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ROLE OF INTRINSIC AND SUPPLEMENTED ANTIOXIDANTS IN FOLLICULAR FLUID: A SHIELD AGAINST OXIDATIVE STRESS IN OOCYTE HEALTH AND EMBRYO DEVELOPMENT

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ABSTRACT

Background: In In Vitro Fertilization (IVF), a form of Assisted Reproductive Technologies (ART), the quality of oocytes and the successful development of embryos are crucial in determining the rate of fertility. The excessive presence of ROS (Reactive Oxygen Species) can cause oxidative stress, which negatively affects follicular fluid (FF) and oocyte maturation. Certain non-endogenous antioxidants, such as catalase, glutathione, and Superoxide Dismutase (SOD), are already present in Follicular fluid, which counterbalances these ROS and protects oocytes. **Method:** In addition to examining the possibility of exogenous supplements of antioxidants, such as vitamins C and E, and Coenzyme Q10 (CoQ10), this review investigates the function of these intrinsic antioxidants in maintaining oocyte health. **Result:** According to current in vivo and in vitro research findings done in mice, pigs, sheep, cows, and 18 patients in the age group(40±1), respectively, targeted antioxidant supplementation may enhance oocyte quality, embryo viability, and pregnancy outcomes. **Conclusion**: However, addressing individual heterogeneity in oxidative stress and optimizing dosage remains challenging. This review highlights how new antioxidant compounds and targeted interventions may enhance reproductive success by promoting cellular resilience in follicular fluid (FF). However, additional research into targeted antioxidant therapy in IVF is necessary.

INTRODUCTION

Delays in childbirth, lifestyle changes, and increased environmental contamination are some of the causes contributing to the recent spike in infertility rates. An estimated 17.5% population of the world's population is expected to experience infertility, according to research, making it a significant global health issue [1-2]. The most critical factor that influences the complex biological process of fertility is Oxidative stress. Reactive oxygen species (ROS) are naturally produced by cellular metabolism; however, when ROS levels increase beyond the capabilities of the antioxidant defense system, the cell may experience oxidative stress. This Oxidative stress in the reproductive system can have adverse effects on the development of embryos, the quality of oocytes, and may produce an overall result of infertility. The ovarian follicle's

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follicular fluid, which surrounds the egg, is crucial for maintaining the oocyte's health and facilitating the early stages of embryonic development. Since antioxidants provide a defense against damage caused by ROS, their role in follicular fluid has been extensively studied [3-4]**.**

Assisted reproductive technology is the primary strategy for overcoming infertility, and both patients and doctors support increasing its success rate. Because oocytes develop in the complex environment of ovarian follicles, the successful application of assisted reproductive technologies (ART) relies on the quality of oocytes. To maintain the health of the oocytes, follicular fluid (FF) plays a crucial role. This review will examine the role of antioxidants in FF, exploring how both endogenous and supplemented antioxidants function as protective agents against oxidative stress [5-6].

Follicular Fluid: A Protective Microenvironment:

The ovarian follicle is the basic anatomical and functional unit of the ovaries, which consists of one oocyte and surrounding granulosa cells. The follicular cavity gets filled with Follicular Fluid (FF) when follicles mature into antral follicles, establishing an essential microenvironment for follicle and oocyte development. This FF is composed of Plasma proteins, granulosa cells, metabolic products, plasma exudates, paracrine growth factors, and various hormones (Table 1). Throughout follicular development, the inclusions of FF are influenced by the hormones and paracrine signals of the Hypothalamic-pituitary-gonadal axis, as well as the indirect effects of numerous diseases, changing dynamically [7-8]**.**

For the proper development and growth of oocytes, the Ovarian follicle's environment plays an important role. The ovarian follicle's specialized medium, referred to as follicular fluid (FF), envelops the oocyte and supplies it with vital nutrients, hormones, cytokines, and other signaling molecules, thereby supporting its growth and development. In addition to these vital nutrients, Follicular fluids consist of abundant Antioxidants, which shield the oocyte from oxidative stress. Age, hormonal changes, and environmental stressors can hurt the composition of follicular fluid. When the production of reactive oxygen species (ROS) exceeds the defenses of antioxidants, this condition is called oxidative stress and is especially hazardous in this microenvironment [9-10].

Table 1: Components of follicular fluid

Component Type	Examples
Hormones	Estradiol, Progesterone, LH, FSH, Androgens
Proteins	Albumin, Immunoglobulins, Growth factors (IGF-1, EGF, TGF-β), Enzymes
Lipids	Cholesterol, Prostaglandins, Fatty acids
Carbohydrates	Glucose, Lactate, Pyruvate
Electrolytes	Na^+, K^+, Ca^{2+}, Cl^-, Mg^{2+}, HCO_3^-
Cytokines & Chemokines	IL-6, IL-8, TNF-α, MCP-1
Reactive Oxygen Species (ROS) & Antioxidants	H_2O_2, Superoxide, Glutathione, SOD, Catalase
Metabolites	Amino acids, Urea, Creatinine
Extracellular Vesicles	Exosomes, Microvesicles
MicroRNAs (miRNAs)	miR-21, miR-132, others

Human Follicular Fluid's reactive oxygen species generation and its clinical importance

Reactive oxygen species (ROS) are chemical species, primarily including hydrogen peroxide (H2O2), the Superoxide anion (O2•−), the Hydroxyl radical (•OH), and singlet oxygen (1 O2). These are produced when oxygen is not completely reduced. There are several ways in which the ovary produces ROS. The electron transfer mechanisms of respiration for aerobic metabolism are the primary sources of ROS production, and mitochondria are the most abundant organelles in oocytes. Through a variety of enzymatic processes, electrons that leak from the respiratory chain of mitochondria reduce molecules of

O2 to O2− (primary reactive oxygen species, ROS), which are then transformed into molecules such as H_2O_2, OH, and Hypochlorite (secondary ROS). During this mechanism, complexes of chain I and III mainly generate O2•−(primary ROS) [2]. The SOD1 (Cu–Zn superoxide dismutase) in the space of intermembrane or mitochondrial Mn superoxide dismutase (SOD2) in the matrix of mitochondria eventually transform these O2•− into H2O2 [11].

On the other hand, outside the cell, superoxide dismutase 3 (SOD3) converts O2•− to H_2O_2. In the ER(Endoplasmic Reticulum), both protein and ROS are produced. To restore protein homeostasis, the UPR (Unfolded Protein Response) is activated in response to ER stress caused by the accumulation of misfolded proteins [12].

Xanthine oxidase also contributes to ROS generation by reducing O2 to O2•−, which cytosolic SOD1 then converts to H2O2 and combines with nitric oxide to generate peroxynitrite [13]. Figure 1 illustrates the generation of ROS and its natural breakdown mechanisms.

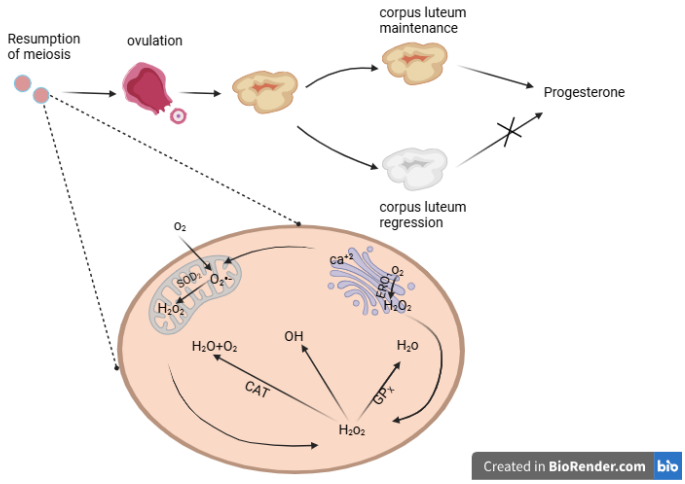

Figure 1: Diagrammatic representation of ROS generation and its natural breakdown mechanisms

Antioxidants in follicular fluid: protection against oxidative stress

Components linked to oxidative stress (OS) are considered among the most crucial elements present in FF that negatively impact the outcomes of assisted reproductive technologies (ART) [14]. OS is a state in which reactive oxygen species (ROS) formation is more than the defences of antioxidants and is especially dangerous for the FF microenvironment. The antioxidants present in FF prevent cellular damage and neutralize ROS, making them a fundamental line of defense. Most essential antioxidants found in FF are SOD (Superoxide Dismutase), catalase, GSH (Glutathione), and vitamins E and C. An essential enzyme of antioxidant called (SOD) catalyzes the transformation of radicals of superoxide into hydrogen peroxide, which is less reactive and can be broken down by other antioxidants. SOD comes in many isoforms, such as manganese SOD (MnSOD) and copper-zinc SOD (CuZnSOD), each with a distinct function in different parts of the cell [15]. Hydrogen peroxide is converted into water and oxygen by another essential enzyme of FF called Catalase, thereby stopping the buildup of potentially dangerous ROS. One of the most critical intracellular antioxidants in FF is GSH, which helps in maintaining the quality of oocytes, depending on its amount. GSH contributes to the regeneration of other antioxidants and directly scavenges ROS, including peroxides and hydroxyl radicals. Vitamins like vit. E (tocopherol) and vit. C (ascorbic acid), apart from enzymatic antioxidants, is also essential for shielding the oocyte from oxidative stress. Water-soluble antioxidant vitamin C scavenges reactive oxygen species (ROS) in the aqueous phase of follicular fluid, while fat-soluble antioxidant vitamin E shields cellular membranes from lipid peroxidation [16].

In a study, Liu et al. found that polycystic ovarian syndrome (PCOS) is linked to higher OS markers in serum and FF. Additionally, OS biomarkers of FF provide more accurate estimates of embryo quality than serum indicators, demonstrating a stronger connection with embryo quality [17]. FF isolated from fertilized oocytes had a greater antioxidant capacity than unfertilized oocytes, which directly impacts the embryo development. Increased ROS in FF is associated with poorer oocyte quality, poorer fertilization, and poorer embryo quality, despite moderate ROS levels being necessary for signaling during ovulation and other physiological processes. Excessive oxidative stress may adversely impact oocyte health and, consequently, fertility outcomes, especially in ART, such as in vitro fertilization (IVF). Apart from antioxidants present endogenously, melatonin, Coenzyme Q10 (CoQ10), and vitamins C and E have also been studied for their ability to provide a protective shield to oocytes against oxidative stress. Studies have shown that when individuals with FF are supplemented with these antioxidants, they can improve mitochondrial function, decrease DNA damage, and increase the chances of a healthy pregnancy and embryo implantation.

Vitamin E is another antioxidant that shields cellular membranes from lipid peroxidation. Vitamin C, for example, is a potent water-soluble antioxidant that directly scavenges ROS. As for the oocyte's energy-demanding maturation phase, CoQ10 promotes mitochondrial energy generation [18].

Despite the positive effects, the use of antioxidants in ART remains complex, and further study is needed to determine the optimal types of antioxidants, doses, and treatment durations. Apart from this promising finding regarding these antioxidant supplements, the danger of over-supplementation is "reductive stress," a condition in which high antioxidant levels may interfere with cellular signaling, potentially hindering oocyte development. A one-size-fits-all strategy is further complicated by the individual diversity in oxidative stress levels among ART recipients, underscoring the need for customized antioxidant therapy.

Antioxidant imbalance and its impact on embryo development

Oxidative stress is particularly harmful during the early phases of embryonic development. During fertilization, the embryo's cells are incredibly active and energy-demanding and are also highly active in the initial rounds of cell division. During this time, any imbalance in ROS levels can result in mitochondrial malfunction, DNA damage, and altered cellular signaling, all of which may hinder the healthy growth of the embryo. Suppose ROS accumulates in the early stages of the embryo. In that case, it may lead to chromosomal defects, impaired cell division, and apoptosis (programmed cell death), all of which have a detrimental effect on the viability of the developing embryo [19-20]. By regulating ROS levels and promoting cellular functions necessary for healthy development, antioxidants present in follicular fluid play a crucial role in protecting the embryo during these early phases. Antioxidants in the follicular fluid help in preserving the ideal oxidative conditions for fertilization and the embryo's early growth that follows. Moreover, the ability of antioxidants to regulate the cellular redox state is crucial for the early differentiation of the embryo and implantation.

Overexposure to ROS may lead to abnormal development of the embryo by interfering with the signaling pathways that determine gene expression and cell fate. Hence, it is necessary to maintain a balance between ROS and antioxidants for both the embryo's early development and its capacity to implant in the uterine lining and create a healthy pregnancy. For the follicular fluid to successfully transition from oocyte maturation to embryo development and ultimately achieve a favorable pregnancy outcome, it must have an environment rich in antioxidants [21]. Figure 2 illustrates balanced and imbalanced antioxidant function in the human follicle.

Follicular fluid oxidative stress biomarkers

Enzymatic antioxidant systems, such as GSH-Px (Glutathione peroxidase), superoxide dismutase (SOD), and CAT (catalase), and antioxidant’s non-enzymatic systems, such as glutathione, melatonin, vitamins C and Comprises the body's antioxidant system. The TAC, or Total Antioxidant Capacity, is often used to indicate the combined antioxidant capacity of both enzymatic and non-enzymatic antioxidants. If there is an imbalance between the antioxidant system and the oxidation system, Reactive oxygen species(ROS) can damage DNA, lipids, and proteins either directly or indirectly. This can result in mutations of genes, denaturation of proteins, and peroxidation of lipids, ultimately impairing regular physiological processes. Thus, the biomarkers mentioned in Table 2 are frequently used for evaluating oxidative damage. These were found to be the most widely used oxidative stress markers in female reproduction as per the compiled research conducted in this area from 2012 to 2022 by Michalina et al [22]. According to research conducted by Iman et al., proper instruments for assessing clinical characteristics in IVF patients may be biomarkers of OS including 8-Oxodeoxyguanosine (8-OHdG), Total Antioxidant Capacity(TAC), and Malondialdehyde (MDA) because they are linked to hormones of reproduction and Assisted Reproduction Technology pregnancy outcomes [23-24]**.**

For oocyte development, it is crucial to directly measure the quantity of ROS in the follicular fluid, or microenvironment. physicians and patients are provided with essential predictive information about the condition of granulosa cells and oocytes before oocyte fertilization. By examining ROS levels in FF, a patient's reproductive potential can be assessed, which will help in choosing the most effective Assisted Reproductive Technology methods to improve success rates. ROS analysis can also help physicians use more targeted treatment approaches by identifying potential reasons for unsuccessful fertility, such as a decline in oocyte quality or damaged granulosa cells [25]. In Table 2, several oxidative stress biomarkers are listed.

Table 2:Tabular representation of various oxidative stress biomarkers and their role

Oxidative stress marker	Description	Relationship with oocyte quality & reproductive outcomes	Key findings	Potential predictive value	Ref.
Total Antioxidant Capacity (TAC)	Measure of overall antioxidants (e.g., vitamin C, SOD, CAT) in the body	Inconclusive, with some studies showing a correlation with successful fertilization	Higher TAC in follicular fluid observed in successfully fertilized oocytes; no clear correlation with pregnancy outcomes	Limited evidence, not a reliable predictor for fertilization or pregnancy outcomes	[26], [27], [28]
Malondialdehyde (MDA)	Product of lipid peroxidation; indicates oxidative stress	Negative correlation with oocyte maturation, fertilization rates, and embryo quality	Higher MDA levels in older women and women with PCOS; negative impact on blastocyst formation	Potential marker for embryo quality, especially in older women and those with PCOS	[29] [30], [28], [31]
8-Oxodeoxyguanosine (8-OHdG)	Oxidative derivative of deoxyguanosine; marker of DNA damage	Negative correlation with oocyte maturation, fertilization rates, and embryo quality	Higher 8-OHdG levels linked to increased oocyte degeneration and lower fertilization rates	Reliable indicator for assessing oocyte quality and IVF outcomes	[29] [32], [33] [34]
Advanced Oxidation Protein Products (AOPP)	Protein oxidation products formed by ROS; reflect protein damage	Negative correlation with oocyte maturation, fertilization, and embryo quality	Elevated in non-pregnant women and women with endometriosis; associated with poor IVF outcomes	Possible marker for oocyte quality and IVF success, especially in endometriosis patients	[35] [36] [37]
Superoxide Dismutase (SOD)	Enzyme that neutralizes superoxide radicals and protects oocytes	Positive correlation with fertilization rates and embryo quality	Lower SOD activity in follicular fluid linked to poor fertilization and oocyte quality in older women	Important for oocyte quality; may influence reproductive success, especially in aging women	[38] [39]
Glutathione (GSH)	Antioxidant that protects cells from oxidative damage	No clear correlation with fertilization rates or pregnancy outcomes	Some studies show lower GSH levels in women with endometriosis and low fertilization rates	Complex relationship; potential impact on embryo development, but inconsistent findings	[40] [41]

MEASURES TO IMPROVE OS ON FOLLICULAR FLUID

The increase in levels of Reactive Oxygen Species and oxidative stress in follicular fluid may negatively impact the growth and quality of oocytes, which may, in turn, affect the outcome of pregnancy. In recent years, it has been demonstrated that an abundance of small molecules with antioxidant properties can mitigate oxidative stress damage. Here, we give a summary of how antioxidants like melatonin, coenzyme Q10, resveratrol, and NAC are used to enhance embryo development in oocyte maturation in vitro, oocyte cryopreservation, and ART procedures for the elderly and patients with ovarian dysfunction.

Melatonin

The neuroendocrine hormone melatonin is secreted by the pineal gland, which has antioxidant qualities. It encourages oocyte

growth, enhances mitochondrial activity, and lowers oxidative stress, hence aiding in the regulation of ovarian and reproductive processes in mammals. According to studies, Melatonin also improves the maturation of oocytes, rate of fertilization, and blastocyst formation, especially in humans, mice, and pigs. Additionally, it may help in enhancing the results of assisted reproductive methods like IVF. However, several studies have found slight improvement, and concerns exist about its long-term effects [42]. Table 3 shows the effect of melatonin in different animals.

Table 3: Effect of melatonin in different animals

Species	Protocol	Dose	Treatment Outcome
Mouse	Melatonin in IVM medium	10^{-6}M/L of melatonin	Enhanced oocyte maturation, blastocyst rates, and embryo implantation [43-46].
Pig	Melatonin in IVM and culture media	10^{-5} M/L of melatonin	Increased oocyte maturation rates and blastocyst formation [47-49].
Sheep	Melatonin in culture medium		Increased oocyte maturation and blastocyst formation [50**].**
Cow	Melatonin in IVM medium	10^{-9}M/L of melatonin	Enhanced embryo development and reduced apoptosis [51],[52]

Coenzyme Q10 (CoQ10)

For both antioxidant defense and mitochondrial energy generation, Coenzyme Q10 (CoQ10) is necessary. With age, CoQ10 levels decrease, which may affect reproductive health. When CoQ10 supplements are given during pregnancy, they enhance pregnancy outcomes, oocyte quality, and mitochondrial function. However, despite its beneficial effects on fertilization and embryo quality in older women, some studies provide contradictory results, particularly regarding clinical pregnancy rates [53].

Resveratrol

Resveratrol, which is a polyphenolic substance, acts as an antioxidant and enhances mitochondrial activity by activating SIRT1A (Table 4). It aids in embryo development by improving oocyte quality and lessening oxidative stress. The benefits of resveratrol have been demonstrated in both humans and animals, particularly in enhancing the quality and rates of oocyte and embryo maturation. Its effects are dose-dependent, though, and greater dosages could have negative consequences [54].

Table 4: Effect of resveratrol in different animals:

Species	Protocol	Dose	Treatment Outcome
Mouse	Resveratrol in IVM medium	1.0 μM/L	Improved oocyte maturation and quality [55]
Pig	Resveratrol in IVM medium	2 μM /L	Enhanced mitochondrial function, increased ATP levels, and improved oocyte quality [56]
Cow	Resveratrol in maturation medium	1.0 μM /L	Reduced ROS levels and improved oocyte quality [57]

Clinical implications and future directions of antioxidant therapy in IVF

Antioxidant therapy has shown considerable promise in improving oocyte quality and embryo development, thereby enhancing the success rates of in vitro fertilization (IVF). By mitigating oxidative stress, antioxidants support oocyte maturation, reduce DNA damage, and improve mitochondrial function.

With many crucial considerations and future research directions, the implementation of antioxidants in clinical research for in vitro fertilization remains an evolving field, despite numerous essential considerations. In research performed by Alice Luddi *et al.,* ovarian stimulation was initiated on the first or second day of either spontaneous or induced menstruation, using recombinant gonadotropins at a daily dosage of 150–300 IU. Following the ultrasound-monitored ovarian response, the dose was modified. A daily Gonadotrophin-releasing hormone (GnRH) antagonist was given after the dominant follicle grew to a diameter of 14 mm, until at least three follicles were larger than 18 mm, at which point ovulation was induced with a Human chronic gonadotrophin(hCG) injection. After the hCG injection,

oocyte retrieval was carried out 34–36 hours later. After aspirating and centrifuging the follicular fluid, only samples devoid of visible blood were chosen [58]. On the day of oocyte retrieval, blood serum samples were also taken for comparison.

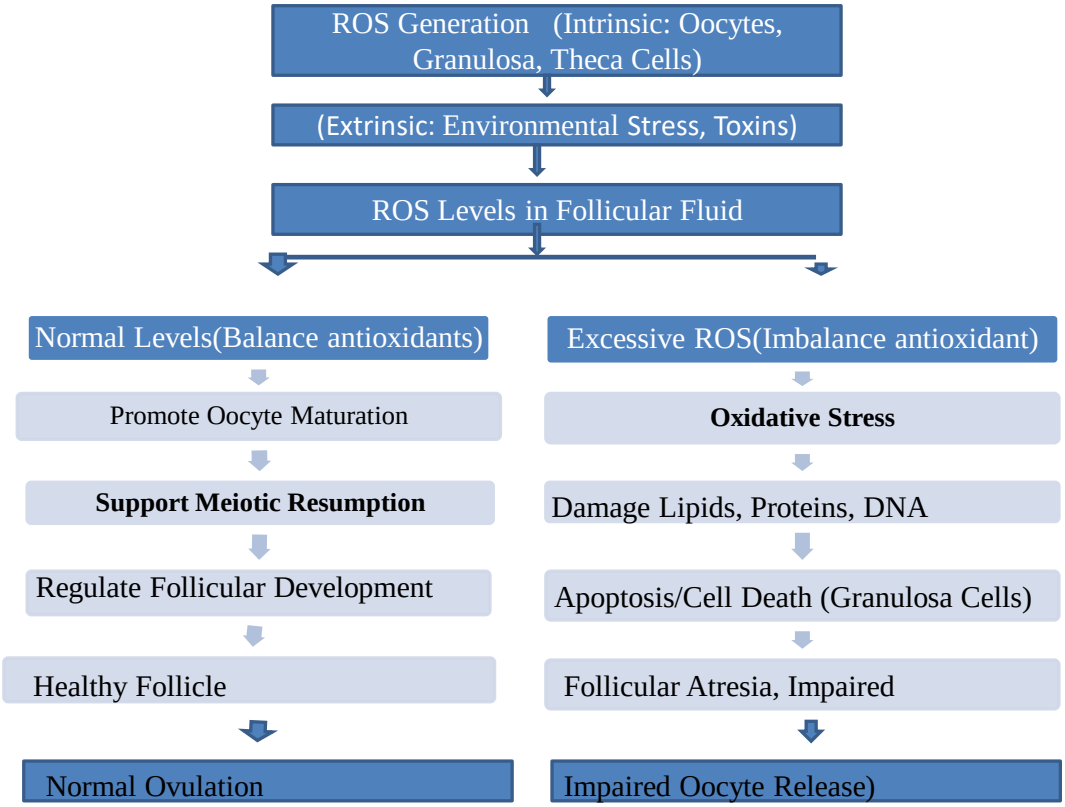

Figure 2: Illustration of balanced and imbalanced antioxidant function in the human follicle in an arrow diagram

Potential for Targeted Antioxidant Therapies in IVF

Antioxidant-targeted treatments in IVF have rapidly emerged as a key component of customized therapy. Oxidative stress is a primary cause of poor oocyte quality and embryo development; hence, antioxidant supplements could significantly enhance the results of ART. CoQ10, vitamins C and E, and glutathione are specific antioxidants that, in recent research, have been shown to lower oxidative damage and are therefore linked to better oocyte maturation, higher pregnancy rates, and higher-quality embryos.

Sadeghi *et al.* (2023) found that CoQ10 supplementation enhanced oocyte quality, particularly in older mothers, a demographic that frequently faces higher levels of oxidative stress [59-61]**.** Improving the clinical use of antioxidants can be achieved by Customizing treatments for each patient. To create more effective, individualized antioxidant regimens, it may be feasible to analyze the oxidative stress profiles in follicular fluid. By ensuring that each patient's unique needs are met, this strategy may increase IVF success rates and reduce the risks associated with excessive use of antioxidants. Women with high levels of oxidative stress, for example, may benefit from higher antioxidant dosages, whereas other women may require only moderate supplementation.

FUTURE RESEARCH DIRECTIONS

Personalized Antioxidant Treatment Based on Oxidative Profiles

Based on each patient's unique oxidative stress profile, the creation of customized antioxidant therapies is one of the most exciting directions for further study. Differences in oxidative stress levels among individuals may enable the tailoring of antioxidant therapy to maximize results.

For proper clinical judgment, research should focus on identifying reliable indicators of oxidative stress in follicular fluid, blood, and oocytes. Analyzing particular indicators in follicular fluid, such as malondialdehyde (MDA) or 8-hydroxydeoxyguanosine (8-OHdG), for example, may help determine the amount of oxidative stress and guide the appropriate use of antioxidants.

New antioxidants optimized for follicular fluid development: The creation of novel antioxidants tailored explicitly for the follicular fluid environment is an intriguing topic for further study, without disrupting the delicate balance of antioxidants that support oocyte health. It's crucial to comprehend how to improve the natural defenses of the follicular fluid. Research into new chemicals or natural antioxidants, such as polyphenols and flavonoids, may offer additional benefits compared to conventional antioxidants like vitamins C and E. To increase the bioavailability and targeted distribution of antioxidants to the ovaries or oocytes, new drugs, such as nanoparticles, could be investigated.

Long-Term Safety of Antioxidant Supplementation

Although the promise of enhancing IVF outcomes has been shown, further research is necessary to assess the long-term effects of antioxidants on oocyte health, embryo development, and overall reproductive outcomes. Possible adverse effects of long-term antioxidant supplementation should be looked into in research, including how it may affect fetal development, folliculogenesis, and ovarian reserve. To determine the optimal times and durations for antioxidant therapies, it's also essential to conduct research. Zhang *et al.* (2023) found that short-term use of antioxidants may enhance IVF results. In contrast, long-term supplementation may not be as beneficial and may even disrupt the normal redox balance of the reproductive system [62].

Combination with Other Fertility Treatments

Future studies should also investigate the synergistic benefits of antioxidants when combined with other reproductive treatments. For example, antioxidants, if combined with hormone therapy, ovarian stimulation procedures, or lifestyle changes (such as nutrition, exercise, and stress management) in women undergoing IVF, may offer more varied advantages. Examining this combination of medicines may result in improved IVF success rates and more beneficial treatment plans.

CONCLUSION

In conclusion, reducing oxidative stress in follicular fluid through the use of antioxidants plays a crucial role in protecting oocyte health and enhancing embryo development. As one of the primary defenses against reactive oxygen species (ROS), critical endogenous antioxidants like glutathione, catalase, and superoxide dismutase (SOD) maintaines DNA integrity, mitochondrial function, and general cellular resilience in oocytes. Exogenous antioxidants, such as vitamins C and E and CoQ10, when administered correctly during assisted reproductive technologies (ART) like IVF, have positive effects in both clinical and in vitro environments, contributing to increased oocyte maturation and embryo quality. Studies over the last five years have demonstrated the benefits of targeted antioxidant supplements in enhancing IVF results. Still, it has also drawn attention to the challenges in determining the proper dosages and managing the risks associated with the overuse of antioxidants, which can lead to oxidative stress. Based on each patient's unique oxidative profile, Assisted Reproductive Technologies could be improved by personalized treatment with antioxidants, which might reduce oxidative damage.

Future studies on new antioxidant chemicals and the combination of therapies could also offer creative and special methods for reproductive health. Together, these insights, when taken as a whole, highlight the medicinal potential of antioxidants as a supplement to reproductive therapies, creating new possibilities to enhance clinical results and assist those who are infertile.

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AUTHOR CONTRIBUTION

Nidhi Gairola was responsible for the conceptualization, supervision, and project administration and contributed to the writing, review, and editing of the manuscript. Himanko Gogoi was involved in the design of the methodology, investigation, data analysis, and writing the original draft. Janhvi Dubey contributed to the investigation and writing of the original draft. Jasvinder Singh Khatiyaan worked on the literature review and contributed to the writing review. Harsh Chaudhary assisted with the literature review, data interpretation, and writing review. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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