FOLLICULAR FLUID COENZYME Q10 LEVEL IN WOMEN UNDERGOING INTRACYTOPLASMIC SPERM INJECTION (ICSI)

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Abstract

Background: Female infertility has a complex pathophysiology that we still don't fully understand. One of the most studied pathogenetic pathways is how old mothers affect their children. The impact of oxidative stress on female infertility is unknown, but both of these conditions are linked to it. Inadequate antioxidant defenses relative to reactive oxygen species (ROS) generation characterizes oxidative stress. ROS, despite being present only in physiological states, serve a crucial regulatory role in the female reproductive system. When there are too many of them, they could have a negative effect on fertility. Coenzyme Q10 (CoQ10) is a lipid-soluble quinone that aids in the body's energy generation cycle by aiding ATP synthesis and functioning as a potent antioxidant that prevents lipid peroxidation and DNA oxidation. Despite a lack of clear data, CoQ10 supplementation has long been used to improve infertility outcomes and is related with a higher clinical pregnancy rate (CPR). The impact on ART clinical outcomes, including live birth rate (LBR) and miscarriage rate (MR), are also unclear. Oocyte quality is significantly affected by the oocyte's surroundings. Because the oocyte finishes developing in the FF, its biochemical characteristics may be crucial to the quality of both the oocyte and the embryo that develops from it. The flow of nutrients, oxygen, and other growth agents between oocytes and cumulus cells is bidirectional. Previous studies linked the presence of these components in the follicle to better oocyte quality and higher conception rates.

Keywords: Women receiving intracytoplasmic sperm injection (ICSI), follicular fluid coenzyme Q10, and miscarriage rate.

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1. INTRODUCTION

In 1988, intracytoplasmic sperm injection (ICSI) was utilized for the first time with human gametes, namely in cases where fertilization had failed with conventional IVF or when there were insufficient sperm cells. In 1992, authorities in Belgium discovered the country's first pregnancy. A single sperm is injected into the cytoplasm of a mature egg as part of an in vitro fertilization (IVF) cycle.[1] The quality of the oocytes utilized in IVF cycles is still a major problem because it is mostly affected by the woman's age and ovarian reserve. There are two nuclear and two cytoplasmic components in the complex process of oocyte maturation. Oocyte maturation is highly dependent on the cytoplasmic organelle known as the mitochondria, which regulates the energy-producing oxidative phosphorylation process. Oocyte quality and DNA quantity could be negatively impacted by disturbances in oxidative phosphorylation.[3]The inner mitochondrial membrane houses four complexes that work together to produce ATP through oxidative phosphorylation.[4] These include cytochromes, flavoproteins, coenzyme Q10, and NAD-linked dehydrogenase. The structure of coenzyme Q10 (Ubiquinone) is very close to that of vitamins E and K. Both complexes I and II are involved in oxidizing tricarboxylic acid byproducts and transferring electrons to coenzyme Q10.It is complex III (cytochrome reductase) that receives the electrons transferred by Coenzyme Q10.Due to its function in cellular development and energy metabolism. The transfer of electrons from complex I to complex IV involves ATP synthase. Coenzyme Q10 (CoQ10) supplementation has been demonstrated to improve

bovine embryo growth, hatching, cell proliferation, and ATP content when used in in vitro fertilization. Energy consumption is a crucial element during oogenesis and oocyte maturation.[6,7]

Coenzyme Q10 (CoQ10) is a popular antioxidant supplement for a wide range of medical disorders. Coenzyme Q10 has also been found to boost sperm count, sperm quality, and the likelihood of conception.[8] CoQ10 supplementation was associated with lower rates of aneuploidy and a higher pregnancy rate, as reported by **Bentov et al.**, however these results are not clinically relevant.[9]

Ovarian responsiveness to stimulation and embryological parameters have both been proven to improve with pre-treatment with CoQ10 in women with low ovarian reserve undergoing IVF-ICSI cycles. More research is needed to see whether there's a change in clinical outcomes, however, because study results have been inconsistent.10 We analyzed the effect of FF CoQ10 levels on the prevalence of clinical pregnancies.

Intracytoplasmic Sperm Injection

In vitro sperm injection (ICSI) is a method wherein a single sperm is injected into the cytoplasm of a fertilized egg. In males with inadequate semen characteristics or who had no or low fertilization rates with conventional IVF, this technique, conducted as part of the IVF cycle, provides an efficient method for helping conception. When conventional in IVF failed to result in a pregnancy, or when there were insufficient sperm cells, ICSI was initially utilized on human gametes in 198811,12. [13]

In 1992, authorities in Belgium discovered the country's first pregnancy. This method has consistently shown higher fertilization rates and the production of more embryos with higher implantation rates compared to earlier micromanipulation methods. [1]

Indications for ICSI

Patients with surgically inoperable bilateral tubal occlusion or obstruction were the first to benefit from conventional IVF. The desire of infertile individuals to reproduce, combined with advancements in ovarian stimulation and embryology laboratory procedures, has led to the use of IVF in cases with endometriosis, moderate male-factor infertility, and unexplained infertility. 14,15 However, IVF is not an option for a few thousand people with severe male-factor infertility; these people would have to rely on donor sperm or adoption instead. [16]

When it comes to helping men who have obstructive azoospermia (OA) or non-obstructive azoospermia (NOA) conceive, ICSI has emerged as the treatment of choice. [17]

Several non-male factor indications have led to the widespread use of ICSI. Fertilization of low-quality or dysmorphic oocytes detected through morphologic analysis is one such sign. Researchers have also suggested using ICSI in poor responders to make the most of the fertilized oocytes that can be injected. [18,19]

Furthermore, ICSI needs to be performed quickly to avoid the disadvantages of in vitro aging post-ovulatory aging oocyte, so that early ICSI users could boost pregnancy rates dramatically by strictly maintaining laboratory conditions. Because of this, ICSI shouldn't be done if any of the aforementioned technological requirements can't be fulfilled. [20]

Rescue ICSI, in which unsuccessfully fertilized oocytes are reinseminated, is also discouraged. High rates of polyploidy and developmental arrest are common in embryos created through rescue ICSI. [21,22]

Sperm Retrieval for ICSI

Ejaculated semen is the most common source from which to collect sperm. For males with obstructive or nonobstructive azoospermia, ejaculatory dysfunction, or problems from cancer treatment, surgical removal of spermatozoa from the testicles or reproductive tract combined with ICSI is a successful treatment. [23]

Using fresh or cryopreserved-thawed testicular sperm did not significantly improve fertilization or clinical pregnancy rates in a 2014 meta-analysis of comparative studies of ICSI in men with azoospermia due to spermatogenic dysfunction. Despite the fact that healthy babies have been born, there are major genetic implications to these practices. [24]

Spermatozoa Selection

Ability to properly prepare spermatozoa and choose normal, viable spermatozoa for injection are crucial to the success of ICSI. They're crucial because artificial insemination, in which spermatozoa are injected directly into an egg, sidesteps natural selection and could introduce a faulty paternal genome. [25]

In vitro fertilization (ICSI) is highly successful if the spermatozoa used have outwardly normal morphology and motility. [26]

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2. TECHNIQUE

In vitro fertilization (ICSI) calls for high-tech equipment and the expertise of a skilled embryologist. Gametes should be shielded from environmental factors including temperature and pH changes that might disturb spindles and lead to chromosome aberrations. [27]

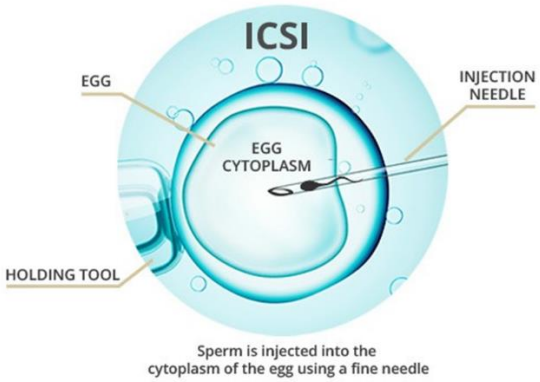

Fig. (1): illustrates the process of injection of the sperm in ICSI procedure

Equipment for ICSI [28,29]:

- **Microscope**

An inverted microscope with high quality optics (eg, Nomarski or Hoffman) should be used for the most accurate visualization of gametes and microtools, thereby decreasing oocyte damage while optimizing fertilization rates. A sophisticated heated optical system, such as polarization microscopy, can be used to monitor spindle position during injection and reduce spindle damage during the ICSI procedure. [30]

- **Controlled Ovarian Stimulation**

The initial part of the IVF/ICSI cycle is ovarian stimulation. Many techniques have been used, ranging from no stimulation at all to highly stimulating regimes involving clomiphene citrate, letrozole, and exogenous gonadotropins (FSH and luteinizing hormone (LH)). [31]

- **Oocyte Retrieval**

No matter the stimulation method, mature oocytes can be collected 34–36 hours following hCG injection. Oocytes are retrieved via transvaginal aspiration under ultrasound guidance under intravenous sedation. The ovaries are seen on a vaginal ultrasonography probe & a connected needle guide aids the doctor in aspirating the oocyte and FF from each follicle.**32**

- **Injection**

An injection pipette containing an immobilized spermatozoon is gently pushed through the zona pellucida and through the oolemma into the center of the oocyte. The spermatozoa should be delivered with the smallest possible amount of medium. Negative pressure is then used to break the oolemma, followed by gentle aspiration of the cytoplasm. **33**

- **Embryo Transfer**

Embryos (1-2) are transferred at either the cleavage (day 3) or blastocyst (day 5) post-fertilization. Transferring embryos during the blastocyst stage reduces the risk of having multiples during a pregnancy and increases the number of live births each cycle. There is a risk that fewer viable embryos will be available for transfer if they are transferred at the blastocyst stage since embryos that did not survive in culture until day 5 are discarded. [34]

Embryo Development and Clinical Outcome

Since its development, ICSI's popularity has skyrocketed as a means to overcome virtually all causes of male infertility. The percentage of live births with ICSI rose from 36.4% in 1996 to 76.2% in 2012 in the United States. [35,36]

Follicular fluid

In follicular fluid, germ cells and somatic cells interact in a highly nuanced milieu. It contains a variety of metabolites and facilitates several processes necessary for oocyte development. Follicular wall metabolites, transudate of plasma, and diffusion of serum all contribute. Over time, this substance will be modified by granulosa cells (GCs) and theca cells. Also present are substances produced locally by follicular metabolism and ovarian cell biological processes.[37]

3. APPEARANCE AND PHYSICAL PROPERTIES OF FF

The first evidence of FF is a secretion that builds up between the granulosa cells of developing follicles. Since autoradiographic studies of the synthetic activity of the granulosa cells have led to this conclusion, much attention has been paid to the fluid of growing and mature follicles. It is a transparent, colorless liquid with a mildly acidic pH of 6.8 to 7.5. Follicle fluid volume increases from a few milliliters to about 20 milliliters in mature follicles as the follicle develops. Follicular fluid normally has a low viscosity, allowing oocytes and other molecules to flow freely within the follicle. The fluid also contains small particles, such as granulosa cells and debris from the follicle.[38]

Formation of follicular fluid

The follicle grows as a result of the expansion of the oocyte, the division of follicular cells, and the development of a central cavity or antrum. The replication of granulosa cells has been the subject of numerous in vitro investigations of follicular growth, whereas the extension of the follicular antrum and associated fluid has been the subject of numerous in vivo research employing ultrasonography. Both the enlargement of the follicular antrum and the replication of follicular cells are critical processes, and both are likely induced by some of the same hormones and growth factors.[39]

Composition and cytology of follicular fluid

Several proteins from various functional groups, such as insulin-like growth factors, receptors, anti-apoptotic proteins, or metalloproteinases, are present in FF, according to a proteomic analysis. The

expression of 17 proteins changed in FF upon HCG injection, making them biomarkers that demonstrate how the follicular milieu varies in response to infertility problems.[40]

Variations of FF properties in Different Patient Groups

Several studies have investigated the composition and properties of FF in different patient groups; including fertile, infertile women and different age groups in addition to different pathologies like PCOs and endometriosis.

With advancement of the age, ovarian reserve decreases which associated with changes in the bio media of FF. **Hashemitabar et al** studied difference in FF composition between young age groups (20-32) and old (38-42), Using matrix-assisted laser desorption/ionization time-of-flight/time-of-flight (MALDI-TOF/TOF) mass spectrometry, proteins were identified. Twenty-three of the protein sites were significantly altered between the young and old groups. MALDI-TOF-TOF-MS confirmed the presence of 19 protein spots. MASCOT analysis revealed that the elderly had five distinct proteins that had been downregulated. Serotransferrin, hemopexin precursor, complement C3, C4, and kininogen were shown to be among them. Several protein markers were identified with promising implications for infertility diagnosis.[41]

Patients with ovarian endometriosis have higher amounts of lactate, beta-glucose, pyruvate, and valine in FF compared to healthy controls, whereas those with deep infiltrating endometriosis have lower levels of glucose, citrate, creatine, and amino acids like tyrosine and alanine. The presence or absence of ovulatory endometriosis could potentially be distinguished by differences in the amounts of amino acids such threonine and glutamine.[42]

Coenzyme Q10

Coenzyme Q10 (CoQ10) is a chemical molecule that serves as an electron carrier/proton translocator in the respiratory chain (energy-promoting agent that leads to ATP synthesis in the mitochondrial membrane) and as an antioxidant, making it vital to human health. [43,44]

Coenzyme Q10 and Oocyte quality

Coenzyme Q10 (or ubiquinone, or ubidecarenone) is a nutrient that functions similarly to vitamins and is soluble in fat. Its name implies that it can be found in any human cell. Its primary localization is in mitochondria, however other cellular components including membranes and lipoproteins also contain it. The creation of cellular energy is CoQ10's fundamental function. CoQ10 is an element of the electron transport chain (ETC) along the inner mitochondrial membrane, which is responsible for converting metabolic products (carbohydrates, lipids, and proteins) into ATP. [45]

The inevitable deterioration in oocyte quality with aging is a significant challenge when treating elderly patients. Increased point mutations and deletions in mitochondrial DNA were the primary cause of this reduction. [46]

Coenzyme Q10 Mechanism of action

CoQ10 is a lipophilic molecule found in the hydrophobic region of all cell membranes, making it fat-soluble. Located in the inner mitochondrial membrane, its primary function is to transport electrons and protons down the respiratory chain during ATP biosynthesis. [47]

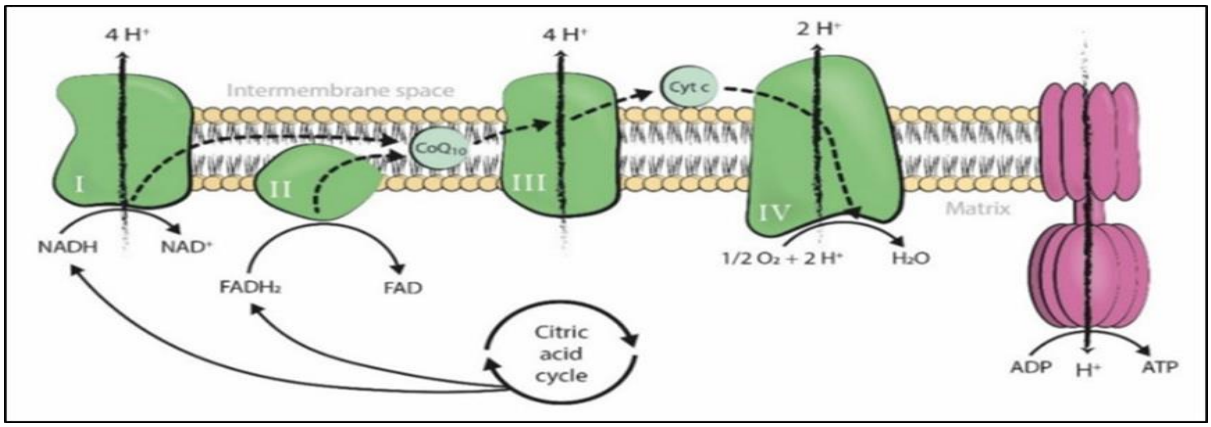

Fig. (2): illustrates in brief steps of mitochondrial respiratory chain (Complexes I, II, III, and IV; CoQ10, and cytochrome c) and the F1-F0 ATPase, all are located in the inner mitochondrial membrane. NADH and FADH2 transmit electrons to complexes I and II, respectively. This action creates an electrochemical gradient through the chain's electron flow and proton pumping oxygen molecules produces ATP molecules by action of ATPase. ADP: adenosine triphosphate. ATP: adenosine triphosphate. Pi: inorganic phosphate. H+: hydrogen ion (proton). NADH: nicotinamide adenine dinucleotide, reduced form. FADH2: flavin adenine dinucleotide, reduced form. NAD+: nicotinamide adenine dinucleotide, oxidized form. FAD: flavin adenine dinucleotide, oxidized form. O2: oxygen. H2O: water. Cyt c: cytochrome c. CoQ10: coenzyme Q10. [48]

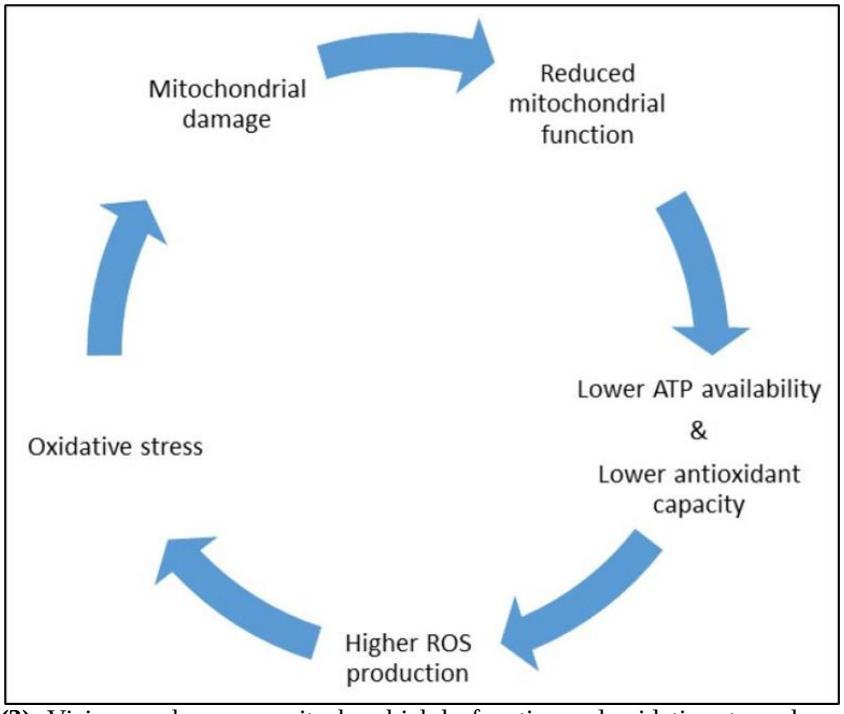

Fig. (3): Vicious cycle among mitochondrial dysfunction and oxidative stress damage. [48]

CoQ10 and Female Fertility

- **Deterioration of Female Reproductive Capacity**

Fertility in women is one of the many important physiological functions that declines with age. [49]
Reproductive potential peaks about age 25 in women and then rapidly declines from ages 32–37, with the end of a woman's reproductive life occurring around age 50.

Because of the high energy and oxygen demands of the developing oocyte and embryo, oxidative phosphorylation capacity is a factor in female fertility. [51]

- **Coenzyme Q10 and the Female Reproductive Tract**

Women's ovarian reserves rapidly fall with age for a variety of causes, but one that stands out is damage to their oocytes due to a loss in their DNA repair systems. [52]
The ovary, like all other tissues, produces CoQ10 in the mitochondrial inner membrane. High numbers of mitochondria in mature normal oocytes give the oocyte a significant ability to synthesize high amounts of CoQ10, and the oocyte also exhibits appropriate exogenous absorption of this coenzyme. Therefore, CoQ10 deficiency may result from the progression of mitochondrial dysfunction. [3,53]

- **Effect of Supplementation of CoQ10**

CoQ10 supplementation in older animals and humans improves fertility because it decreases reactive oxygen species (ROS) and oocyte chromosome aneuploidies while increasing ovarian reserve and ATP production in the mitochondria. [54]

Coenzyme Q10 in the Follicular Fluid

It has been hypothesized that changes in the intrafollicular environment may influence centrosome-based microtubule assembly, which in turn may contribute to the observed age-related rise in aneuploidy. [55]
The effects of reduced antioxidant enzyme (ROS) generation and mitochondrial dysfunction were studied by **Quinzi et al.** Increased reactive oxygen species (ROS) generation, lipid oxidation, and cell death were all linked to moderate CoQ10 depletion. 56 Recent research has linked CoQ10 administration to less reactive species in mouse ovaries. [57]
Coenzyme Q10 (CoQ10) is essential for oocyte energy production due to its function in the oxidative phosphorylation pathway. Thus, infertility in dams was linked to decreased ATP production and increased meiotic spindle abnormalities as reported by **Ben-Meir et al.** [49].
Compared to dysmorphic oocytes, mature oocytes had considerably higher quantities of CoQ10/concluded protein **(Turi A. et al., 2012).**
Patients with poor ovarian reserve and advanced age present the greatest challenges to IVF/ICSI centers. Although there is some evidence that CoQ10 may help treat patients with low ovarian reserve, this evidence is inconclusive at best. CoQ10 supplementation was not associated with an increased likelihood of pregnancy in clinical trials involving those patients. [59,60]
CoQ10 treatment improved reproductive success, delayed follicle loss, and increased mitochondrial energy in a model of aging. [61]

4. CONCLUSION

Finally, Coenzyme Q10 (CoQ10) levels in follicular fluid were discovered to be predictive of embryo quality and pregnancy success. In addition to the higher fertilization rates and increased embryo production and implantation rates shown by Intracytoplasmic Sperm Injection over previous micromanipulation techniques, Our results suggest that women undergoing in vitro fertilization may benefit from taking CoQ10 as a supplement. To establish firm findings about the clinical administration of CoQ10 in female infertility, bigger, randomized, and controlled research are required.

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