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Physiology

CO-Enzyme Q10 Modulates Semen Parameters in Diabetic Male Rats on Combined Anti-Retroviral Therapy

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Abstract **Original Research Article**

Infertility affects roughly one in six couples worldwide, with male reproductive health increasingly compromised by chronic illnesses, environmental stressors, and pharmacological interventions. Human immunodeficiency virus (HIV) and its treatment with combination antiretroviral therapy (cART) are known to negatively impact male fertility, while oxidative stress plays a central role in this decline. Coenzyme Q10 (CoQ10), a mitochondrial cofactor with antioxidant properties, has been explored as a therapeutic option, though findings remain inconsistent. This study examined the impact of CoQ10 supplementation on sperm viability and morphology in male Wistar rats exposed to cART and diabetic conditions. A total of 102 adult rats were divided into control and treatment groups, receiving either cART alone, CoQ10 at varying doses (10, 30, or 50 mg/kg), or a combination of both. A second study phase included diabetic rats treated similarly. Semen samples were assessed for viability and normal morphology. Results showed that cART and diabetes each significantly reduced sperm health. However, CoQ10—especially at the low dose (10 mg/kg)—markedly improved both viability and morphology, even in the presence of cART or diabetes. High doses of CoQ10 (50 mg/kg), however, offered limited benefit and, in some cases, worsened outcomes. These findings suggest that low-dose CoQ10 supplementation could protect against reproductive toxicity induced by cART and diabetic stress. CoQ10 appears most effective at lower concentrations, supporting its potential use as an adjunct therapy to preserve male fertility in patients undergoing antiretroviral treatment.

Keywords: Coenzyme Q10, cART, male fertility, sperm morphology, HIV, oxidative stress, diabetes, antioxidant therapy.

INTRODUCTION

Human fertility is declining due to various endogenous and exogenous factors, including environmental contaminants, diet, and lifestyle. Globally, one in six couples experiences infertility, with male factors contributing to about half of cases (Eisenberg *et al.*, 2023). Male infertility can arise from pre-testicular, testicular, and post-testicular causes. Pre-testicular factors include hypogonadotropic hypogonadism, substance abuse, and chronic alcoholism (Scroppo *et al.*, 2023). Testicular causes range from congenital anomalies (e.g., Klinefelter's syndrome) to trauma, tumors, or varicocele. Post-testicular factors include vas deferens obstruction, ejaculatory duct issues, and erectile dysfunction (Abdalla *et al*., 2023).

Human immunodeficiency virus (HIV) weakens the immune system, progressing to acquired immune deficiency syndrome (AIDS) if untreated, the resulting immune suppression increases vulnerability to opportunistic infections and comorbidities, worsening health outcomes (Prabhu & van Wagoner *et al.*, 2023). AIDS has claimed over 36.3 million lives, with Sub-Saharan Africa being the most affected region (Haacker, M. 2024).

The WHO recommends immediate treatment with a combination of three or more antiretroviral drugs to suppress viral replication and boost immunity, measured by CD4 cell count (Jain *et al.*, 2024). While combination ART (cART) doesn't cure HIV, it significantly improves health outcomes and life expectancy and has transformed HIV into a manageable chronic condition (Nuwagaba *et al.*, 2024).

Combination antiretroviral therapy (cART) has not only improved life expectancy for people living with

HIV, it has enabled new family planning options for couples. However, HIV-infected couples face challenges like hypogonadotropic hypogonadism, reduced libido, and lower pregnancy rates (Patra *et al.,* 2023). Globally, reproductive organ toxicity linked to highly active antiretroviral therapy (HAART) is rising, with testicular damage being a major concern (Akhigbe *et al.,* 2024). Germ and somatic cells show varying sensitivity to HAART, leading to testicular atrophy, germ cell loss, multinucleated gonocytes, Sertoli cell vacuolization, and Leydig cell apoptosis or hyperplasia — all contributing to infertility (Ogunlade *et al.,* 2022).

While clinical attention to HAART's effects on male fertility is limited, research has shown altered sperm quality in men undergoing HAART treatment. It remains uncertain whether HIV itself or cART contributes more to male infertility, as studies show conflicting results (Akhigbe *et al.,* 2024). Given the importance of semen quality for reproductive success, it's essential to investigate cART's impact on spermatogenesis.

With rising rates of diabetes and obesity, infertility has become a growing global concern, affecting up to 15% of couples trying to conceive. While studies link obesity and diabetes to reproductive dysfunction and reduced semen quality, the underlying pathways remain unclear (Lotti *et al.,* 2023). Several mechanisms have been proposed, including impaired Hypothalamus-Pituitary-Gonadal axis function, increased reactive oxygen species (ROS), sperm DNA fragmentation, and biochemical imbalances (He *et al.,* 2021). Evidence suggests that hormonal changes, antioxidant depletion, and inflammatory markers contribute to subfertility in obese and diabetic men (Madhu *et al.,* 2022). This study aims to explore the individual and combined effects of obesity and diabetes on male reproductive function.

Coenzyme Q10 (CoQ10) is a vital cofactor in mitochondrial oxidative phosphorylation, driving ATP production and plays an important role in energy metabolism in tissues with high energy demands. In its reduced form, ubiquinol, acts as an antioxidant and helps regenerate other antioxidants in the body (Mantle *et al.,*2024). Beyond energy production, CoQ10 influences gene expression, membrane stability, and cellular signaling. Naturally present in mitochondria, it protects cells from damage, abnormal growth, and apoptosis through its antioxidant, energy-boosting, and anti-inflammatory properties (Nie *et al.,* 2023).

Coenzyme Q10 has shown potential in treating male infertility, though published data remain inconsistent (Alahmar *et al.,* 2022). This study aims to evaluate the efficacy of CoQ10 in improving key semen parameters, including sperm count, motility, forward motility, and morphology.

MATERIALS AND METHODS

Drugs and Chemicals

Coenzyme Q10 (100 mg) and Combination Anti-retroviral Therapy (Dolutegravir/Lamivudine/Tenofovir disoproxil 50 mg /300 mg /300 mg) were the drugs administered to the experimental animals.

Chemicals used in the study include biochemical reagent kits (Fortress), Diethyl ether (Sigma), Ethanol 95% (Sigma), Formalin 10% and Chloroform (BDH Chemicals Ltd).

Animals

One hundred and two adults male Wistar rats weighing 170-200g were obtained from the Animal House of the Department of Experimental Pharmacology and Toxicology, University of Port Harcourt. The animals were allowed three weeks of acclimatization to laboratory conditions and handling. The animals were housed in cages with plastic bottom and wire-mesh top and fed with normal rat chow. They were supplied with tap water ad libitum throughout the experimental period. The Animal House was adequately ventilated under standard conditions (ambient room temperature, 28 ± 2ºC with a natural lighting condition). The animals were fed with Finisher feeds (Top Feed Ltd).

Ethical consent was sought and obtained from the University of Port Harcourt Research Ethics Committee.

Study Design

The research was conducted in two phases. The first phase (Study I) investigated the effects of Coenzyme Q10 (CoQ10) supplementation, both alone and in combination with combined antiretroviral therapy (cART). The experimental design included the following groups:

Normal Control Group (NC): Rats received standard rat chow with no treatment.

cART Group (CT): Rats were administered 70 mg/kg body weight of cART daily.

CoQ10 Supplementation Groups: Rats received CoQ10 at low (10 mg/kg body weight), medium (30 mg/kg body weight), or high doses (50 mg/kg body weight) daily.

Test Groups: Rats received a combination of cART and CoQ10 at the following doses:

cART + CoQ10 Low Dose (10 mg/kg)

cART + CoQ10 Medium Dose (30 mg/kg)

cART + CoQ10 High Dose (50 mg/kg)

The second phase (Study II) examined the combined effects of CoQ10 supplementation and cART in diabetic rats. The experimental groups included:

Normal Control Group (NC): Rats received standard rat chow with no treatment.

cART Group (CT): Rats were administered 70 mg/kg body weight of cART daily.

Diabetes Mellitus Group (DM): Diabetes was induced in rats via intraperitoneal administration of alloxan at 50 mg/kg body weight and sustained throughout the study.

Test Groups: Diabetic rats received a combination of cART and CoQ10 at varying doses:
cART + CoQ10 Low Dose + DM (10 mg/kg)
cART + CoQ10 Medium Dose + DM (30 mg/kg)
cART + CoQ10 High Dose + DM (50 mg/kg)

Induction of Diabetes

Diabetes was induced experimentally by a single dose intraperitoneal administration of 50 mg/kg body weight of a freshly prepared alloxan monohydrate.2 Forty-eight hours later, Rat with blood glucose levels above 200 mg/dL were considered diabetic and used in this study. Prior to initiation of this experiment, the animals were fasted for 8 to 12 hours but allowed free access to water until the end of this experiment.

Sperm collection and Analysis

The epididymis was lacerated to extract the semen and the semen was emulsified with 0.5% eosin. The sample was examined under a microscope using 10x and 40x objective lenses. Ten to twelve fields were analyzed to assess viable cells, determined by the percentage of unstained cells compared to stained ones. Additionally, ten to twelve fields were examined to evaluate the percentage of normal cells compared to abnormal ones, as well as the proportions of actively motile, sluggish, and dead cells. The sperm count was performed using a counting chamber. The semen was diluted at a 1:20 ratio with formal saline. The chamber was assembled and filled with the diluted sample. The sperm cells were counted in four sets of 16 squares, and the total count was multiplied by 100,000 to calculate the sperm count.

Sperm Quality Analysis

After euthanizing the animals, a 2 mm section of the right epididymal tail was carefully excised. The tissue was incubated in 5 mL of TALP (Tyrode's - Albumin - Lactate - Pyruvate) solution at 37°C for 10 minutes to allow the sperm to disperse (Paui *et al.*, 2024).

Sperm Motility (Percentage of Motile Sperm)

To assess sperm motility, 5 µL of the supernatant containing sperm cells was placed between a slide and cover slip. The sample was observed under a phase-contrast microscope (CX31, Olympus Optical, São Paulo, Brazil) at 100x magnification. Sperm movement was evaluated in three different fields, and the percentage of motile sperm was calculated as the average across these fields (Hassan *et al.*, 2021).

Sperm Count (Million Sperm/mL)

For sperm concentration analysis, 10 µL of the sperm suspension was diluted in 990 µL of a paraformaldehyde and sodium citrate solution. A 10 µL aliquot of the diluted sample was loaded onto a Neubauer hemocytometer and observed under a light microscope at 400x magnification. Sperm cells were counted, and the concentration was calculated based on the chamber's dimensions, expressed as millions of sperm per milliliter (O'Brien *et al.*, 2022).

Sperm Morphology (Percentage of Normal Cells)

To evaluate sperm morphology, 20 µL of the sperm suspension was smeared onto a slide, air-dried, and stained with eosin-nigrosin (1% eosin Y and 5% nigrosin). After drying, the slides were examined under a light microscope (CX31, Olympus Optical, São Paulo, Brazil) at 400x magnification. A total of 200 sperm cells were assessed per slide, with morphological abnormalities recorded for the head, midpiece, and tail. The percentage of normal cells was calculated based on the total sperm counted (Gacem *et al.*, 2021).

Data presentation And Analysis

A coding guide was developed along with the data collection tool in order to facilitate its analysis. Using the coding guide, the data collected was carefully analyzed using one-way analysis of variance (ANOVA) using SPSS software. Data was presented as mean ± SEM. Treated groups were compared with control group using Turkey test and differences between groups were considered significant at $p < 0.05$.

RESULTS

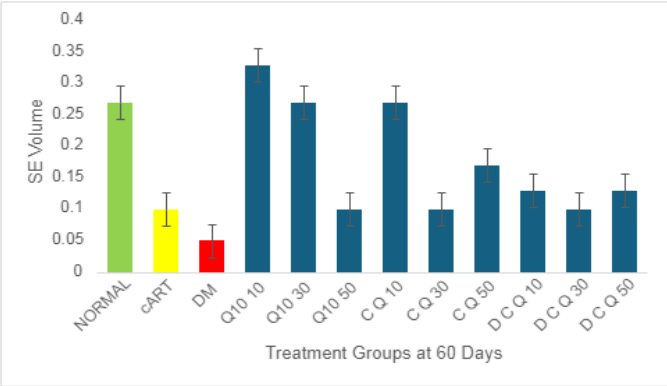

Fig 1: Changes Associated with Semen Volume After Q10 Supplementation

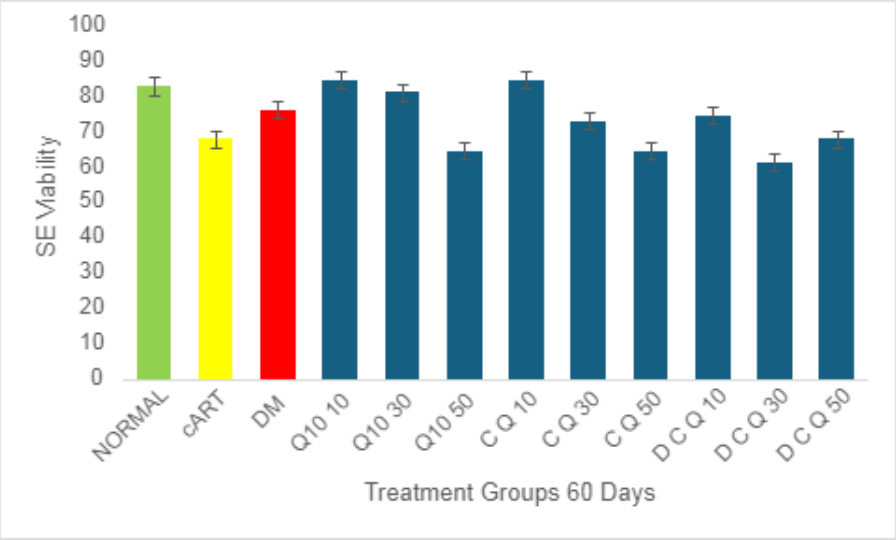

Fig 2: Changes Associated with Semen Viability After Q10 Supplementation

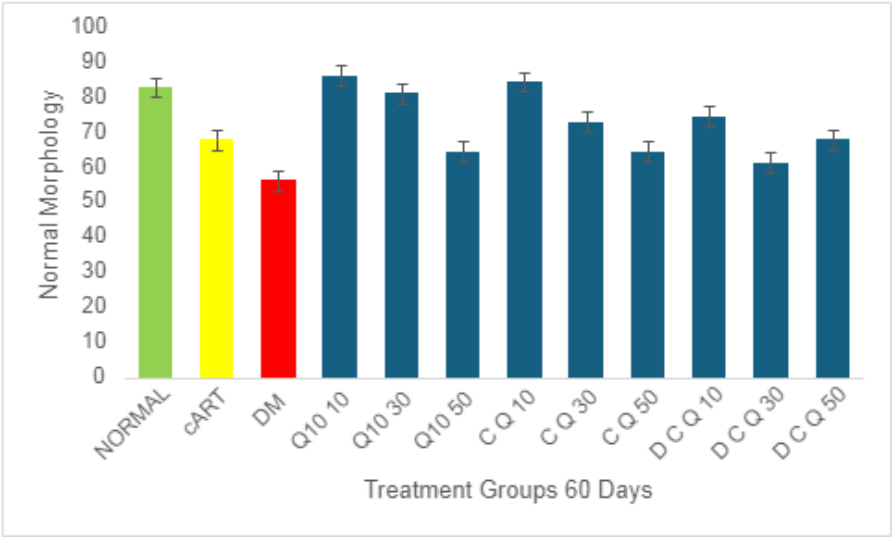

Fig 3: Changes Associated with Normal morphology After Q10 Supplementation

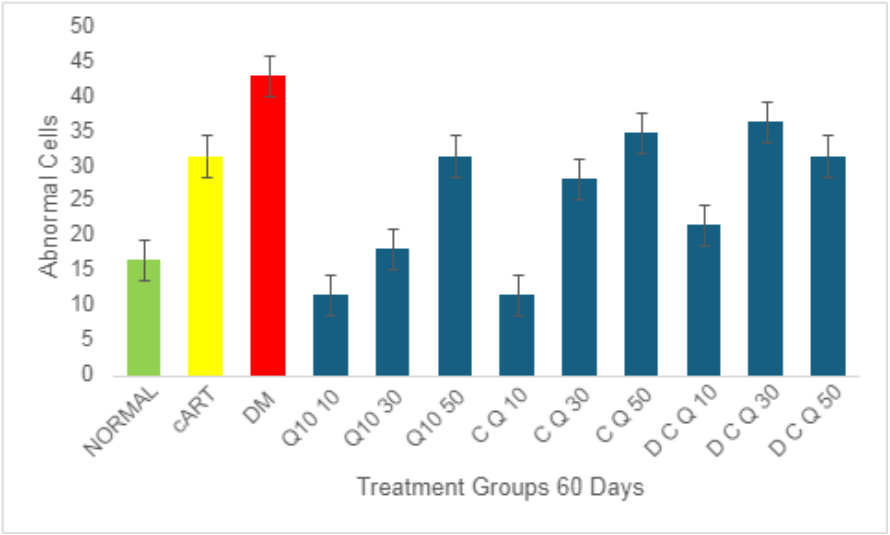

Fig 4: Changes Associated with Abnormal Cells After Q10 Supplementation

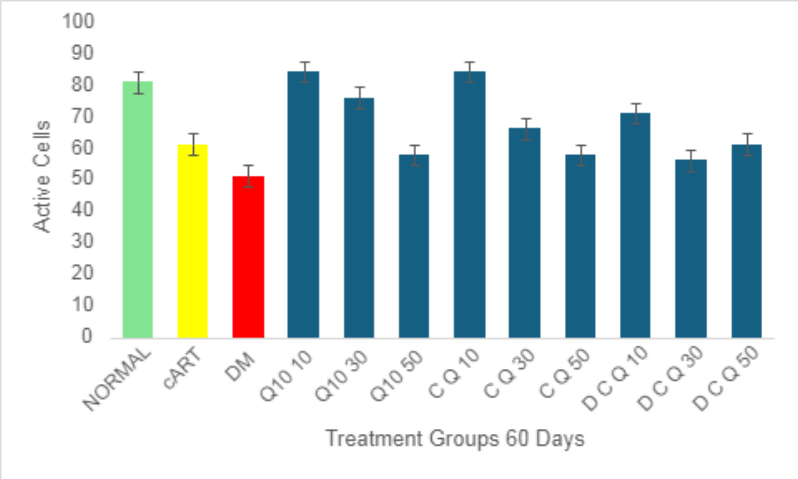

Fig 5: Changes Associated with Active Cells After Q10 Supplementation

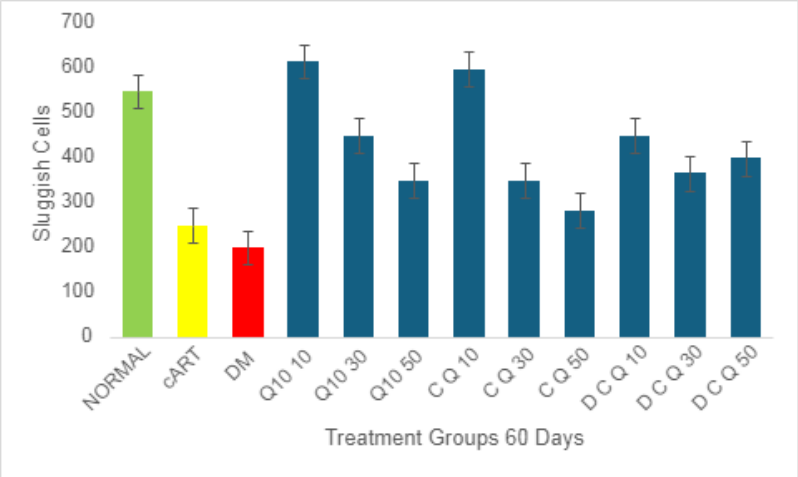

Fig 6: Changes Associated with Sluggish Cells After Q10 Supplementation

Semen Associated Changes: Viability and Normal Morphology Assessment

The evaluation of cell viability and normal morphology percentages across groups highlighted distinct patterns in response to cART, DM, and Q10 interventions. The normal group displayed both high viability and normal morphology at 83.33%. In contrast, the cART group demonstrated reductions to 68.33% for both metrics, indicative of compromised cell integrity under antiretroviral therapy. The DM group showed relatively preserved viability at 76.67%, but normal morphology was notably reduced to 56.67%, reflecting specific structural impairments under diabetic conditions.

Q10 supplementation improved outcomes significantly, particularly in the Q10 10 group, which recorded the highest values for viability (85.00%) and normal morphology (86.67%). Q10 30 showed slightly reduced, but still robust, viability and morphology at 81.67%, while Q10 50 resulted in decreased values of 65.00% for both metrics, suggesting a dose-dependent effect. Combination therapy with cART yielded similar trends; C Q 10 achieved high viability and morphology rates of 85.00%, while C Q 30 and C Q 50 reported declines to 73.33% and 65.00%, respectively. Diabetic combination groups exhibited moderate improvements, with D C Q 10 achieving 75.00% viability and morphology, while D C Q 30 and D C Q 50 had further reductions to 61.67% and 68.33%, respectively. These results suggest that lower doses of Q10, particularly in Q10 10 and C Q 10, are effective in restoring both viability and morphological normalcy across experimental conditions.

Semen Associated Changes: Abnormalities and Motility Patterns

The study analyzed abnormalities and motility percentages (active and sluggish) across various experimental groups, highlighting the impact of cART, DM, and Q10 interventions. The normal group exhibited low abnormalities (16.67%) with high active motility (81.67%) and sluggish motility at 550.00. The cART group displayed increased abnormalities (31.67%) and reductions in both active motility (61.67%) and sluggish motility (250.00). The DM group had the highest abnormalities (43.33%) and experienced a marked decline in active motility (51.67%) and sluggish motility (200.00), reflecting significant motility impairment.

Q10 supplementation improved outcomes substantially, especially in the Q10 10 group, which recorded the lowest abnormalities (11.67%), highest active motility (85.00%), and elevated sluggish motility (616.67). Q10 30 showed moderate improvements, with abnormalities at 18.33%, active motility at 76.67%, and sluggish motility at 450.00. However, Q10 50 demonstrated diminished effects, with abnormalities increasing to 31.67% and active motility decreasing to 58.33%. Combination therapy with cART showed a similar trend, with C Q 10 yielding the most favorable outcomes (11.67% abnormalities, 85.00% active motility, and 600.00 sluggish motility). C Q 30 and C Q 50 reported increased abnormalities (28.33% and 35.00%, respectively) and decreased active motility (66.67% and 58.33%). Diabetic combinations exhibited mixed effects, with D C Q 10 performing best (21.67% abnormalities, 71.67% active motility, and 450.00 sluggish motility), while D C Q 30 and D C Q 50 experienced higher abnormalities (36.67% and 31.67%, respectively) and reduced active motility. These findings highlight Q10 10 and C Q 10 as optimal doses for preserving motility and minimizing abnormalities under various stressors.

SEMEN

Physiological and environmental factors affect male reproductive health, these factors include metabolic disorders, medication use, and oxidative stress (Rotimi, *et al.*, 2024: Bhattacharya *et al.,* 2024:). Key parameters such as semen volume, pH, sperm viability, morphology, and motility are good indicators of potent men. The present study investigated the effects of diabetes mellitus (D) and combination antiretroviral therapy (cART) on these reproductive markers, it also tries to investigate the potential benefits of coenzyme Q10 (Q10) as a protective agent. The findings revealed significant abnormalities in sperm quality in DM and cART groups, including reduced semen volume, lower sperm viability, and increased abnormal morphology. Q10 supplementation, particularly at lower doses, appeared to enhance sperm function, suggesting potential mitigation oxidative stress and improving overall reproductive health.

The results from the DM and cART groups showed impairments in semen quality. The semen volume was significantly reduced in DM and cART subjects when compared to normal group. the reduction may be linked to metabolic dysregulation and oxidative stress, both have been implicated in testicular dysfunction and impaired seminal fluid production. In addition, the pH of semen in the DM group was significantly lower when compared to other groups, suggesting possible acidosis, which can negatively impact sperm motility and viability, these results are comparable to results obtained from Huang, *et al*., (2024).

The DM and cART groups also showed abnormality in sperm viability and morphology. The DM group showed a sharp decline in normal morphology, while cART also showed a decline in normal sperm forms. These findings suggest that both conditions contribute to structural sperm defects, maybe due to increased oxidative damage and disrupted spermatogenesis, these results are supported by Longo *et al.,* (2024) and Owembabazi *et al.,* (2024).

The study showed that Q10 supplementation had promising protective effects, with lower doses (Q10 10, Q10 30, and C Q 10) demonstrating the most significant improvements in sperm parameters. The Q10 10 and C Q 10 groups exhibited semen volumes similar to the normal group, suggesting that Q10 may may have the potential to enhance seminal fluid production, Elsayed *et al., (*2024) observed similar trends. Furthermore, sperm viability and normal morphology were highest in these groups, indicating reduced oxidative damage and improved testicular function, results are comparable with Nazari *et al., (*2024).

Motility parameters also showed considerable improvement with Q10 treatment. The Q10 10 and C Q 10 groups exhibited the highest percentages of actively motile sperm and the lowest percentages of sluggishly motile sperm, suggesting enhanced mitochondrial function and energy production in sperm cells, Aliabadi *et al.,* (2024) published similar findings. Interestingly, higher doses of Q10 (Q10 50 and C Q 50) did not provide the same level of benefit, with sperm viability and motility parameters declining, possibly indicating a threshold beyond which excessive supplementation may not be advantageous.

Potential Mechanism

The negative effects recorded in DM and cART groups can be attributed to oxidative stress and metabolic dysfunction. Diabetes induces excessive reactive oxygen species (ROS) production which leads to lipid peroxidation, DNA damage, and impaired sperm function (Venditti *et al.,* 2024). Also, chronic hyperglycemia disrupts hormonal balance, reducing testosterone levels and impairing spermatogenesis (Minas, *et al.,* 2024). Similarly, cART drugs have been associated with mitochondrial toxicity, oxidative stress, and testicular dysfunction, which may explain the observed declines in sperm quality Baysal *et al.,* (2024).

CONCLUSION

Q10's protective effects may likely be explained by its powerful antioxidant and mitochondrial cofactor capacity. By neutralizing ROS and enhancing cellular energy production, Q10 may have improved sperm viability, morphology, and motility (Ahmed *et al.,* 2024). Its potential to stabilize mitochondrial membranes and reduce lipid peroxidation (Rizzardi *et al.,* 2024) may explain the improvements observed in actively motile sperm in the Q10 10 and C Q 10 groups. Q10 has been shown to support testosterone biosynthesis, this

may also contribute to enhanced spermatogenesis and overall semen quality (Ghewade, *et al.,* 2024).

Expand clinical trials to investigate Q10's long-term effects in managing DM- and cART-related complications across different patient populations.

Examine dose optimization for Q10 supplementation to determine the most effective concentration for various physiological functions.

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