

# Impact Of Oxidative Stress, Antioxidant Enzymes, And Trace Elements In Determining Ivf Treatment Cycles

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## ABSTRACT

Infertility is a complex reproductive disorder influenced by multiple biochemical and physiological factors, with in vitro fertilization (IVF) being a widely used assisted reproductive technique. The present study was conducted to evaluate the relationship between oxidative stress, antioxidant enzyme activity, trace element levels, and IVF outcomes. This observational study included patients undergoing IVF treatment, and follicular fluid and blood samples were collected during oocyte retrieval. IVF outcome parameters, including fertilization rate and cleavage rate, were assessed along with embryo development characteristics. The results demonstrated that fertilization rate ( $65.19 \pm 1.81\%$ ) and cleavage rate ( $62.78 \pm 1.85\%$ ) were significantly higher in embryo transfer (ET) done groups compared to ET not done groups ( $p < 0.001$ ). However, no significant difference was observed between IVF positive and IVF negative groups for fertilization and cleavage rates. Analysis of trace elements indicated no significant association of blood cadmium ( $0.1261 \pm 0.02 \mu\text{g/dL}$ ) and lead ( $3.66 \pm 0.26 \mu\text{g/dL}$ ) levels with reproductive outcomes. Serum copper levels also did not show statistically significant variation, although slightly higher values were observed in reproductive impairment cases. Follicular fluid zinc levels ( $0.43 \pm 0.02 \text{ mg/L}$ ) showed a positive trend with fertilization efficiency, though not statistically significant ( $p = 0.0632$ ). Elevated antioxidant enzyme activity, including superoxide dismutase and glutathione-S-transferase, was observed in cases with poor ovarian response, suggesting a compensatory mechanism against oxidative stress. Increased oxidative stress was associated with poor embryo morphology, delayed cleavage, and higher fragmentation rates. Thus, oxidative stress and follicular biochemical environment play an important role in IVF outcomes, while trace elements such as zinc may contribute to improved fertilization efficiency.

**Keywords:** IVF Outcome, Oxidative Stress, Antioxidant Enzymes, Trace Elements, Fertilization Rate

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## INTRODUCTION

Infertility is a significant global health concern that affects millions of couples worldwide and has profound psychological, social, and medical implications. It is estimated that approximately 10–15% of couples of reproductive age experience infertility, defined as the inability to achieve pregnancy after one year of regular unprotected intercourse. The causes of infertility are multifactorial and may include female factors, male factors, combined causes, or unexplained infertility. In recent decades, assisted reproductive technologies (ART) have revolutionized infertility management and significantly improved the chances of conception for affected couples. Among these technologies, in vitro

fertilization (IVF) is one of the most widely used and effective treatment options for various infertility conditions (Vitagliano et al., 2023). IVF involves a series of complex clinical and laboratory procedures including controlled ovarian stimulation, oocyte retrieval, fertilization in a laboratory environment, embryo culture, and embryo transfer into the uterus. Despite technological advances in reproductive medicine, the success of IVF treatment remains variable and is influenced by multiple biological and clinical factors such as maternal age, ovarian reserve, hormonal profile, oocyte quality, embryo development, and uterine receptivity. Among these determinants, oocyte competence and embryo quality are considered critical factors influencing successful fertilization, implantation, and clinical pregnancy outcomes (Sun et

al., 2023; Zhao et al., 2024). The follicular microenvironment plays an essential role in supporting oocyte maturation and developmental competence. Follicular fluid provides a specialized biochemical milieu containing hormones, cytokines, growth factors, metabolites, and nutrients that regulate folliculogenesis and oocyte maturation. Variations in the biochemical composition of follicular fluid can influence the quality of retrieved oocytes and subsequent embryo development. Previous studies have demonstrated that the follicular environment contributes significantly to oocyte competence and embryo viability during assisted reproductive procedures (Li et al., 2021). Embryo development during IVF culture is another critical determinant of treatment success. After fertilization, embryos undergo a sequence of cleavage divisions before reaching the blastocyst stage. Morphological assessment remains one of the most widely used methods in clinical IVF laboratories for selecting embryos with the highest implantation potential. Embryo transfer decisions are typically based on the evaluation of parameters such as cleavage rate, blastomere symmetry, fragmentation, and overall morphological quality. However, despite improvements in embryo culture techniques and laboratory protocols, variability in embryo development and implantation success remains a significant challenge in ART programs (Tran et al., 2022). Additionally in embryological parameters, patient-related characteristics such as age, duration of infertility, ovarian response to stimulation, and the number of retrieved oocytes have also been shown to influence IVF outcomes. Female age is considered one of the most important predictors of IVF success, as increasing maternal age is associated with declining ovarian reserve and reduced oocyte quality. Several clinical studies have reported that implantation and live birth rates decrease significantly with advancing maternal age due to increased chromosomal abnormalities and reduced ovarian function (Vitagliano et al., 2023; Zhang et al., 2022). Therefore, identifying clinical and laboratory indicators associated with successful IVF outcomes is crucial for improving treatment strategies and optimizing patient management. Given the complexity of factors involved in assisted reproduction, comprehensive evaluation of patient characteristics, ovarian response, and embryological outcomes is necessary to better understand the determinants of IVF success. The present study aims to analyze key clinical and embryological parameters associated with IVF treatment cycles and to investigate their relationship with fertilization and embryo development outcomes. Understanding these relationships may contribute to

improved patient management and provide valuable insights for optimizing IVF protocols in clinical practice.

## **MATERIALS AND METHODS**

### **Study Design**

The present investigation was conducted as a laboratory-based observational study involving patients undergoing in vitro fertilization (IVF) treatment. The study aimed to evaluate the relationship between oxidative stress parameters and IVF outcomes, as oxidative imbalance has been recognized as a critical determinant of oocyte competence and embryo development (Agarwal et al., 2012; Ruder et al., 2008).

### **Study Population**

Patients undergoing IVF treatment at the collaborating fertility clinic were included in the study. Individuals with severe systemic illness or metabolic disorders were excluded to minimize confounding factors affecting oxidative stress levels, as systemic oxidative imbalance may significantly influence reproductive outcomes (Agarwal et al., 2012).

### **Sample Collection**

Biological samples associated with the follicular microenvironment were collected during routine oocyte retrieval procedures performed under controlled ovarian stimulation protocols. Follicular fluid represents a crucial biochemical environment influencing oocyte maturation and fertilization potential (Revelli et al., 2009). Samples were immediately processed and stored under appropriate laboratory conditions to preserve biochemical integrity.

### **Preparation of Samples**

The collected samples were centrifuged to remove cellular debris and obtain clear supernatant for biochemical analysis. Proper sample processing is essential to prevent artificial oxidative alterations during laboratory handling (Wiener-Megnazi et al., 2004).

### **Estimation of Oxidative Stress Markers**

Oxidative stress levels were evaluated using standard spectrophotometric biochemical assays. Indicators of lipid peroxidation and reactive oxygen species generation were analyzed to determine the extent of oxidative damage, as elevated oxidative stress has been associated with impaired IVF outcomes (Agarwal et al., 2005; Wiener-Megnazi et al., 2004).

### **Assessment of Antioxidant Status**

Total antioxidant capacity and enzymatic antioxidant activity were measured using established biochemical methods. These assays provided insights into the defensive mechanisms protecting the oocyte microenvironment from oxidative injury, which plays a key role in successful fertilization and embryo development (Revelli et al., 2009; Ruder et al., 2008).

### **Evaluation of IVF Outcome**

IVF treatment outcomes were evaluated using standard embryological and clinical assessment criteria followed at the collaborating fertility centre. The assessment criteria used for evaluating IVF outcome parameters are summarized in Table 1

### **Fertilization Rate**

Fertilization was assessed approximately 16–18 hours after insemination or intracytoplasmic sperm injection (ICSI) by observing the presence of two pronuclei (2PN) and two polar bodies under an inverted microscope, which is considered the standard criterion for normal fertilization (Alpha Scientists in Reproductive Medicine & ESHRE Special Interest Group, 2011). The fertilization rate was calculated as:

Fertilization Rate (%) =  $\frac{\text{Number of fertilized oocytes}}{\text{Total Number of Mature oocytes}} \times 100$

Only normally fertilized oocytes were considered for further embryo culture and developmental assessment (Gardner et al., 2004).

### **Embryo Development**

Embryonic development was evaluated during the in vitro culture period using standard morphological assessment criteria. Cleavage stage embryos were examined under an inverted phase-contrast microscope at defined time intervals following fertilization. Morphological assessment of embryos remains a widely accepted approach for evaluating developmental competence in assisted reproductive technologies (Alpha Scientists in Reproductive Medicine & ESHRE, 2011).

### **Cleavage Stage Progression**

Embryo cleavage was monitored from Day 2 to Day 3 post-fertilization. Developmental progression was assessed based on the timely division of blastomeres according to established embryological standards. Embryos demonstrating appropriate cleavage kinetics were considered developmentally competent, whereas delayed or arrested cleavage was categorized as abnormal development (Hardarson et al., 2001).

### **Assessment of Blastomere Number and Cytoplasmic Fragmentation**

Blastomere number and degree of fragmentation were evaluated as key indicators of embryo developmental competence during in vitro culture. The number of blastomeres in each embryo was recorded at defined observation points, with normally developing embryos expected to exhibit 2-4 cells on Day 2 and 6-8 cells on Day 3 post-fertilization. Embryos showing significant deviation from these expected cleavage patterns were considered to have impaired developmental potential. Cytoplasmic fragmentation was assessed by estimating the proportion of anuclear cytoplasmic fragments relative to the total embryo volume and was graded as mild (<10%), moderate (10–25%), or severe (>25%). Increased levels of fragmentation were regarded as indicative of reduced embryo viability and compromised developmental competence (Van Royen et al., 1999).

### **Statistical Analysis**

Statistical analysis was performed using IBM SPSS (Version XX). Continuous data were expressed as mean  $\pm$  SD, and categorical data as frequency and percentage. Normality was checked using the Shapiro–Wilk test. Student’s t-test or Mann–Whitney U test was used for two-group comparisons, and one-way ANOVA (with Tukey’s post hoc test) for multiple groups. Correlations were analyzed using Pearson’s or Spearman’s test. Categorical variables were tested using Chi-square or Fisher’s exact test. A p-value < 0.05 was considered significant.

## **RESULTS AND DISCUSSION**

### **IVF Outcome Parameters**

The baseline characteristics of the study population were evaluated to understand the distribution of infertility types and contributing factors. The majority of participants were diagnosed with primary infertility (57.2%), followed by secondary infertility (28.1%) and unexplained infertility (14.7%), as presented in Table 3. Additionally, the prevalence of male factor infertility was observed in 30.1% of cases, while 69.9% of participants did not exhibit male factor involvement (Table 4). These findings indicate that female-related and unexplained causes constitute a significant proportion of infertility cases, consistent with previous epidemiological observations in assisted reproduction studies (Vitagliano et al., 2023; Zhao et al., 2024).

Analysis of IVF outcome parameters revealed significant differences in fertilization and cleavage rates based on embryo transfer (ET) status. Cycles in

which embryo transfer was performed demonstrated a significantly higher fertilization rate ( $65.19 \pm 1.81\%$ ) compared to cycles where embryo transfer was not carried out ( $50.76 \pm 3.10\%$ ,  $p < 0.001$ ). Similarly, the cleavage rate was significantly higher in the ET done group ( $62.78 \pm 1.85\%$ ) compared to the ET not done group ( $49.43 \pm 3.40\%$ ,  $p < 0.001$ ), as shown in Table 2. These findings suggest that optimal early embryonic development is a key determinant for embryo selection and transfer decisions, aligning with established embryological assessment criteria (Alpha Scientists in Reproductive Medicine & ESHRE, 2011; Gardner et al., 2004). In contrast, no statistically significant difference was observed between IVF-positive and IVF-negative outcomes. Although slightly higher fertilization ( $68.92 \pm 2.81\%$ ) and cleavage rates ( $65.19 \pm 3.07\%$ ) were noted in IVF-positive cases compared to IVF-negative cases ( $62.24 \pm 2.44\%$  and  $61.08 \pm 2.29\%$ , respectively), these differences did not reach statistical significance ( $p > 0.05$ ), as indicated in Table 2. This observation suggests that while fertilization and early cleavage are essential indicators of embryo viability, they may not independently predict clinical pregnancy outcomes, which are influenced by additional factors such as uterine receptivity and implantation dynamics (Zhang et al., 2022; Tran et al., 2022). Overall, these findings emphasize that early embryonic developmental competence plays a critical role in IVF treatment progression, whereas successful implantation and pregnancy outcomes depend on a complex interplay of embryonic and maternal factors (Gardner et al., 2004; Zhao et al., 2024).

#### Trace Metal Levels and Reproductive Outcome

Assessment of trace metal concentrations indicated that blood cadmium and lead levels did not show a statistically significant association with reproductive impairment or IVF outcome parameters (Table 4). Similarly, serum copper levels did not demonstrate significant variation across IVF outcome groups, although a trend toward higher copper concentration was observed in IVF positive cases (Table 5). Follicular fluid zinc levels showed variability in relation to fertilization efficiency, with comparatively higher zinc concentrations observed in cases demonstrating improved fertilization rates (Table 6). Trace elements are known to play essential roles in reproductive physiology, particularly in oocyte maturation and enzymatic regulation. The observed trend of higher zinc levels in better fertilization outcomes aligns with previous findings suggesting that zinc is crucial for meiotic progression and oocyte competence (Revelli et al., 2009). However, the

absence of statistically significant associations for heavy metals suggests that environmental toxicant exposure may not be a primary determinant of IVF success in the present cohort, or that larger sample sizes may be required to detect subtle effects.

#### Antioxidant Enzyme Activity in Relation to IVF Outcome

The activity of antioxidant enzymes in follicular fluid was evaluated to evaluate their relationship with IVF outcomes, particularly focusing on the role of superoxide dismutase (SOD) were analysed in relation to IVF-positive and IVF-negative groups. The results indicated that SOD activity was comparable between IVF-positive ( $3.76 \pm 0.42$ ) and IVF-negative ( $3.96 \pm 0.25$ ) groups, with no statistically significant difference ( $p > 0.05$ ). Similarly, GST activity was slightly higher in the IVF-positive group ( $4.94 \pm 0.44$ ) compared to the IVF-negative group ( $4.16 \pm 0.35$ ); however, this difference was also not statistically significant ( $p > 0.05$ ), as shown in Figure 3. These findings suggest that although antioxidant enzyme activity tends to be higher in successful IVF cycles, the variation is not sufficient to establish a direct predictive relationship with IVF outcome.

The observed trend of increased GST activity in IVF-positive cases may reflect a relatively enhanced antioxidant defense mechanism, which could contribute to improved oocyte quality and fertilization potential. Previous studies have demonstrated that oxidative stress within the follicular environment can adversely affect oocyte maturation, fertilization, and embryo development (Zehra Wiener-Megnazi et al., 2004; Ashok Agarwal et al., 2012). Antioxidant enzymes such as SOD and GST play a crucial role in neutralizing reactive oxygen species and maintaining cellular integrity during folliculogenesis and early embryogenesis.

However, the lack of statistical significance in the present study indicates that antioxidant enzyme levels alone may not serve as independent predictors of IVF success, and their role is likely influenced by multiple interacting factors, including oxidative stress status, environmental exposures, and individual physiological variability. Similar observations have been reported in studies suggesting that while oxidative balance is essential, IVF outcomes are multifactorial and cannot be attributed to a single biochemical parameter (Zhang X et al., 2022; Zhao J et al., 2024). Overall, the findings highlight the importance of maintaining redox balance in the follicular environment, while also emphasizing that successful IVF outcomes depend on a

complex interplay of biochemical, cellular, and clinical factors rather than a single biomarker.

### **Oxidative Stress and Embryo Development**

Embryos derived from cases with increased oxidative stress parameters demonstrated comparatively poorer morphological characteristics, including delayed cleavage progression and increased fragmentation. Conversely, balanced oxidative status was associated with improved embryo development and higher fertilization efficiency. Reactive oxygen species play dual roles in reproductive physiology. While physiological levels are necessary for normal cellular signaling, excessive oxidative stress can lead to lipid peroxidation, DNA damage, and impaired embryogenesis. The present findings support previous evidence indicating that oxidative stress negatively affects embryo quality and implantation potential (Ruder et al., 2008). These observations highlight the importance of maintaining optimal redox balance in IVF treatment.

### **CONCLUSION**

This study highlights the significant role of oxidative stress and antioxidant defense mechanisms in determining IVF outcomes. Increased oxidative stress was associated with poor embryo quality, delayed cleavage, and higher fragmentation, indicating its negative impact on embryonic development. Elevated antioxidant enzyme activity in poor ovarian responders suggests a compensatory response to oxidative imbalance. Although trace elements such as cadmium, lead, and copper did not show significant associations with IVF outcomes, follicular fluid zinc exhibited a positive trend with fertilization efficiency. These findings emphasize the importance of maintaining redox balance within the follicular microenvironment to improve reproductive success. Further large-scale studies are recommended to validate these observations and explore targeted antioxidant-based therapeutic strategies in assisted reproduction.

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### **Conflict of Interest**

The authors have no conflict of interest.

### **Data Availability Statement**

The data is available on request to the corresponding author of this manuscript.

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**Tables and Figures:**

Table 1 Various assessment Criteria

| Parameter                | Assessment Criteria              | Normal Range              | Clinical Significance             |
|--------------------------|----------------------------------|---------------------------|-----------------------------------|
| <b>Fertilization</b>     | Presence of 2PN & 2 polar bodies | 16–18 h post-insemination | Indicates normal fertilization    |
| <b>Cleavage Stage</b>    | Timely blastomere division       | Day 2–3                   | Reflects developmental competence |
| <b>Blastomere Number</b> | Cell count per embryo            | 2–4 (Day 2), 6–8          | Predictor of embryo               |

|                           |                           | (Day 3)             | viability                         |
|---------------------------|---------------------------|---------------------|-----------------------------------|
| <b>Fragmentation</b>      | % cytoplasmic fragments   | <10% optimal        | High fragmentation → poor outcome |
| <b>Embryo Development</b> | Morphological progression | Continuous cleavage | Indicates embryo quality          |

Table 2. Fertilization and Cleavage Rates in Relation to Embryo Transfer and IVF Outcome

| Parameter                     | ET Done (n=37) | ET Not Done (n=66) | p-value   | IVF Positive (n=54) | IVF Negative (n=83) | p-value |
|-------------------------------|----------------|--------------------|-----------|---------------------|---------------------|---------|
| <b>Fertilization Rate (%)</b> | 65.19 ± 1.81   | 50.76 ± 3.10       | 0.0001*** | 68.92 ± 2.81        | 62.24 ± 2.44        | 0.0796  |
| <b>Cleavage Rate (%)</b>      | 62.78 ± 1.85   | 49.43 ± 3.40       | 0.0002*** | 65.19 ± 3.07        | 61.08 ± 2.29        | 0.773   |

\*\*\*p < 0.001 indicates highly significant difference.

Table 3. Distribution of Infertility Type in Study Population

| Type of Infertility     | Number of Subjects (n) | Percentage (%) |
|-------------------------|------------------------|----------------|
| Primary Infertility     | 116                    | 57.2           |
| Secondary Infertility   | 57                     | 28.1           |
| Unexplained Infertility | 30                     | 14.7           |

Table 4. Prevalence of Male Factor Infertility

| Male Factor Infertility | Number of Subjects (n) | Percentage (%) |
|-------------------------|------------------------|----------------|
| <b>Yes</b>              | <b>61</b>              | <b>30.1</b>    |
| <b>No</b>               | <b>142</b>             | <b>69.9</b>    |

Table 5. Blood Heavy Metal Levels and Reproductive Impairment

| Parameter | Reproductive Impairment Yes | Reproductive Impairment No |
|-----------|-----------------------------|----------------------------|
|           |                             |                            |

|                              |                          |                         |
|------------------------------|--------------------------|-------------------------|
| <b>Blood Cadmium (µg/dL)</b> | 0.1261 ± 0.02<br>(n=112) | 0.1109 ± 0.01<br>(n=48) |
| <b>Blood Lead (µg/dL)</b>    | 3.66 ± 0.26<br>(n=105)   | 3.48 ± 0.42<br>(n=45)   |

Values expressed as Mean ± SE.

Table 6. Serum Copper Levels and IVF Parameters

| Parameter                             | Serum Copper (µg/L) | p-value |
|---------------------------------------|---------------------|---------|
| Reproductive impairment – Yes (n=129) | 0.8706 ± 0.03       | 0.7823  |
| Reproductive impairment – No (n=70)   | 0.7861 ± 0.04       |         |
| Fertilization rate <50% (n=77)        | 0.8724 ± 0.04       |         |

Table 7. Follicular Fluid Zinc Levels and IVF Outcome

| Parameter                             | Follicular Fluid Zinc (mg/L) | p-value |
|---------------------------------------|------------------------------|---------|
| Reproductive impairment – Yes (n=142) | 0.43 ± 0.02                  | 0.0632  |
| Reproductive impairment – No (n=58)   | 0.40 ± 0.01                  |         |
| Fertilization rate <50% (n=77)        | 0.38 ± 0.02                  |         |

Fig: 01 Comparison of Fertilization Rate Between ET Done and ET Not Done Groups

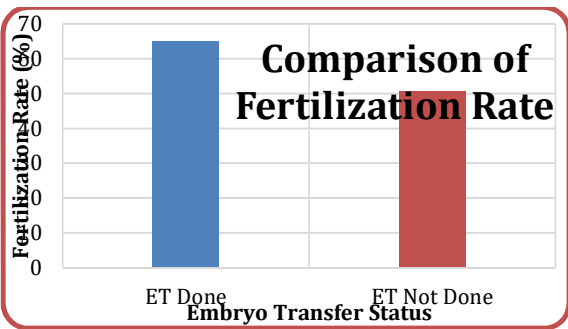
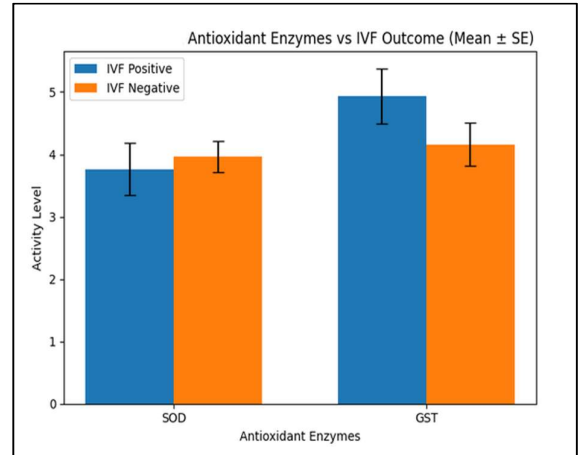


Fig: 02 Comparison of fertilization and cleavage rates between IVF-positive and IVF-negative groups



mean ± SE. No statistically significant difference was observed ( $p > 0.05$ ).

Fig: 03 Antioxidant Enzymes vs IVF Outcome

