

## Review

# Fortification methods of coenzyme Q10 in yogurt and its health functionality—a review

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## 1. Abstract

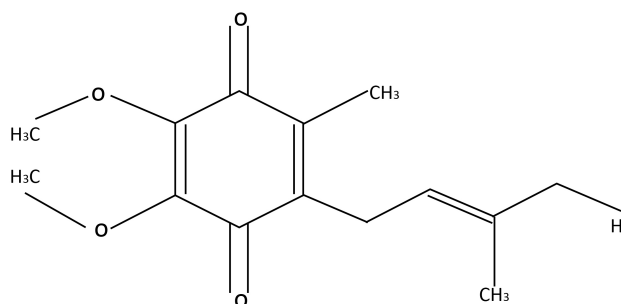
Coenzyme Q10 (CoQ10) is an antioxidant, fat-soluble component present in the mitochondrial cells. It provides beneficial results in the treatment of male infertility. In the current scenario, the sedative lifestyle, diet and stress in human lead to excessive free radicals (ROS), leading to health ailments. The review is conducted to compare the effect of different fortification methods of CoQ10 in the Yogurt. The study showed that nanoparticles form of CoQ10 in yogurt showed higher bioaccessibility rates in humans, and the microencapsulation of CoQ10 showed a low amount of Ubiquinone released during its shelf life. The functional Yogurt produced by the *Monascus*-fermented soybean powder (MFSP) co-fermentation has been shown to have high free radicals scavenging activity. Thus, the review observes that each fortified sample is useful in its way

as CoQ10 supplements. Further studies must be done for accurate conclusions on its effect on male infertility, and other fortification media can be explored.

## 2. Introduction

Coenzyme Q10 (CoQ10) belongs to non-enzymatic lipophilic antioxidants, which play a rudimentary role in mitochondrial bioenergetics. The chemical designation of CoQ10 is given as 2, 3-dimethoxy-5-methyl-6-decaprenyl-1, 4-benzoquinone [1]. The structure comprises a substituted benzoquinone moiety to which ten isoprenoid units are attached. The CoQ10 is fat-soluble vitamin-like; present in nearly all the tissues (heart, kidney, and liver) and a molecule redox in nature which biochemically co-occurs in a reduced form (Ubiquinol-10) and an

oxidized form (Ubiquinone-10) predominantly in the lungs and brain. The CoQ10 structure is presented in Fig. 1 (Ref. [2, 3]).



**Fig. 1.** CoQ10 (Ubiquinone 10) is a 1,4-benzoquinone also known as ubiquinone or coenzyme Q [2, 3].

CoQ10 is essential for converting energy from carbohydrates and fatty acids to (ATP) in mitochondrial energy metabolism, responsible for a multitude of physiological functions. The main role of CoQ10 in mitochondria is to regulate the electron transport in the respiratory chain.

It transfers electrons between the complexes I and II followed by II and III. It also transfers protons necessary for ATP synthesis from fatty acids to the inner mitochondrial membrane in the Q cycle [4]. In the tissues, protection to DNA damage, inhibition of lipid peroxidation, and protein oxidation are provided by the reduced form of CoQ10, as it is a powerful antioxidant in its reduced form. It also functions in close liaisons with other antioxidants [5].

## 2.1 Health benefits of coenzyme Q10

### 2.1.1 Aging

Aging is an oxidative stress mechanism that increases the reactive oxygen species and lowers the human body's metabolic functioning. The aging process also is found to reduce the CoQ10 naturally present in our body due to oxidative stress [6]. This further leads to tissue fibrosis, which affects all the major organs' functioning and the person bears a high risk of cardiovascular-related mortality [7]. A clinical study of 4-year period concluded that a combination of selenium and CoQ10 enhanced the overall physical activity and also reduced the chances of acquiring diseases caused due to chronic oxidative stress [8]. The study on healthy humans who consumed CoQ10 supplemented diet inhibited aging-related diseases and improved cellular bioenergetics [9].

### 2.1.2 Cardiovascular disease

The primary cause of cardiovascular diseases is the increase of oxidative stress in the body leading to metabolic dysfunction. One of the main reasons for heart failure is the loss of Contractile function. This occurs due to the energy loss in the mitochondrial cells which is caused

by low levels of CoQ10 in the body. Study on supplementation of CoQ10 showed improved health status even in cardiomyopathy patients with high severity rate [10]. Supplementing CoQ10 in the diet also showed no adverse hemodynamic profile [11]. The statistical study on dietary CoQ10 supplementation's effect reported the statistical data of an increase in the stroke volume and cardiac output for heart failure patients. The study also stated that the consumption of CoQ10 is useful for managing the health of heart failure patients [12]. The systolic function of the heart improved with CoQ10 consumption. It was also found to reduce the thickening of arterial walls and decreased the occurrence of Ventricular tachycardia [13]. Consumption of CoQ10 in the diet by young patients suffering from idiopathic dilated cardiomyopathy showed positive diastolic function changes and improved cardiac output [14].

### 2.1.3 Diabetes mellitus

Type II diabetes has become a concern worldwide, and supplementation of CoQ10 acts as an antioxidant aid to reduce the secondary ailments of diabetes. The dietary CoQ10 scavenges the ROS species, preserves the pancreatic cells' mitochondrial function, and maintains the body's glycemic index [15]. Consumption of Ubiquinone lowers the excess renal hydrogen peroxide produced in the mitochondria caused due to type II diabetes. Thus, it was found that CoQ10 supplementation is renal protective and inhibits any effects due to the mitochondrial changes in type II diabetic patients [16]. The antioxidant enzymes catalase and glutathione peroxidase were found to increase statistically in type II diabetic patients who consumed CoQ10 in their diet. It was also found to reduce the Hemoglobin A1c in the blood and maintain high-density level (HDL) cholesterol [17].

### 2.1.4 Male infertility

Infertility is the abridgement of the inability of any living organism to produce progeny of its kind and it is a major clinical complication, affecting an individual medically and psychologically. This inability is afflicted in 15% of the reproductive-aged couples. Male only comprise about one-fourth of the total cases and on the other side 50% cases are comprised of both male and female factors. The environmental, dietary, medical, genetic, and physiologic factors could lead to Male infertility. Out of a multitude of factors oxidative stress is one of the major factors reported to contribute to male infertility. Oxygen performs an influential part in maintaining the levels of reactive oxygen species (ROS) is fundamental for the functioning of the normal cells. The implication of infertility in 40% men according to historical studies, has determined ROS as an etiologic factor. At the same time, 30%–80% of male infertility subjects also found to have higher ROS levels [18–21]. The spermatozoa are prone to be damaged by oxidative stress and ROS expected to play a substantial pathogenic

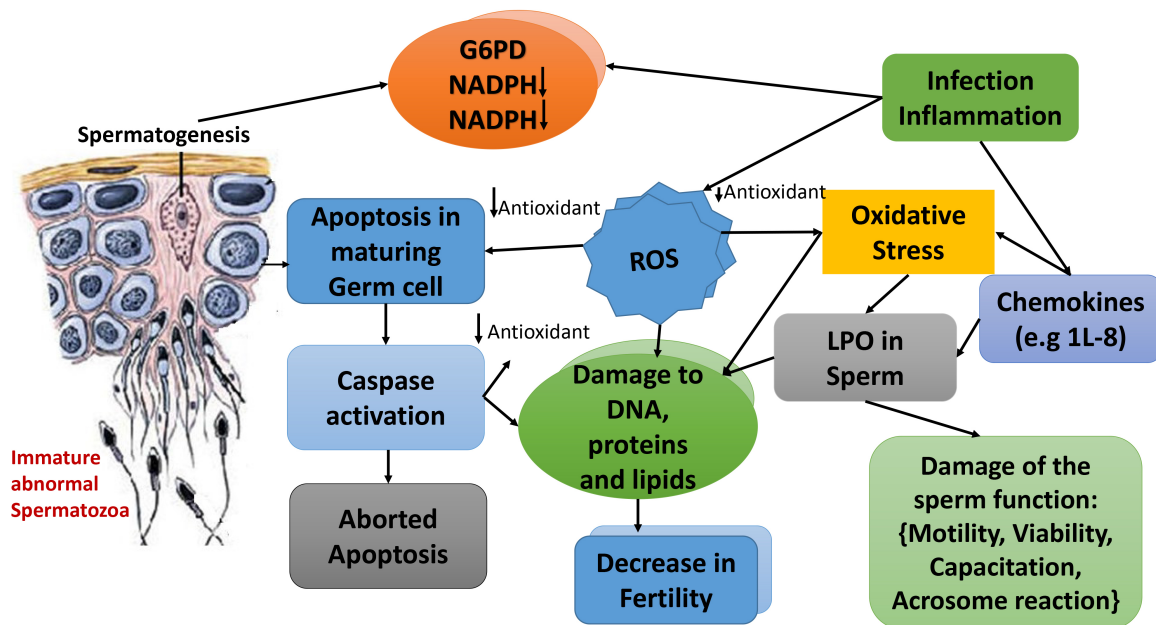


Fig. 2. Effect of oxidative stress on Human Semen [23].

role. The spermatozoa plasma membrane has a high concentration of polyunsaturated fatty acids (PUFA) with a low concentration of cytoplasmic scavenging enzymes which makes it highly vulnerable to peroxidation under the elevated concentration of ROS in the seminal fluid. However, due to membrane damage caused by peroxidation, the permeability is altered with calcium and adenosine triphosphate (ATP) depletion and increased sodium inflow, thus calcium-dependent enzymes getting activated along with a deluge of proteins and lipid disruption, structural DNA alteration, enzyme inactivation and further leads to apoptosis. Therefore, the spermatozoa are said to face “oxygen-paradox” like all other aerobic cells. The ROS is available as free radicals and examples of ROS forms that have been known to cause hindrance in sperm survival include hydroxyl ions, superoxide, hydrogen peroxide, peroxy radicals and hypochlorite ions [22]. A detailed explanation on the effect of the Reactive oxygen species (ROS) on the sperm cells due to oxidative stress is illustrated in Fig. 2 (Ref. [23]).

50% of CoQ10 is present in the mitochondria making it available to free radicals, which are primarily formed during oxidative phosphorylation. For adequate motility, the sperm cells require a high expenditure of energy, and this high energy requirement is fulfilled by the presence of a large number of mitochondria [24]; on the other hand; it could play a critical role in the protection of membrane from oxidative stress and hence maintains the sperm integrity [25]. In an adult human body, the endogenous synthesis and nourishment replace 0.5 g CoQ10 daily. While the total CoQ10 in the body is reported to be 2 g. The average rate at which turnover of CoQ10 occurs in the body is estimated to be nearly four days [26]. Hence, the outside sources

become essential with the increased disability of the synthesis’s internal machinery to produce CoQ10. The ability to produce CoQ10 starts gradually plunging after 20, but a rapid decline is observed after 40 [27]. The suggested daily intake of CoQ10 from external nutritional sources varies from 30–100 mg to 60–1200 mg for the healthy population in some medical conditions when used as a coadjutor therapy [5, 28, 29].

## 2.2 Coenzyme Q10 and its administration in functional food

The requirements of CoQ10 can be administered by functional foods, an idea that Japan initiated in the early 1980s [30]. Amidst the various definitions of functional foods, such foods’ main objectives should improve health and well-being. With the increasing popularity of CoQ10 based on its health benefits and nutrition, the fortification of CoQ10 with food products may lead to a trailing start to the studies on various fortified food products. In the United States, CoQ10 is considered as a food component where the FDA does not require the approval of products that contain CoQ10; approval becomes mandatory only for specific health claims. Because of its molecular structure and physical form, the fortification of CoQ10 is not an easy process. In its unadulterated form, CoQ10 exists in crystalline structure due to its high molecular weight ( $M_r = 863$ ) and lipophilic nature. Further, it is insoluble in water, which is a significant limitation for food fortification [31].

## 2.3 Coenzyme Q10 and fortification

CoQ10 intake from exogenous sources is absorbed in the small intestine, and efficient absorption occurs in the presence of high-fat meals. The process of micelle forma-

tion and emulsification are imperative for fat assimilation. The process is aided by bile secretions and the pancreas [32, 33]. The intestinal mucosal cells uptake CoQ10 and, after intestinal absorption, are blended to chylomicrons and transported across the body. The lymphatic system facilitates the transport of CoQ10 to the circulation and gets integrated into low-density lipoprotein (LDL) cholesterol in the liver and finally gets deposited in the tissues [34]. To improve the bioavailability of CoQ10 have done by researchers in oral formulations prepared for better bioavailability of CoQ10. McClements [35] stated that compounds like CoQ10, which falls under the category of bioactive lipophilic components, can be incorporated into beverages or foods which could be easily consumed since it increases the palatability, desirability, and bioavailability. The effect of grape juice on the transport of CoQ10 by Caco-2 cells was investigated by Itagaki [27], which studied the combined effect of CoQ10 and grape juice and established that this combination could improve the absorption of CoQ10 as grape juice inhibits the function of P-glycoprotein. Various products enriched with CoQ10 were studied and were proved to be stable. However, no published studies compare the bioavailability of CoQ10 between enriched foods and varying concentrations of CoQ10. Yogurt serves as an effective medium for the fortification of CoQ10 as it is on the higher side of lipid content and allows for the CoQ10 to get soluble in the media. Also, Yogurt contains a certain amount of CoQ10 it which increases the bioavailability rates.

### 3. Methodology

#### 3.1 Methods of CoQ10 preparations

The fortification of yoghurt with coenzyme Q10 was done by [36, 37] and these preparations were then compared to evaluate the differences in the bioavailability of the enriched yoghurts. Table 1, Ref. [36–40] delineates the methodology of fortification of yoghurts with CoQ10.

Pyo and his team [37] showed the comparison between enriched yoghurt samples. This study gives us an insight into the various yoghurt fortification methods such as emulsification with sunflower oil, nanoparticle synthesis along with CoQ10 and  $\gamma$ -Cyclodextrin complex-based CoQ10. It is an extensive study based on the efficiency percentage, loss due to the samples' processing changes, and bioaccessibility. This study concluded that the CoQ10 infused with nanoparticles showed a better efficiency during fortification in the yoghurt than other compared methods. The better stability in the nanoparticles' case can be attributed to its smaller particle with the increase in the rate of dissolution of the infused product [41]. The study of the CoQ10 infused with nanoparticles recorded that the stability of the nanoparticles and were found to be stable in a homogenous dispersion at the freeze-dried storage conditions [42].

The microencapsulation of CoQ10 in yoghurt was reviewed based on Table 2, Ref. [36–38, 43], and it can be observed that the fortified yoghurt has higher stability, gel firmness, pH, and increased storage time [36]. The release of the CoQ10 from the yoghurt is a significant factor in the fortification process. The release of CoQ10 from microcapsules was observed by HPLC using n-hexane and found to be relatively high during the first day of storage. The microencapsulated samples can be quickly released during the early stages by the penetration effect. The released CoQ10 in the yoghurt was stable during the storage conditions [44]. The review on the functional yoghurt production based on fermentation of *Monascus*-fermented soybean powder (MFSP) is different from other studies because the enrichment was done on the already available CoQ10 in the MFSP by fermentation. The MFSP was added to the starter culture, and the development of the yoghurt and the enrichments took place. The MFSP consists of bioactive substances such as isoflavones, gamma-Aminobutyric acid (GABA), and Ubiquinone (CoQ10), which are not expressed [45, 46].

These bioactive components could be activated by the fermentation process and express their activity in the yoghurt sample. The levels of isoflavones, GABA, and Ubiquinone and antioxidant activity before and after the fermentation process were extensively studied. The levels of free isoflavones in the MFSP containing yoghurt increased during the first 48 h of the fermentation process, and GABA levels were increased during the fermentation process about 13.8 times [37]. The ubiquinone (CoQ10) content during the fermentation process increased about 32.3 times higher than the standard. Further, the traces of the CoQ10 were found to be present during fermentation of about 13.4  $\mu\text{g/g}$  content [37]. The CoQ10 content was maximum for the first 20 days of fermentation in the sample [45, 46]. The increase in the ubiquinone concentration was due to the result of the fermentation of the red soybeans. The enriched yoghurt's antioxidant property with MFSP also showed high radical scavenging activity, including DPPH for the fermented yoghurt.

#### 3.2 Diverse clinical studies with CoQ10

##### 3.2.1 Coenzyme Q10 safety assessment

The demand for the consumption of CoQ10 has seen an elevation in recent years. In most European countries and the United States, CoQ10 is used as a common dietary supplement [47]. CoQ10 has been sanctioned as a drug for congestive heart failure in Japan in the year 1973 [12]. Furthermore, it has been approved as a food and cosmetic material in Japan in 2001 and 2004 [45, 46]. Thus, with the growing consumption of CoQ10, a safety assessment of the compound becomes necessary, hence with available literature evidence, the safety data has been presented in the review.

**Table 1. Distinction between the methodologies adopted for the preparation of yoghurt enriched with CoQ10.**

Type of CoQ10 preparation	Reference	Methodology
Emulsified CoQ10	[39]	In 0.56 g of sunflower oil, a weighted quantity of 60 mg CoQ10 was dissolved. Monoglyceride (170 mg) and aqueous solution of skim milk (1.46 g) were incorporated into the mixture. An equivalent weight of 7.75 g water was added to the emulsion, after which it was homogenized at 64.01 g for 20 min. The emulsion was then stored at a temperature of $-40^{\circ}\text{C}$ .
$\gamma$ -Cyclodextrin complex based CoQ10	[39]	At a temperature of $70^{\circ}\text{C}$ , 4.5 mL of water was used to dissolve 200 mg of $\gamma$ -Cyclodextrin, 40 milligram of CoQ10 was dissolved in 0.5 mL of diethyl ether and homogenization of the sample was carried out at 711.2 g for 30 s in a homogenizer. The mixture was cooled on a magnetic stirrer within a duration of 30 min at room temperature and drying was carried out at $55^{\circ}\text{C}$ in an oven. The complex was hence dissolved in 4 mL water and stored at $-40^{\circ}\text{C}$ .
CoQ10 infused with nanoparticle	[39, 40]	At room temperature, 400 mg of PLGA (50:50) was dissolved in 16 mL of ethyl acetate with continuous stirring for 2 h. The resultant organic phase was emulsified with 40 mL of an aqueous phase containing 1% DMAB (weight/volume). The resultant emulsion at room temperature was stirred for 1 h, and homogenization at 1600.2 g for 5 min was carried out in an ultrasonic homogenizer for 2 min at 50% amplitude. Then 240 mL water was added to the resulting emulsion with constant stirring, which resulted in nano-precipitation. For evaporation of ethyl acetate, agitation of the sample was continued at 1000 rpm at $40^{\circ}\text{C}$ , centrifugation at 1000 g at the $10\text{--}15^{\circ}\text{C}$ led to the nanoparticles' isolation. The nanoparticles were washed thrice with deionized water. 6 mL 1% Tween twenty solutions were used to dissolve the nanoparticles, and it was kept at a storage temperature of $-40^{\circ}\text{C}$ .
Microencapsulation of CoQ10	[36]	B-Immunoglobulin and Arabic Gum were prepared at 1–4% (w/v) were separately prepared in deionized water and stirred for about 2 h using a magnetic stirrer and were stored at $4^{\circ}\text{C}$ to allow hydration. The CoQ10 powder was dissolved olive oil, mixed at 200 rpm in an incubator for a day. The oil in water emulsion was prepared using coenzyme Q10 mixture to the $\beta$ immunoglobulin, which is considered the oil phase was homogenized at 14000 rpm for 2 min. The Arabic gum mixture was added to the prepared oil in water emulsion and was stirred for 15 min at 350 rpm. The pH of the emulsion was adjusted, and the microcapsules were collected and stored $-45^{\circ}\text{C}$ .
Functional yogurt co-fermented with Monascus-fermented soybean powder (MFSP)	[45, 46]	This method does not involve the fortification process. However, the content of CoQ10 in the yoghurt was enriched by the fermentation process. The ratio of 1:1 (weight/weight) of skim milk powder and fermented soybean powder (SMP+MFSP) of about 100 g was dissolved in 1000 mL of purified water, and 100 g of sucrose was added. It was homogenized and then heated at $90^{\circ}\text{C}$ . After cooling to $37^{\circ}\text{C}$ , <i>L. delbrueckii</i> , <i>L. bulgaricus</i> , and 5% of the seed culture solution (starter) mixed with <i>S. thermophilus</i> in equal proportions (v/v) were added to the mix and incubated for 48 h. The yoghurt with (SMP+MFSP) has an enriched amount of isoflavones, GABA, and Ubiquinone contents.

### 3.2.2 The acceptable daily intake (ADI) of CoQ10

The acceptable daily intake (ADI) is defined as the dose consumed by an individual over their lifetime without the involvement of substantial risk of health hazard. The ADI can be calculated from the toxicity studies of animals conducted throughout an extensive duration using the No observed adverse effect level (NOAEL) by the equation [50, 51].

$$\text{ADI} = \frac{\text{NOAEL}}{\text{Safety factor of 100 fold (10 species differences} \times 10 \text{ individual difference)}}$$

The study reported no dangerous occurrence in the body mass, food consumption, and mortality in the rats. Also, no changes in the clinical pathology were observed. The elimination half-lives of CoQ10 ranges were found to be 10.7 to 15.2 h. Based on the rats' 52-week study duration as mentioned in Fig. 3 (Ref. [52]), the NOAEL was

determined to be 1200 mg/kg day. Therefore, the ADI was 12 mg/kg/day based on the calculation from NOAEL. It implies that the ADI of a human being weighing 60 kg to be 720 mg/day [52].

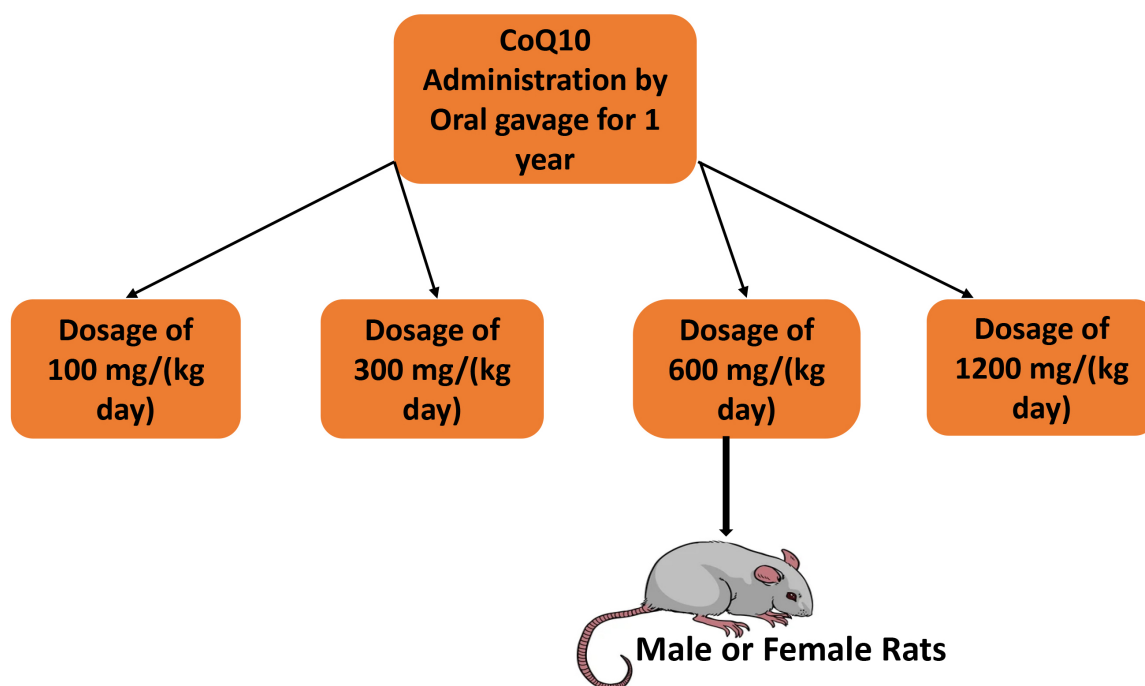
### 3.2.3 Observed safe level (OSL) of CoQ10

The observed safe level (OSL) is often expounded as an individual's safety with compelling evidence upon the ingestion of the highest dosage of any compound or drug. It stands rigid even if there are no established adverse effects reports at any hierarchy. In a study conducted on the effect of CoQ10 on patients with symptoms of early Parkinson's disease (PD), a vast group size with  $n = 80$  was considered [53]. The study administered dosages in the range from 300 to 1200 mg/day of CoQ10. The clinical trial was designed in a placebo-controlled and doubled-blind approach with a duration of 16 months. At the end of the trial period, no adverse effects were observed in the subjects with any dose



**Table 2. The physiochemical nature of CoQ10 fortified yoghurts observed based on the study by [36, 37, 43, 48, 49].**

Physiochemical property	Observation
Acidity and pH	Microencapsulated CoQ10 yoghurt The acidity was higher in the fortified sample due to the production of lactic acid during storage conditions. The pH remained the same in the fortified sample [36].
	Fermented MFSP yoghurt The acidity of the yoghurt with MFSP increased during the fermentation process leading to a decrease in the sample's pH. It occurs due to the acid bacteria's metabolism in the culture increases the amount of organic acid in the sample [37].
Whey separation	Microencapsulated CoQ10 yoghurt The whey separation is low in fortified yoghurt because the presence of $\beta$ immunoglobulin and Arabic gum absorbs the water in the sample and reduces the incidence of whey separation [36]. The proteins have a wide dynamic, and the relaxation of the protein-protein bonds point towards the yoghurt gel matrix and fat globules rearrangement. This phenomenon has a profound effect on whey separation in yoghurt [36, 38].
Gel firmness	Microencapsulated CoQ10 yoghurt The gel firmness of the fortified yoghurt increased during the storage time; it is due to the increase of the total solids content, which provides a route to escalating linkages between particles causing the firmness in the gel [43].
Viable cells	Fermented MFSP yoghurt The viable cells increased during the first 24 h of the fermentation period and were followed by a slight decrease in the starter culture's viable cells. The viable cells increase the acidity as well as the scent of the yoghurt [48, 49].

**Fig. 3. CoQ10 administration in rats to determine ADI and NOAEL [52].** Adapted data from reference to convert as a figure.

group. Thus, the study established the OSL of CoQ10 to be 1200 mg/day, as the trial provided efficacious corroboration.

### 3.3 Effect of CoQ10 in neurodegenerative disease

Most literature studies provide rigorous evidence that oxidative stress and mitochondrial dysfunction are the major contributors to neurodegenerative disease. In Friedreich's ataxia (FA), amyotrophic lateral sclerosis (ALS), and Parkinson's Disease (PD), and aloft in the levels of 8-hydroxy-2'-deoxyguanosine (8OHdG) along with

oxidative markers has been illustrated, as reported in the studies conducted [54–59]. In PD, most genes are associated with the functioning of Mitochondria, and hence a strong correlation can be drawn about the PD-related neurodegeneration with mitochondria and oxidative stress. The greatest medical challenge is posed by nitroxidative stress and cytokines that cause inflammation which eventually are the greatest inducers of neurodegenerative diseases. Several research has shown that inflammatory cytokines and mitochondrial dysfunction are the promoters of neurodegenera-

tion. This results in the Ab peptide accumulation in case of Alzheimer's disease. While in case of Parkinson's Disease (PD) due to  $\alpha$ -synuclein mutation in the nigrostriatal system, there is a continuous loss of dopaminergic neurons [60].

### 3.3.1 Parkinson's disease (PD)

The safety and efficacy of CoQ10 were studied in a random, double-blind, parallel-group for 16-months. Eighty subjects enrolled for the study who were able to perform their daily activities were chosen. These patients also simultaneously attended movement disorder school. The subjects were administered oral dosages of CoQ10 in concentrations 300 mg/day, 600 mg/day, and 1200 mg/day for 16 months until they reached a point where a rescue treatment became vital [61]. The UPDRS (Unified Parkinson Disease Rating Scale) was employed to measure the difference in total scores between the end of the study and the baseline as reported in the literature [53]. A slower decline was experienced by those who consumed higher dosages of CoQ10 at the end of the study. Parkinson's has another feature, which is known as Visual Dysfunction. This disorder is associated with a decrease in the concentration of CoQ10 in the body as reported in the literature evidence of the study conducted, a double-blind, placebo-controlled, clinical trial where CoQ10 in the concentration of 360 mg/day for four weeks was administered to 38 patients [62]. At the end of the study period, evaluation of the results was determined by the Farnsworth-Munsell 100 Hue test, and a positive result was observed in the patients, where the visual symptoms showed improvement.

### 3.3.2 Huntington's disease

No literature or evidence leads to a better understanding of the disease, but it is believed that CoQ10 deficiency is a concern and has been identified to play a pivotal role in the disease process [63]. Various literature and studies have identified Huntington's disease as an autosomal dominant disorder where a condition of neuronal degeneration occurs, which has a proclivity towards basal ganglia, brainstem, and cerebral cortex [57]. According to previously conducted studies, it has been established that the patients with Huntington's disease, when treated with CoQ10, show a reduced lactate level, which otherwise is found at peak levels in such patients. Trials were conducted to evaluate the safety and efficacy of CoQ10 on patients with Huntington's disease [64]. The study was conducted as an open-label trial upon ten patients for six months with CoQ10 doses ranging between 300–1200 mg/day. The results exhibited no noteworthy changes in the patients.

## 3.4 The role of CoQ10 in male infertility treatment

The sperm cells undergo impairment due to the production of excessive reactive oxygen species (ROS), and hence it negatively affects male fertility. CoQ10, also known as Ubiquinone, is an isoprenylation benzoquinone responsible for the transport of electrons from complexes

I and II to complex III in the respiratory chain of the mitochondria, which is vital for the stability of complex III [65]. In addition to facilitating the electron transport, CoQ10 is also a potent antioxidant, membrane stabilizer, and regulates the transition pore permeability of mitochondria [47]. The various causes of male infertility as well as the treatment mechanism of CoQ10 for it has been pictorially illustrated in the Fig. 4. The three main improvements in the sperm due to CoQ10 consumption are increased sperm motility, increased sperm count and improved sperm morphology which thereby improves the fertility of the men.

As per a study conducted, 212 men with idiopathic oligoasthenoteratospermia, a male infertility type, were randomly administered 300 mg CoQ10 daily via oral ingestion for a time duration of 26 weeks followed by a 30-week duration without treatment [66]. The results showed significant improvement in sperm motility and density. The determination of sperm morphology Kruger classification was employed where a positive correlation was found between sperm count and sperm motility. In another study, 40 male patients aged between 26–40 years, diagnosed with oligoasthenozoospermia—a kind of male infertility, were supplemented with Carni-Q-Nol, which is a combination of 440 mg L-carnitine fumarate + 30 mg ubiquinol + 75 IU vitamin E + 12 mg vitamin C in each softsules [67]. During the initial three months, two softsules were administered, and during the second 3 months, three softsules were administered daily. After the treatment period of 3 and 6 months, sperm density was observed by 48.9% and 80.9%, respectively. The sperm pathology decreased by 25.8% after three months of treatment. Male infertility is caused due to the formation of the free radicals in the body because of various external factors, and so the CoQ10 naturally present in the mitochondrial sperm cells is reduced [68]. The consumption of CoQ10 in functional foods is safer and more advisable than consuming them as medications to avoid any side effects. Since CoQ10 is a compound known to have high lipophilicity, high molecular weight, and heat-sensitive in nature, yoghurt is considered the best medium for the fortification of CoQ10 [48]. The consumption of CoQ10 by people affected with idiopathic as then azoospermia leads significant increase in the ubiquinone and ubiquinol levels in semen with an inverse relationship between the ratio of Ubiquinone to ubiquinol [61]. With the supplementation of CoQ10 for two weeks to healthy volunteers, the total antioxidant capacity and the plasma CoQ10 concentrations in the human body were observed to be elevated [69]. Thus, based on this, consuming the coenzyme Q10 fortified products provides more antioxidant effect to improve male fertility by reducing the free radicals in the spermatozoa.

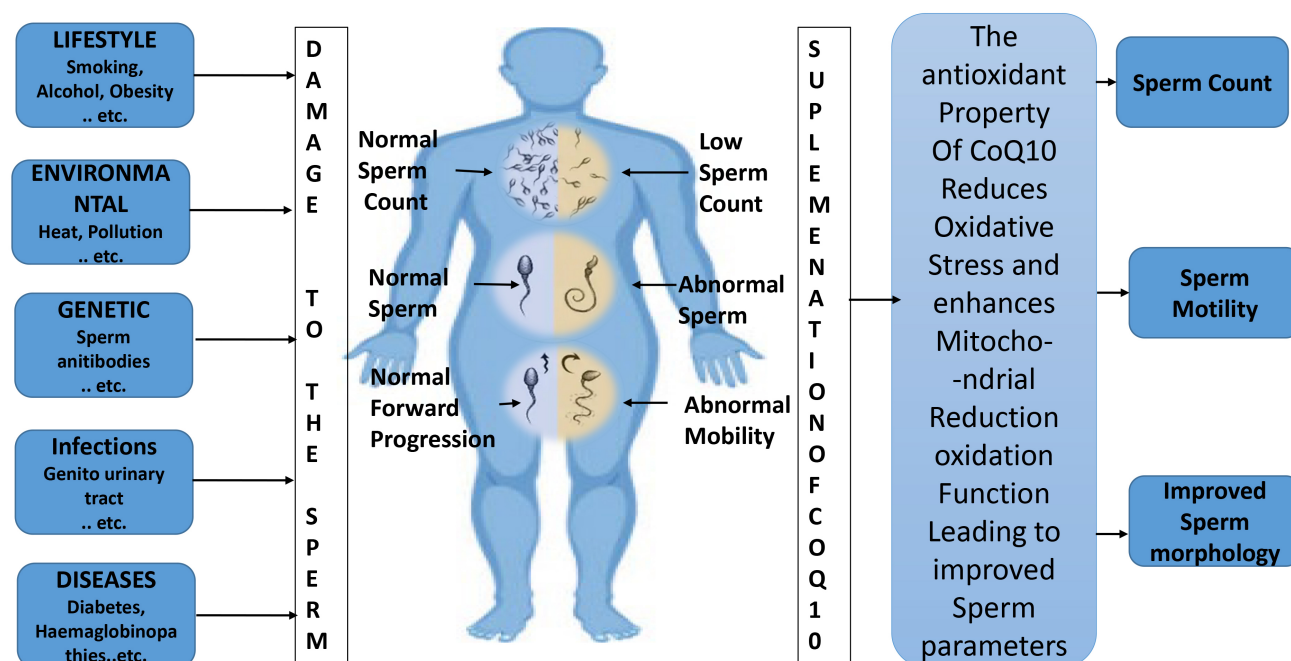


Fig. 4. Schematic representation of causes of male infertility and effect of treatment by CoQ10.

#### 4. Conclusions

CoQ10 acts as an antioxidant and is present sparsely in the mitochondrial cells of the spermatozoa. This review was done to study the various methods for the fortification of yoghurt with coenzyme Q10 and its effect of reducing the oxidative stress of the human focused on male infertility. The coenzyme Q10 is a lipophilic and heat-sensitive component; hence yoghurt is the best medium for its fortification. The consumption of CoQ10 as a supplement, along with yoghurt enhances the effect against the oxidative phosphorylation in the mitochondria. Since the spermatozoa have high mitochondrial cells and this CoQ10 is said to believe to reduce oxidative stress. The study considered various fortifying methods to inoculate CoQ10 in the yoghurt. The methods studied were emulsification of CoQ10 with oil, the formation of  $\gamma$ -Cyclodextrin/CoQ10 complex, nanoparticles formation of the CoQ10, microencapsulation of the CoQ10 with  $\beta$  Immunoglobulin and Arabic gum, and the production of Co-Fermentation of monascus -Fermented soybean powder (MFSP) with yoghurt. Each method has its unique features and limitations. The CoQ10 in  $\gamma$ -Cyclodextrin/CoQ10 complex has been studied to have more efficiency and less loss during the processing changes. The CoQ10 infused in nanoparticles showed promising results based on the bioavailability and amount of CoQ10 retained after *in-vitro* digestion, proving that it will be present in the body even after digestion. The microencapsulated yoghurt samples showed better results in maintaining the pH at the required level and providing firmness of the sample. The release of CoQ10 was observed in the sample during the whole process and shelf life. The

HPLC analysis of the microencapsulated yoghurt showed only a meager amount of CoQ10 in the yoghurt during its shelf life. The utilization of monascus-fermented soybean powder is an innovative concept to fortify the CoQ10; by observing this method, it can be recommended to be consumed as a fortified coenzyme Q10 product. The MFSP method is slightly acidic due to the high number of acidic bacteria supporting the lactic acid metabolism. In conclusion, the intake of CoQ10 fortified yoghurt possibly the best supplement for oxidative stress and improving humans' health. Further extensive preclinical and clinical research using these supplements needs to be done to test the benefits and optimize the dosage of these fortified products. Further research on fortification in water-based mediums can be explored to improve fortification efficiencies as well.

#### 5. Author contributions

SS and SD equally contributed in literature search, idea formation and writing the paper. NG provided her knowledge and guidance in writing this paper. MME, MWQ, SBC and BB involved in reviewing and organizing the MS.

#### 6. Ethics approval and consent to participate

Not applicable.



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## 9. Conflict of interest

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## 10. References

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