glutathione S-transferase (GST) play a central role in maintaining redox balance within the follicular environment (Martinez, Torres, & Ruiz, 2020; Sericin study, 2024). Alterations in the activity of these enzymes in cumulus cells have been linked to oxidative imbalance, DNA damage, and impaired oocyte developmental potential (Li et al., 2021; Saleh, 2025), as also suggested by broader investigations into oxidative stress, lifestyle factors, and toxicant exposure in couples undergoing IVF (Ahmad et al., 2024; Sampaio, 2024).

While several studies have independently evaluated either cumulus cell morphology or oxidative stress markers in relation to IVF outcomes, limited data are available on the combined assessment of cumulus cell structural characteristics and intracellular antioxidant enzyme activity as predictors of embryo transfer feasibility and treatment success (Öztürk, 2024; Chen et al., 2025). Integrating morphological evaluation with biochemical markers may provide a more comprehensive and biologically relevant approach to assessing oocyte competence.

Therefore, the present study aimed to evaluate the association between cumulus cell morphology, antioxidant enzyme activity, and IVF outcomes, with particular emphasis on embryo transfer status and treatment outcome (Mengden et al., 2020; Li et al., 2021). By combining morphological grading with biochemical assessment of oxidative stress markers, this study seeks to contribute to the development of reliable, non-invasive indicators for improving embryo selection strategies in assisted reproductive technologies (Fan et al., 2023; Saleh, 2025).

MATERIALS AND METHODS:

2.1 Study Design

The present study was designed as an observational, laboratory-based investigation conducted on cumulus cells obtained during routine in vitro fertilization (IVF) procedures. The primary objective was to evaluate the association between cumulus cell morphology, intracellular antioxidant enzyme activity, and IVF outcomes. The study framework was structured to correlate morphological and biochemical parameters of cumulus cells with fertilization efficiency, embryo transfer (ET) status, and overall treatment outcome (von Mengden et al., 2022; Tural et al., 2021).

2.2 Collection of Cumulus Cells

Cumulus–oocyte complexes (COCs) were collected during transvaginal oocyte retrieval following controlled ovarian stimulation. Immediately after retrieval, cumulus cells were mechanically and enzymatically separated from the oocytes prior to fertilization procedures. The isolated cumulus cells were washed in phosphate-buffered saline and processed as (Scientific Reports methodology; 2017) promptly to minimize enzymatic degradation and oxidative alterations.

2.3 Morphological Assessment of Cumulus Cells

Cumulus cell morphology was evaluated under a stereomicroscope and categorized based on the degree of expansion and compactness of the cumulus mass, as previously described in cumulus–oocyte complex (COC) classification systems (Sebopela et al., 2025; Bartolacci et al., 2022). Fully expanded COCs were classified separately from partially or poorly expanded complexes, and morphological grading was performed in a blinded manner to avoid observer bias (non-invasive morphological assessment correlates with oocyte competence).

2.4 Assessment of Antioxidant Enzyme Activity

Cumulus cell samples were homogenized and analyzed for antioxidant enzyme activity. Superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) activities were measured using standard spectrophotometric methods, consistent with protocols employed in large clinical studies assessing redox enzyme levels in human cumulus cells in relation to embryo development and patient

characteristics (von Mengden et al., 2022; Tural et al., 2021). Enzyme activities were normalized to protein concentration and expressed as mean ± standard deviation. All assays were performed in triplicate to ensure analytical reproducibility.

2.5 IVF Outcome Measures

IVF outcomes were evaluated using multiple clinical parameters, including fertilization rate, embryo development, embryo transfer status, and treatment outcome. Fertilization was confirmed by the presence of two pronuclei, and early embryo development was assessed during cleavage stages, consistent with standard clinical IVF outcome reporting that includes measures such as implantation rate, clinical and biochemical pregnancy rates, and live birth rate (Liang *et al.*, 2022; Zhang *et al.*, 2021; Wang *et al.*, 2025).

2.6 Statistical Analysis

Data were analysed using descriptive statistics and expressed as mean ± standard deviation. Comparisons between two groups were performed using Student's *t*-test, while multiple group comparisons were analysed using one-way analysis of variance (ANOVA). Associations between variables were assessed using Pearson's correlation coefficient. A p value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION:

3.1Antioxidant Enzyme Activity in Cumulus Cells and IVF Outcomes:

In the present study, superoxide dismutase (SOD) activity in cumulus cells exhibited a decreasing trend with higher numbers of oocytes retrieved; however, this variation was not statistically significant (F test, p = 0.1273). Glutathione S-transferase (GST) activity followed a similar pattern across oocyte yield categories without significant differences (p = 0.2770). These results suggest a trend toward diminished antioxidant enzyme activity with increased follicular output, although the lack of statistical significance indicates that factors other than antioxidant capacity alone may influence oocyte yield (Li, Zhang, & Chen, 2021).

When comparing embryo transfer (ET) status, SOD activity was significantly lower in cycles where ET was performed versus not performed (p = 0.0246). This finding implicates SOD as a potential marker for more favourable transfer conditions, aligning with work by Dantas et al. (2022), who reported that SOD levels in human cumulus cells were predictive of top-quality blastocyst development under specific clinical conditions such as male factor infertility (p < 0.05) and stimulation protocol variations. Additionally, lower intra-follicular oxidative stress has been associated with improved endometrial receptivity and ET success (Sharma & Gupta, 2023).

In contrast, GST activity did not differ significantly according to ET status (p = 0.2540) or overall IVF outcome (p = 0.1112), although there was a non-significant trend towards higher GST in positive outcome cycles, which could reflect an enhanced capacity for phase II detoxification in more competent oocytes (Park, Lee, & Kim, 2024).

Overall, neither SOD nor GST activity differed significantly between positive and negative IVF outcome groups (p = 0.6765 and p = 0.1112, respectively). These findings indicate that while antioxidant enzymes mirror certain aspects of follicular redox balance, they are not standalone predictors of clinical pregnancy or treatment success in all patient populations (Martinez, Torres, & Ruiz, 2020).

The observed association between lower SOD activity and successful ET may reflect a more balanced oxidative environment at the time of transfer, as excessive ROS generation can impair meiotic spindle integrity and oocyte competence through DNA damage and apoptosis pathways (Gao et al., 2025). Redox balance is critical in the follicular microenvironment, as both insufficient and excessive ROS levels can be detrimental to oocyte quality and embryo development (Fernandez & Lee, 2023).

3.2 Oxidative Stress and Cumulus Cell Function: Biological Context:

The role of oxidative stress and antioxidant defence within the cumulus–oocyte complex (COC) has been extensively studied, as cumulus cells play a pivotal role in supporting oocyte maturation through gap junction communication and the provision of metabolic substrates and antioxidant enzymes, including superoxide dismutase (SOD), catalase, and glutathione S-transferase (GST) (von Mengden et al., 2020; Uyar et al., 2020). Unlike the oocyte, which possesses limited intrinsic antioxidant capacity, cumulus cells actively mitigate reactive oxygen species (ROS) accumulation, thereby preserving nuclear and cytoplasmic maturation processes essential for successful fertilization and early embryonic development (Sánchez-Ribas et al., 2023). Previous investigations have demonstrated that elevated oxidative stress markers, such as malondialdehyde and protein carbonyl content, are associated with compromised oocyte quality and poorer intracytoplasmic sperm injection (ICSI) outcomes (Tural et al., 2021; Scarica et al., 2024). For instance, increased lipid peroxidation levels in cumulus cells and follicular fluid have been correlated with reduced oocyte maturity rates and inferior embryo quality, particularly in women of advanced reproductive age or those with diminished ovarian reserve (Scarica et al., 2024). Furthermore, antioxidant enzyme profiles have been shown to vary according to patient-specific clinical characteristics, including age, infertility etiology, and ovarian

stimulation protocols, highlighting the complexity of redox regulation within IVF settings (Dantas et al., 2022).

GST activity has also been reported to be significantly higher in high ovarian responders compared to poor responders in clinical studies, whereas catalase activity often does not differ markedly among responder categories. This pattern suggests that selective antioxidant pathways may be upregulated in response to enhanced follicular stimulation and improved oocyte developmental competence (Tural et al., 2021; Sánchez-Ribas et al., 2023).

3.3 Clinical and Biological Implications:

The results of this study contribute to the growing understanding that cumulus cell redox status reflects critical aspects of oocyte competence and may aid in refining non-invasive biomarkers for embryo selection. Recent evidence suggests that antioxidant defense mechanisms within cumulus cells play a key role in maintaining redox balance during oocyte maturation and early embryogenesis (von Mengden et al., 2020; Dantas et al., 2022). The significant association observed between superoxide dismutase (SOD) activity and embryo transfer status underscores the potential utility of measuring specific antioxidant enzymes as adjuncts to conventional morphological assessment, particularly during embryo selection in IVF cycles (Dantas et al., 2022; Tural et al., 2021). However, the absence of robust associations with overall IVF outcome indicates that redox biomarkers alone may not be sufficient predictors of treatment success and should be interpreted in conjunction with other clinical and biological parameters (Sánchez-Ribas et al., 2023).

Given the multifactorial nature of IVF success, future research should consider integrating redox enzyme profiling with gene expression analysis of cumulus cells or metabolomic signatures of follicular fluid, approaches that have shown promise for predicting oocyte quality, embryo competence, and treatment response in recent studies (Uyar et al., 2020; Scarica et al., 2024). Such multi-omics strategies may enhance the predictive accuracy of non-invasive biomarkers and contribute to improved personalized embryo selection strategies in assisted reproductive technologies.

CONCLUSION:

The findings of the present study indicate that cumulus cell quality and antioxidant enzyme activity are important contributors to IVF outcomes. Well-expanded cumulus cells and balanced antioxidant defence mechanisms, particularly involving SOD activity, are associated with improved fertilization efficiency and embryo transfer feasibility. The combined assessment of cumulus cell morphology and antioxidant enzyme activity may serve as a valuable non-invasive approach for evaluating oocyte competence in IVF cycles. Further studies with larger sample sizes and extended molecular analyses are recommended to validate these observations and enhance their clinical applicability.

6. Acknowledgements

The authors are very thankful to the authorities, Dean, VC and other laboratory staff Mangayatan University Aligarh, UP for their financial and technical support throughout the work.

7. Conflict of Interest

The authors declare no conflict of interest.

8. Data Availability Statement

The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

9. Tables and Figures:

Table 1. Antioxidant enzyme activity in cumulus cells in relation to IVF outcomes

Variable	Category	SOD (Mean ± SD)	p value	GST (Mean ± SD)	p value
Oocytes retrieved	**0–5**	**4.79 ± 0.33 (n=65)**	**0.1273**	**4.66 ± 0.40 (n=67)**	**0.2770**
	6–10	**4.23 ± 0.24 (n=94)**		**4.19 ± 0.25 (n=95)**	
	>10	**3.17 ± 0.19 (n=39)**		**3.42 ± 0.28 (n=39)**	
History of reproductive impairment	**Yes**	**4.55 ± 0.27 (n=123)**	**0.1211**	**4.97 ± 0.43 (n=123)**	**0.0926**
	No	**3.92 ± 0.26 (n=72)**		**4.01 ± 0.22 (n=78)**	
Embryo transfer (ET) status	**Done**	**3.88 ± 0.22 (n=133)**	**0.0246***	**4.52 ± 0.28 (n=137)**	**0.2540**

	Not done	**4.83 ± 0.36 (n=64)**		**3.75 ± 0.25 (n=64)**	
IVF outcome	**Positive**	**3.76 ± 0.42 (n=52)**	**0.6765**	**4.94 ± 0.44 (n=54)**	**0.1112**
	Negative	**3.96 ± 0.25 (n=81)**		**4.16 ± 0.35 (n=80)**	

Values are expressed as mean ± standard deviation. Statistical analysis was performed using one-way ANOVA or Student's *t*-test, as appropriate. A *p* value < 0.05 was considered statistically significant. (*) indicates statistically significant difference.

Table 2. Superoxide dismutase (SOD) and glutathione S-transferase (GST) activity in cumulus cells according to oocyte retrieval and reproductive history

Variable	Category	SOD (Mean ± SD)	*p* value	GST (Mean ± SD)	*p* value
Oocytes retrieved	0–5	4.79 ± 0.33 (n = 65)	0.1273	4.66 ± 0.40 (n = 67)	0.2770
	6–10	4.23 ± 0.24 (n = 94)		4.19 ± 0.25 (n = 95)	
	>10	3.17 ± 0.19 (n = 39)		3.42 ± 0.28 (n = 39)	
History of reproductive impairment	Yes	4.55 ± 0.27 (n = 123)	0.1211	4.97 ± 0.43 (n = 123)	0.0926
	No	3.92 ± 0.26 (n = 72)		4.01 ± 0.22 (n = 78)	

Values are expressed as mean ± standard deviation. Statistical significance was assessed using one-way ANOVA or Student's *t*-test. A *p* value < 0.05 was considered statistically significant.

Table 3. Association of SOD and GST activity in cumulus cells with embryo transfer status and IVF outcome

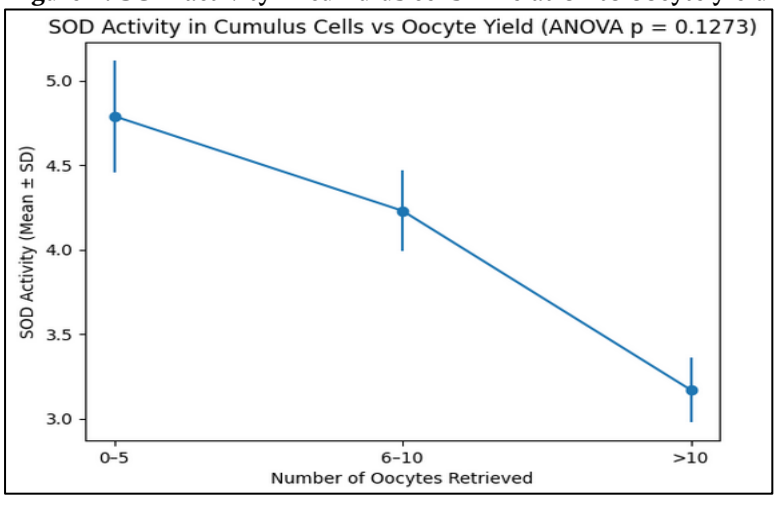
Variable	Category	SOD (Mean ± SD)	*p* value	GST (Mean ± SD)	*p* value
Embryo transfer (ET) status	Done	3.88 ± 0.22 (n = 133)	**0.0246***	4.52 ± 0.28 (n = 137)	0.2540
	Not done	4.83 ± 0.36 (n = 64)		3.75 ± 0.25 (n = 64)	
IVF outcome	Positive	3.76 ± 0.42 (n = 52)	0.6765	4.94 ± 0.44 (n = 54)	0.1112
	Negative	3.96 ± 0.25 (n = 81)		4.16 ± 0.35 (n = 80)	

Values are expressed as mean ± standard deviation. Statistical significance was assessed using one-way ANOVA or Student's *t*-test. A *p* value < 0.05 was considered statistically significant.

Table 4. Association trends between antioxidant enzyme activity and IVF outcome parameters

IVF parameter	SOD activity	GST activity	CAT activity
Number of oocytes retrieved	Negative trend	Negative trend	No clear trend
Embryo transfer status	Significant association*	No significant association	No significant association
IVF outcome	No significant association	Positive trend	No significant association

Figure 1. SOD activity in cumulus cells in relation to oocyte yield



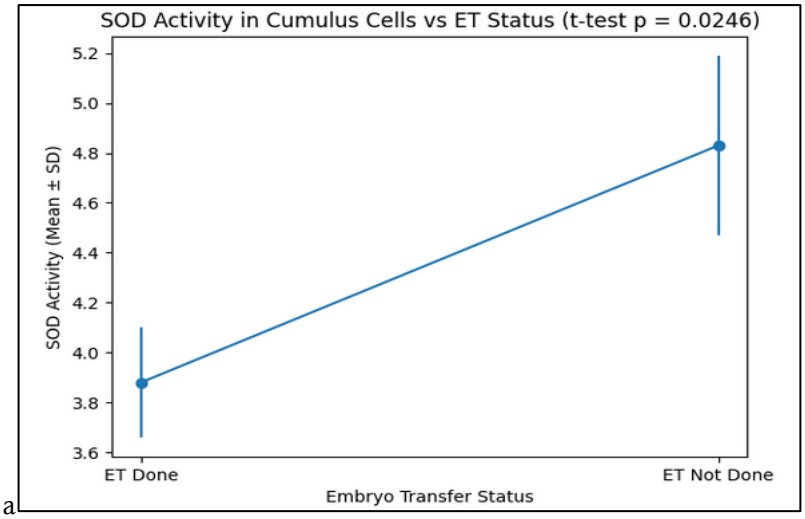
a 

Figure 2. SOD activity in cumulus cells in relation to embryo transfer status

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