Section: Miscellaneous

Original Research Article

ENHANCING FERTILITY WITH PATENTED PROPRIETARY SIRTUINS AND NRF2 ACTIVATOR (EQOQ®) INTERVENTION ON OOCYTE, EMBRYO QUALITY, AND IMPLANTATION RATES IN FEMALES WITH DIMINISHED OVARIAN RESERVE SEEKING FERTILITY TREATMENT AFTER THE AGE OF 30 YEARS: A MULTICENTRE, RANDOMIZED, DOUBLE-BLIND, CONTROLLED CLINICAL TRIAL. (FERTSIRT TRIAL)

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Abstract

Background: Diminished Ovarian Reserve (DOR) and advanced maternal age are pivotal factors in female fertility, significantly impacting assisted reproductive treatments. The aim is to evaluate the effectiveness of the EQOQ®, a novel intervention involving sirtuins and Nrf2 activators on oocyte and embryo quality and enhancing implantation rates in DOR patients over 30 years of age. **Materials and Methods:** A multicentre randomized double-blind controlled clinical trial with a parallel-group design recruited 100 eligible DOR individuals with at least one additional risk factor such as obesity, type 2 diabetes, type 1 diabetes, and hypothyroidism. They were randomly assigned to the EQOQ® intervention group (n=50) or a control group (SOC) (n=50). The EQOQ® intervention spanned six months, with comprehensive assessments of oocyte and embryo quality, implantation rates, and adverse event monitoring. **Result:** The EQOQ® intervention group demonstrated a statistically significant improvement in oocyte quality compared to the control group, marked by a substantial increase in normal morphology (82% ± 2.1%) $p<0.00001$. and improved oocyte maturation (79% ± 4.1%) when compared with the control group which is statistically significant $p<0.0001$. Furthermore, the EQOQ® group exhibited improved embryo quality, exhibiting a higher proportion of high-grade embryos (82% ± 3.1%) $p<0.00001$ and increased blastocyst

formation rates (73.03% ± 4.7%) compared to controls p<0.00001. Most notably, EQOQ® intervention resulted in a significantly elevated implantation rate (92% ± 1.6%) p<0.00001, resulting in improved clinical pregnancy and live birth rates compared to the control group. Adverse events associated with EQOQ® remained minimal and similar to the control group. **Conclusion:** The EQOQ® intervention demonstrated significant efficacy in augmenting oocyte and embryo quality, leading to higher rates of successful implantation and enhanced fertility outcomes in females diagnosed with diminished ovarian reserve (DOR) above the age of 30. EQOQ® holds promise as a prospective therapeutic approach to alleviate age-related fertility challenges, offering hope to couples tackling infertility for healthier pregnancies.

INTRODUCTION

Infertility is a prevalent global issue affecting numerous couples around the world. Various factors, including environmental stressors, unhealthy lifestyles, and delayed family planning, contribute to less-than-optimal fertility outcomes in both men and women.[1] Environmental stressors, poor dietary habits, sedentary lifestyles, insufficient sleep, and substance use can induce oxidative stress and inflammation, negatively affecting fertility. The postponement of family planning and advancing maternal age significantly affects female fertility, as the quality of oocytes diminishes over time. Additional risk factors such as obesity, polycystic ovary syndrome (PCOS), diabetes, hypothyroidism, and hypertension further contribute to impaired oocyte and embryo quality.[2-4] The trend of delayed family planning is on the rise, and as women age, the quality of their oocytes decreases, posing challenges to fertility. Poor oocyte quality is characterized by spindle formation error and an increased risk of chromosomal abnormalities.[5] Meiotic and mitotic aneuploidies drive the arrest of human embryos in vitro as development increasingly relies on embryonic gene expression at the blastocysts stage. A recent study by McCoy et al. published in Genome Medicine from Johns Hopkins University, which involved (909) fertility attempts, represents the largest study of its kind. Their findings revealed that 77.3% possessed whole or segmental aneuploidies of one or more chromosomes.[6] Recent advancements of Sirtuins, known as Silent Information Regulator Proteins have a major role in female meiosis and female reproductive longevity, their expression has been identified in oocytes across various mammalian species, including humans. Sirtuins are present in the whole ovarian follicle, ovarian epithelium and stroma, and luteinized granulosa cells. Sirtuins are a major determinant influencing ovarian aging, and the quality of gametes. As age advances, sirtuin genes are downregulated, and there is a concurrent downregulation of Nrf2 (Nuclear factor erythroid 2-related factor 2). Nrf2 is present in the cytoplasm and nucleus of ovarian cells, mediating its anti-inflammatory role by activating the HO-1 axis, in obese conditions, sirtuins are also downregulated.[7,8] A previous study revealed that comorbid conditions associated with infertility, such as type 2 diabetes (T2D), type 1 diabetes (T1D), and hypothyroidism (HT), are linked to significantly lower levels of sirtuin mRNA expression, resulting into fertility failure in female.[9]

A decrease in sirtuins and Nrf2 levels can result in spindle formation errors, aneuploidies, abnormal distribution of organelles and cortical granules, apoptosis, decreased membrane fusion, reduced oocyte activation, reduced blastocyst yield, lower blastocyst quality, and epigenetic alterations. Populations affected by these factors often experience compromised embryogenesis, reduced embryo quality, and an increased incidence of embryo malformations, ultimately leading to reduced pregnancy rates. To address these challenges, novel interventions are required to enhance oocyte and embryo quality.[7-9]

Studies have demonstrated Nicotinamide Mononucleotide is a potent sirtuins activator, Nicotinamide mononucleotide (NMN), a product of the nicotinamide phosphoribosyl transferase (NAMPT) reaction and a key NAD+ intermediate, can reverse defects in mitochondrial homeostasis, reactive oxygen species (ROS) production, DNA repair, as well as cell survival caused by insufficient NAD. A recent study by Miao et al. showed that the occurrence of aberrant spindle/chromosome structure was considerably higher in aged oocytes compared with controls but declined after NMN supplementation. These results suggest that NMN is able to effectively improve the maturation ability of aged oocytes by maintaining the correct spindle/chromosome structure. NMN supplementation restored the NAD+ levels in maternally aged oocytes, enhancing their maturation rate, fertilization ability, and subsequent embryonic development potential. Through single-cell transcriptome analysis, they further discovered that NMN supplementation improved the quality of aged oocytes by recovering mitochondrial function, which, in turn, reduced the accumulated ROS to suppress apoptosis during aging.[10]

L-Ergothioneine functions as a potent activator of the nuclear factor erythroid 2-related factor 2 (Nrf2), which is essential for cellular homeostasis. Through its interaction with Nrf2 and its downstream effector, Kelch-like ECH-associated protein 1 (Keap1), L-Ergothioneine regulates the expression of key genes involved in cellular processes. This includes the

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modulation of Caspase3 and BCl2, critical regulators of apoptosis, contributing to the maintenance of oocyte reserve. Furthermore, L-Ergothioneine exhibits the ability to decrease follicle death while simultaneously promoting the development of mature follicles, thereby enhancing ovarian function. By binding to the antioxidant response element (ARE) within the promoter regions of target genes, Nrf2-Keap downregulates the expression of inflammatory genes, which in turn prevents embryo dysfunction and reduces spindle formation defects by increasing Cyclin beta 1, these multifaceted actions collectively lead to improved oocyte quality, highlighting the potential therapeutic significance of L-Ergothioneine in reproductive health.[8]

Several preclinical and clinical studies have individually examined the potential benefits of NMN and L-Ergothioneine. Combining sirtuins and Nrf2 may provide a solution to this issue. However, to our knowledge, there have been no human studies combining NMN and L-Ergothioneine in combination. This study marks the first of its kind globally. A patented formulation called EQOQ® has been developed, containing 250 mg of Nicotinamide Mononucleotide (NMN) and 0.5 mg of L-Ergothioneine. This combination acts as an activator of sirtuins and Nrf2, may promising therapy. Nicotinamide mononucleotide (NMN) and L-Ergothioneine together enhance reproductive health by improving oocyte quality, maintaining oocyte reserve, and reducing chromosome errors and spindle formation errors. It promotes successful fertilization, embryo development, and implantation while decreasing DNA fragmentation and the risk of miscarriage. Additionally, it mitigates apoptosis, inflammation, and trophoblast invasion, leading to higher embryo quality and increased live birth rates with Decreased genetic defects. Overall, the synergistic action of NMN and L-Ergothioneine improves various aspects of fertility treatment outcomes.[8-10]

Thus this study aims to assess the impact of EQOQ® on oocyte and embryo quality, encompassing evaluations of morphology, maturation rate, and chromosomal abnormalities. It also involves the quantitative analysis of mature oocytes, evaluating blastocyst formation rates, and assessing embryo fragmentation in comparison to a controlled group (Standard of care). Secondary objectives involve examining, implantation, clinical pregnancy rates, live birth rates between the EQOQ® intervention and control groups, and potential adverse effects of EQOQ®.

MATERIALS AND METHODS

Study design and randomization:

The EQOQ® Study of Enhancement of Fertility Outcomes through Sirtuins & Nrf2 Pathways (FERTSIRT Trial) was structured as a 12-months randomized, double-blind, multicentre controlled clinical trial. One hundred eligible female DOR patients, aged 30 and above, clinically diagnosed with infertility, and voluntarily providing informed consent, were systematically enrolled. The inclusion and exclusion criteria were thoughtfully designed to ensure study relevance to the target population and eliminate confounding variables. The trial included eight study visits conducted at monthly intervals, patient flow throughout the study is mentioned in Figure no 1. Participants were randomly assigned in a 1:1 ratio to receive either the EQOQ® (Nicotinamide Mononucleotide 250 mg and L-Ergothioneine 0.5mg) intervention or placebo control group treatment 3 months before the IVF protocol (Intervention phase), followed by the IVF protocol in both groups (follow-up phase). Randomization occurred over a 12-month period.

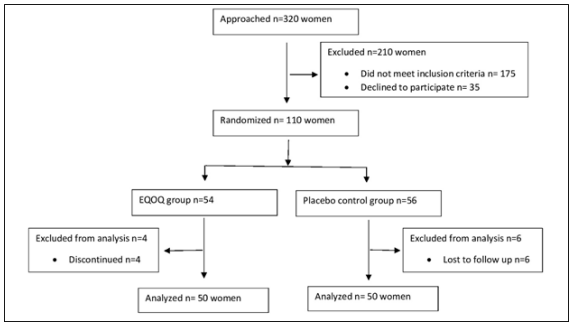

Figure 1: Patient flow throughout the study

Inclusion Criteria

Participants eligible for this study, females aged 30 years or above seeking fertility treatment, participants must meet at least one of the following criteria: a Body Mass Index (BMI) equal to or greater than 30 kg/m² indicating obesity, a confirmed diagnosis of Type-2 Diabetes as documented in medical records or by a physician, or a diagnosed thyroid disorder confirmed through laboratory tests or medical history, or hypertension characterized by a systolic blood pressure of 140 mmHg or higher and a diastolic blood pressure of 90 mmHg or higher. Furthermore, participants should have been diagnosed with Polycystic Ovary Syndrome (PCOS) at least six months before and must have completed treatment for PCOS with no active PCOS symptoms or no active medication for at least six months before enrollment. Additionally, participants must have a confirmed diagnosis of Diminished Ovarian Reserve (DOR) based on standardized criteria such as Anti-Müllerian Hormone (AMH) levels, Follicle-Stimulating Hormone (FSH) levels, and Antral Follicle Count (AFC).

Exclusion Criteria

Participants were excluded from the study age below 30 years or if they were pregnant or breastfeeding at the time of enrollment. Individuals with a prior history of genetic disorders or chromosomal abnormalities were not eligible. Additionally, participants with significant medical conditions that affected fertility or pregnancy outcomes, such as endometriosis, uterine fibroids, blocked fallopian

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tubes, uterine septum, pelvic adhesions, cervical stenosis, tubal blockage, or those who were undergoing chemotherapy or radiation therapy for malignancy, were excluded. Those with newly diagnosed or active Polycystic Ovary Syndrome (PCOS) within the last six months who were receiving treatment for PCOS were also ineligible. Furthermore, participants who were involved in concurrent clinical trials or those who were unable to comply with study requirements or provide informed consent were excluded from the study.

Treatment protocols

Participants in the study received a daily dose of either EQOQ®, containing Nicotinamide Mononucleotide (250 mg) and L-Ergothioneine (0.5 mg), or a placebo-controlled (SOC) for three months before the IVF protocol, at a dosage of one tablet twice daily. Following the three-month intervention phase, patients in both groups underwent the IVF protocol (EQOQ® continued till Fetal heart rate on Doppler ultrasound device) as part of the follow-up phase of the infertility treatment cycle. Female participants attended in-person study visits, where baseline evaluations included medical history, demographic data collection, physical examination, hormonal testing, BMI measurement, and blood pressure measurement. During assisted reproductive techniques such as IVF, assessments were conducted to evaluate oocyte morphology, maturation rate, embryo grading, blastocyst formation, and fragmentation to assess oocyte and embryo quality. Pregnancy outcomes, including clinical pregnancy rates and live birth rates, were monitored for both the intervention and control groups.

Safety assessments involved continuous monitoring and documentation of adverse events and side effects related to the EQOQ® intervention and placebo control group.

Methods to assess oocyte quality: (List of assessment during trial)

Oocyte Retrieval: Participants underwent controlled ovarian stimulation. Oocyte retrieval was performed using transvaginal ultrasound-guided follicular aspiration.

Oocyte Assessment: Oocytes were examined under a microscope for morphological characteristics. Assessments included cytoplasmic granularity, zona pellucida appearance, and the presence of polar bodies.

EQOQ® Intervention: Participants in the intervention group received the EQOQ® intervention, outlining specific details of the intervention and its duration. Placebo Control group participants received standard fertility treatment without the EQOQ® intervention.

Embryo Quality:

Fertilization and Culture:

Fertilization of retrieved oocytes was carried out using standard IVF procedures. Embryos were cultured in a controlled environment, monitoring their development.

Embryo Grading: Embryo quality was assessed based on the number of blastomeres, symmetry, and fragmentation. Grading criteria were established to categorize embryos into quality classes.

EQOQ® Intervention: The EQOQ® intervention was prolonged for the intervention group until the clinical detection of fetal heart sounds. Control group participants followed standard embryo culture and assessment procedures.

Implantation Rates and Clinical Pregnancy

Embryo Transfer: High-quality embryos were selected for transfer. Transfers were performed under ultrasound guidance.

Implantation Assessment: Ultrasound was used to confirm the presence of gestational sacs in the uterine cavity. Implantation rates were calculated based on the number of gestational sacs compared to the number of transferred embryos.

Clinical Pregnancy, Live Birth and Birth Weight: Clinical pregnancy was confirmed by the presence of a fetal heartbeat on a Doppler ultrasound device. The number of clinically pregnant participants was recorded. Follow-up continued throughout pregnancy. Delivery information, including live birth occurrence and birth weight, was recorded.

Statistical analysis:

It included descriptive statistics for baseline characteristics, comparison of primary and secondary outcomes between the EQOQ® intervention group and placebo control group using appropriate statistical tests (e.g., chi-square test), subgroup analysis based on age and other relevant factors, multivariate analysis to adjust for potential confounders, with statistical significance set at $p< 0.05$. By evaluating changes from baseline to specific time points, the study aimed to determine the improvement in oocyte and embryo quality, enhancement in implantation rates, and potential benefits of EQOQ® in enhancing fertility outcomes for females with DOR seeking fertility treatment after the age of 30 years.

RESULTS

The study comprised 100 female participants with Diminished Ovarian Reserve (DOR) actively seeking fertility treatment after surpassing the age of 30. Random allocation placed individuals into either the EQOQ® intervention group or the placebo control group.

Demographic Analysis: [Table 1] presents a comprehensive overview of demographic and clinical characteristics, facilitating a comparison between the EQOQ® Intervention Group and the Placebo Control Group. The findings reveal comparable mean ages of 34.1 and 33.15 years for both groups. However, the EQOQ® Intervention Group exhibits a marginally higher mean BMI (26.6 kg/m²) than the Placebo Control Group (23.97 kg/m²). This is substantiated by a slightly elevated

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count of individuals with obesity in the Placebo Control Group (18) versus the EQOQ® Intervention Group (14). Furthermore, the EQOQ® Intervention Group reports a higher count of individuals with Type-2 Diabetes (28) and Thyroid Disorders (25) compared to the Placebo Control Group (24 and 21, respectively). Conversely, the Placebo Control Group has a slightly higher count of individuals with Hypertension (12) than the EQOQ® Intervention Group (13). Reproductive history, as indicated by mean Gravida and Parity values, is comparable between groups. Nonetheless, the EQOQ® Intervention Group reports a slightly higher count of individuals with prior fertility treatments (17) in contrast to the Placebo Control Group (14). Lastly, the EQOQ® Intervention Group exhibits a higher mean weight (66.52 kg) and height (1.66 m) compared to the Placebo Control Group (60.08 kg and 1.65 m). These baseline characteristics are integral for discerning the composition of study groups, ensuring that observed EQOQ® effects are not confounded by demographic disparities.

Impact of EQOQ® on Oocyte Quality:

The EQOQ® intervention group demonstrated a statistically significant improvement in oocyte quality compared to the placebo control group. Notably, the EQOQ® group exhibited a higher percentage of oocytes with normal morphology (82% ± 2.1%) compared to the placebo control group (55% ± 3.4%), with a p-value of < 0.00001. Additionally, the EQOQ® group displayed a significantly higher oocyte maturation rate (79% ± 4.1%) compared to the placebo control group (37% ± 5.4%), with a p-value of < 0.0001. Moreover, the EQOQ® intervention led to a reduction in chromosomal abnormalities in oocytes, with a mean of 4% ± 2.2%, in contrast to the placebo control group's mean of 13% ± 1.8%, with a p-value of < 0.00001. These findings indicate a statistically significant enhancement in oocyte quality within the EQOQ® (intervention) group compared to the placebo group. [Figure 1]

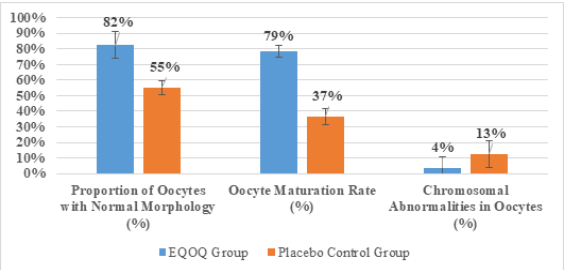

Figure 1: Comparison of Oocyte Quality Characteristics between EQOQ® and Placebo Control Groups

Impact of EQOQ® on Embryo Quality: The EQOQ® intervention group highlighted a significant enhancement in embryo quality compared to the control group. Demonstrating superior embryo grading, the EQOQ® group presented a notably higher percentage of high-quality embryos (82% ± 3.1%) compared to the placebo control group (45% ± 5.6%), with a p-value of < 0.00001. Furthermore, the EQOQ® group displayed a significantly elevated blastocyst formation rate (73.03% ± 4.7%) compared to the control group (43.91% ± 6.2%), with a p-value of < 0.00001. The EQOQ® intervention also resulted in a reduction in embryo fragmentation (4.92% ± 1.2%) compared to the control group (22.35% ± 1.8%), with a p-value of < 0.00001. The data undeniably indicates that the EQOQ® group exhibited a profound and statistically significant improvement in embryo quality compared to the placebo group. [Figure 2]

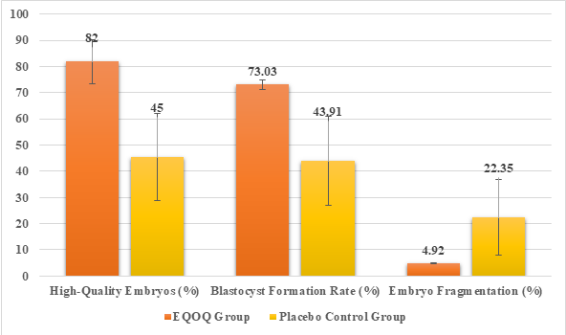

Figure 2: Comparison of Embryo Quality Characteristics between EQOQ® and Placebo Control Groups

Impact of EQOQ® on Implantation Rates:

The EQOQ® intervention group manifested a statistically significant increase in implantation rates compared to the placebo control group. Notably, the EQOQ® experimental group exhibited a significantly higher rate of successful implantations resulting in clinical pregnancy (92% ± 1.6%) compared to the placebo control group (48% ± 2.3%), with a p-value of < 0.00001. (Figure 3)

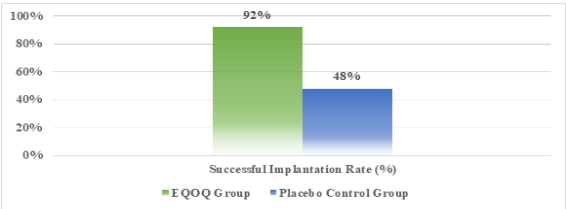

Figure 3: Comparison of Implantation Rate (%) Between EQOQ® and Placebo Control Groups

Impact of EQOQ® on Pregnancy and Live Birth:

The EQOQ® intervention group demonstrated a higher quantity of fully developed oocytes, an increased rate of clinical pregnancy, and superior progression of embryo development compared to the control group. Furthermore, the EQOQ® group exhibited significantly elevated clinical pregnancy rates (66% ± 2.4%) and live birth rates (52% ± 3.8%) compared to the control group (32% ± 3.1% and 16% ± 3.7%, respectively); p < 0.00001. EQOQ® statistically displayed superior reproductive outcomes across various key parameters compared to the control group. EQOQ® was also associated with a higher yield of fully developed oocytes retrieved,

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an increased rate of clinical pregnancy, and more advanced embryo development. [Figure 4]

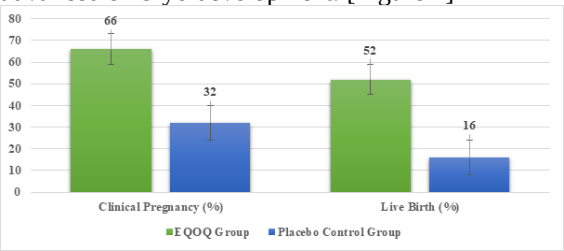

Figure 4: Comparison of Pregnancy and Live Birth Rates between EQOQ® and Placebo Control Groups

Overall, the EQOQ® intervention demonstrated notable efficacy in augmenting the quality of oocytes and embryos, leading to heightened rates of successful implantation and enhanced fertility outcomes in women experiencing diminished ovarian reserve (DOR) seeking fertility treatment post the age of 30. The outcomes of this study suggest that EQOQ® holds substantial promise as a prospective therapeutic intervention for mitigating infertility challenges within this specific demographic.

Table 1: Demographic distribution of the participants of EQOQ® and control group.

List	Variable	EQOQ® Intervention Group	Control Group
Age	Mean	34.1	33.15
	Standard Deviation	2.66	3.07
	Minimum	30	30
	Maximum	39	41
Gender	Count of Female	50	50
BMI (kg/m²)	Mean	26.6	23.97
	Standard Deviation	2.61	1.14
	Minimum	22.5	21
	Maximum	29.5	26.8
Obesity	Count of Yes	14	18
Type-2 Diabetes	Count of Yes	28	24
Thyroid Disorder	Count of Yes	25	21
Hypertension	Count of Yes	13	12
Gravida	Mean	1.7	1.4
	Standard Deviation	0.88	0.76
	Minimum	0	0
	Maximum	3	3
Parity	Mean	0.7	0.9
	Standard Deviation	0.78	0.94
	Minimum	0	0
	Maximum	2	2
Previous Fertility Treatments	Count of Yes	17	14
Weight (kg)	Mean	66.52	60.08
	Standard Deviation	6.15	3.35
	Minimum	57.2	54.5
	Maximum	80.7	69.3
Height (m)	Mean	1.66	1.65
	Standard Deviation	0.05	0.04
	Minimum	1.58	1.58
	Maximum	1.75	1.71
AMH (ng/mL)	Mean	0.598	0.604
	Standard Deviation	0.22	0.20
	Minimum	0.3	0.3
	Maximum	0.9	0.9
FSH (mIU/mL)	Mean	13	13
	Standard Deviation	1.43	1.42
	Minimum	11	11
	Maximum	15	15

Table 2: Recorded Adverse Events in EQOQ® and Placebo control Groups

Adverse Event	EQOQ® Group	Placebo Control Group	Severity
Nausea	3	6	Mild
Vomiting	1	8	Mild
Diarrhea	2	4	Mild
Headache	2	5	Mild
Exhaustion	1	4	Mild
Anxiety	3	11	Mild
Depression	0	4	Mild

Adverse Events: Potential risks associated with the EQOQ® and Placebo Control Group intervention were assessed in depth in the study, with an eye on objectivity and precision. Participants who received a placebo control group as part of the intervention reported a higher frequency of adverse events, including as gastrointestinal distress (nausea, vomiting, and diarrhea), headache intensity, exhaustion levels and mood changes (anxiety, depression). In contrast, the EQOQ® intervention

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demonstrated a favorable safety profile, as evidenced by the low frequency and mild severity of adverse events compared to the Placebo Control Group.

Adverse events reported in the placebo control group can be attributed to psychological factors, treatment expectations, physiological responses to standard treatment, hormonal fluctuations, and potential baseline health disparities. The emotional burden of fertility concerns and the absence of a specific intervention may contribute to increased stress and adverse events in the control group, emphasizing the potential benefits of interventions like EQOQ® in improving both reproductive outcomes and overall patient experience [Table 2].

DISCUSSION

The study aimed to assess the efficacy of EQOQ® in enhancing fertility outcomes among females diagnosed with Diminished Ovarian Reserve (DOR) undergoing fertility treatment beyond the age of 30 years. The results demonstrated a statistically significant and favorable impact of EQOQ® on both oocyte and embryo quality, leading to increased rates of successful implantation and improved outcomes in terms of clinical pregnancy and live birth. These findings are consistent with previous scientific literature indicating that advancing maternal age is linked to a decline in oocyte quality, resulting in compromised fertility outcomes.[1-5] However, the EQOQ® intervention seemed to mitigate the detrimental of ageing on oocyte quality.

The study's findings reveal that the EQOQ® intervention group displayed a higher percentage of oocytes with normal morphology, an increased rate of oocyte maturation, and a reduction in chromosomal abnormalities.[11] Compared to the control group receiving a placebo. This observation suggests that EQOQ® may positively impact the structural characteristics, developmental stage, and genetic stability of oocytes, ultimately enhancing overall oocyte quality.[12] Furthermore, the favorable effects of EQOQ® extended beyond oocyte quality to encompass the progression of embryo development. In the EQOQ® study's intervention group, there was a greater percentage of high-quality embryos, an increased rate of blastocyst formation, and reduced embryo fragmentation compared to the control group. The study results imply that EQOQ® plays a significant role in improving embryo quality, a crucial factor for the successful establishment of pregnancy through implantation.

Moreover, the significant enhancement in implantation rates observed in the EQOQ® intervention group constitutes evidence supporting the positive influence of EQOQ® on the likelihood of successful conception. The observed rise in the number of successful implantations leading to clinical pregnancy within the EQOQ® group is a promising revelation, indicating that EQOQ® holds the potential to improve fertility outcomes in females diagnosed with diminished ovarian reserve (DOR) who are undergoing fertility treatment after the age of 30 years. Secondary endpoints, including the quantification of fully developed oocytes obtained the rate of successful fertilization, the rates of clinical pregnancy, and the rates of live births, offer additional substantiation for the efficacy of EQOQ® in enhancing fertility outcomes within this specific demographic.

The noted reduction in adverse events linked to the EQOQ® intervention highlights the safety profile of this innovative method, a crucial consideration in the domain of fertility treatments. The research outcomes align with and contribute to the existing body of literature examining the impact of aging on female reproductive capacity and potential interventions to improve fertility outcomes.[13,14]

Nevertheless, it is imperative to recognize that while the study provides valuable insights into the potential benefits of EQOQ®, it carries certain limitations. The sample size employed in the study might have been insufficient to detect more subtle effects, and the study duration may not have adequately captured the long-term consequences of the EQOQ® intervention. Additionally, the research was confined to female individuals displaying specific risk factors, such as obesity,[15] type-2 diabetes,[16] thyroid disorder,[17] or hypertension.[18] These factors could have influenced the generalizability of the findings to broader populations.

The potential advantages of integrating the EQOQ® intervention into fertility treatment are multifaceted. Firstly, it holds the promise to improve the quality of both oocytes and embryos, thereby increasing the likelihood of successful implantation and achieving favorable outcomes in terms of clinical pregnancy and live birth rates. This improvement encompasses the structural, developmental, and genetic characteristics of oocytes, enhancing their overall quality. Furthermore, EQOQ® positively influences the quality of embryos, a pivotal factor for the successful initiation of pregnancy through implantation. This results in a higher number of successful implantations, particularly beneficial for females with diminished ovarian reserve (DOR) undergoing fertility treatment after the age of 30. Importantly, the EQOQ® intervention appears to be associated with a reduction in adverse events, underscoring its favorable safety profile.

Nonetheless, it is crucial to recognize certain limitations in this study. These limitations encompass the possibility of sample size constraints that might impede the detection of subtle effects, the potential for the study's duration to inadequately capture long-term consequences, and the restriction to females with specific risk factors, which could influence the generalizability of the findings to broader populations.

In summary, the research contributes to the existing body of knowledge concerning interventions aimed at improving fertility outcomes in females diagnosed with Diminished Ovarian Reserve (DOR) beyond the

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age of 30 years. The administration of EQOQ® has shown promising effectiveness in enhancing the quality of oocytes and embryos, leading to increased rates of successful implantation and a higher likelihood of achieving pregnancy. The findings from this investigation warrant further exploration of EQOQ® as a potential therapeutic intervention for addressing age-related fertility challenges and have the potential to pave the way for more individualized approaches.

CONCLUSION

The study establishes that EQOQ® (Nicotinamide Mononucleotide 250 mg and L-Ergothioneine 0.5mg) treatment along with standard treatment can enhance fertility outcomes in females diagnosed with Diminished Ovarian Reserve (DOR) undergoing fertility treatment after the age of 30. The EQOQ® intervention demonstrates significant improvements in oocyte and embryo quality, leading to improved rates of implantation, clinical pregnancy, and live births. Previous Scientific studies indicate that treatment with Nicotinamide Mononucleotide and L-Ergothioneine activates the Sirtuins Nrf2 pathways thereby enhancing oocyte, embryo quality increase oocytes activation, sperm binding, improved embryo grade, less DNA fragmentation hence improve embryo quality and decrease genetic defects. Importantly, it is notable that this study represents the first trial conducted in India. However, further research is essential to validate these findings in larger trials and explore the enduring impacts of EQOQ® intervention. Additionally, investigating the molecular mechanisms underlying the positive effects of EQOQ® on oocyte and embryo quality would provide valuable insights into its mode of action. The results of this study carry significant clinical implications, offering a hopeful perspective for couples facing fertility challenges and offering hope to couples struggling for successful pregnancies despite encountering fertility challenges.

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