Assessment of in vitro fertilization and early embryonic development using SMART medium enriched with coenzyme Q10

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Abstract:

Background

Zygote produce from once a sperm fertilizes an egg cell. Then, the zygote (unicellular) will begin chain of cellular cleavages to produce multicellular mass, its embryo, the differentiated to different tissues and organism. The development of the embryo is called embryogenesis.
Coenzyme Q10, is an antioxidant produced in the body. It boosts cellular energy and may enhance the immune system. CoQ10 is present and measurable in seminal fluid,the concentration of CoQ10 directly correlates with both sperm count and motility. It is beneficial in the prevention and treatment a wide range of health problems.

Objectives

The present study was aimed to investigate the possibility of using coenzyme Q10 to improvein vitro fertilization (IVF), and early embryonic development (ED) in mice as a model for human being

Methods

Superovulation program was achieved to mature healthy female mice with age 10-12 weeks and weight 24-26 gm. After sacrificing female, oocytes were collected and incubated within CO2 incubator for less than 1 hour. Sperm were collected from vas deference of males. Sperm parameters were assessed after 30 min. of incubation. Mature oocytes were divided into three groups according to theconcentrations of CoQ10 including G1 (control group; SMART medium only), G2 (treated group; SMART medium enriched with 20 M CoQ10) and G3 (treated group; SMART medium enriched with 40 M CoQ10). IVF technique was performed for 3 groups, and assessment of IVF (%), embryonic development stage (%) and abnormal embryo morphology (%) for each embryo stage.

Results

Results of the present study appeared significant increment ($P<0.05$) in the percentages of IVF for both treated groups as compared to the control groups. Also, significant increase ($P<0.0P$) in the IVF (%) was observed when using 40 M CoQ10 as compared to 20 M CoQ10. Non significant differences ($P>0.05\%$) in the 8-cells embryo stage were assessed among control and treated groups

Conclusion

From the results of the present study it wasconcluded that the coenzyme Q10 (40μM) enriched to the culture medium improved percentage of in vitro fertilization and no effect on embryonic development.

Key word:Fertilization,Embryo,Smart,coenzyme Q10

Introduction

Coenzyme Q10 (CoQ 10) is essentially a vitamin or vitamin-like substance. CoQ10 likewise is found in small amounts in a wide variety of foods and is synthesized in all tissues (1). The biosynthesis of CoQ10 from the amino acid tyrosine is a multistage process requiring at least eight vitamins and several trace elements. Coenzymes are cofactors upon which the comparatively large and complex enzymes absolutely depend for their function (2).Coenzyme Q10 (CoQ10) is a component of the mitochondrial respiratory chain, play role both in energy metabolism and as antioxidants for cell membranes and lipoproteins (3) . Coenzyme Q10 biosynthesis is markedly active in testis (4), reduced levels of CoQ10 in seminal plasma and sperm cells of infertile men (5).

In vitro fertilization (IVF) is a procedure that involves retrieving oocytes and spermatozoa from the female and male respectively, and placing them together in a laboratory dish to facilitate fertilization. Fertilized eggs are then allowed to develop in vitro and after several days are transferred into a females uterus where implantation and embryo development can occur (6).

Different types of culture media are used in the programs of IVF based on animal and human studies. Culture media are classified into two types based on composition. The first one is a simple salt solution formulated. (7).

Very limited information was mentioned in the literatures about the effect of CoQ10 on the in vitro fertilization (IVF) and early embryonic development. Therefore; the aim of the study is to investigate the effect of Coenzyme Q10 supplied to culture medium on rate of fertilization and embryonic development in mice as a model for human being.

Materials and Methods:

Animals:

Healthy adult mice of one hundred ten females and forty male mice with age of 10-12 weeks old and 25-27 gm body weight were obtained from the Animal House unit at the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies /AL-Nahrain University. The animals were housed in a plastic cage of measuring (29×15×12) cm, its floor covered with wooden shave. Each cage contains four females. The animals were examined clearly in every week, abnormal and sick mice were excluded from the experiment. Thecages were cleaned and sterilized with 70% ethyl alcohol once a week regularly.

Preparation of CoQ10:

A powder of Coenzyme Q10 (Coenzyme Q10,M.W. 863.358,ultimate Nutrition co; Japan)was used for preparation of CoQ10. Stock solution was prepared by dissolving 0.069 g in 10 ml of SMART medium. For preparation of low concentration treated group (G2; 20µm), 0.25 ml of stock solution was diluted with 7.75 ml of SMART medium. Then, each one ml contains 20 µm of CoQ10. However, addition 0.5 ml of stock solution to 705 ml of SMART medium to prepare high concentration group (G3; 40 µm). Therefore, each ml contains 40µm of CoQ10.

Superovulation program (SOP) and ova collection:

Superovulation is a routine procedure for producing greater yields of oocytes. Superovulation program starts by injecting female mice with 7.5 I.U. of PMSG (intraperitoneally), Also second injection with 7.5 I.U. of PMSG after (24) hours, After (47-48) hours from 2nd injection, 3rd female mice was injected with 15 I.U. of hCG. Sacrificing female and oocytes were recovered (14-16) hours post-hCG by flushing the oviducts.

Female mice were sacrificed, isolate of the oviducts. For oocytes flushing, the ampulla was teared to release the oocytes imbedded within cumulus masses. Then, transferred to the CO2 incubator for incubation for 30-60 min, then they were transferred to a four well-culture dish(5-7 oocytes in each well) containing 1 ml of SMART medium (pH=7.3-7.5) and kept at 36.5°C with 5% CO2 and 95% humidity.

Sperm collection and assessment:

Spermatozoa were collected and assessment as mention in details by Fakhrildin(8).

Technique of in vitro fertilization:

The mature oocytes were washed twice in SMART medium and transported to 4-well culture dish (5-6 oocytes/ well) containing 1mL of the SMART medium either a lone (control group) or supplemented with different concentrations of CoQ10(G2;20µM or G3;40µM). The motile spermatozoa were added to the oocytes at the concentration of approximately 5×104 motile sperm/oocyte. Sperm and oocytes were covered with liquid paraffin and incubated at 36.5°C in a moist atmosphere 5% CO2 with high humidity (95%) for 24h at CO2 incubator. The percentages of IVF were recorded for every group.

Early embryonic development:

Early embryonic development can be divided into several stages: zygote(one cell),2-cells embryo,4-cells and 8-cells embryo. Embryo development is characterized by various morphological features that occur after fertilization (9). The first visible sign of fertilization, the extrusion of 2ndpolr body and then appearance of 2nd pronuclear in the cytoplasm of the oocyte, can be observed 18-22 hours after insemination in vitro(10).

Assessment early embryonic development rate was by recording the number of zygotes, two cells stage and four cells stage embryos at every 24 hour after insemination.

Results

In this study, 40 male and 110 female mice were used. The number of oocytes were collected from superovulated female mice was 1556. The morphologically normal oocytes were 1440 and abnormal oocytes were 116. Percentage of

morphologically normal oocytes is (92.54 %) and morphologically abnormal oocytes is (7.49%). There was significant different (P<0.05) between both types of oocytes.

There is a significant difference (P<0.05) in the IVF (%) between control(G1) and both treated groups. There is significant difference (P<0.05) in the percentages of IVF amongG1, G2 and G3. On the other hand, significant difference (P<0.05) in IVF (%) was assessed between G2 and G3 groups. However, G3 shows higher percentage of fertilization as shown in figure(1).

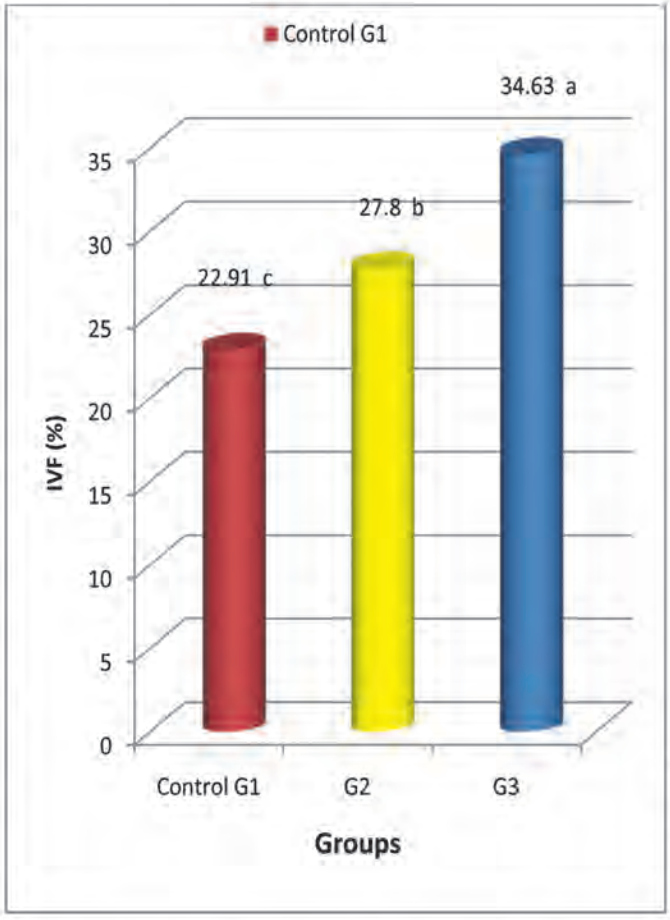
The control group appeared, highest percentage (54.05%) for 2-cells embryo stage, with second degree for 1-cell embryo (zygote). While, the lowest percentage for embryonic development was recorded for 8-cells stage. There was no significant difference (P>0.05) between 1-cell embryo stage, 2-cells embryo stage and 4-cells embryo stage. However, there was significant difference (P<0.05) between 1-cell embryo stage and 8-cells embryo stage, also between 2-cells embryo stage and 8-cells embryo stage. No significant difference(P>0.05) between 4-cells embryo stage and 8-cells embryo stage as shown in table (1).

The results of embryonic development using SMART medium supplemented with 20 µM of Coenzyme Q10 was presented in the table (1). Highest percentage for embryonic development was noticed for one cell stage. In this group, was lowest percentage for 8-cells stage embryonic development. However, non significant difference (P>0.05)were reported between 4-cells and 8-cells stages. Significantly difference(P<0.05) was observed between 1-cell stage and 2-cells stage, also for 4-cells and 8-cells stages.Significantly difference(P<0.05) was assessed between 2-cells stage with other stages of embryonic development in this group.

From table(1), group three (G3) with highconcentration (40µM) of coenzyme Q10 showed higher percentage of embryonic development was 2- cells stage, and second degree for one cell embryo. While, the lowest percentage for embryonic development was 8 cells stage.From same table, non significant differences (P>0.05)were reported between 4-cells and 8-cells stages. In contrast, sigsignificant differences(P<0.05) were observed between 1-cell stage and 2-cells stage also for 4-cells and 8-cells stages. However, significant difference(P<0.05) was noticed between 2-cells stage with other stages of embryonic development in this group.

Table(1):Early embryonic development

Study groups	Early embryonic development (%)			
	1-cell	2-cells	4-cells	8-cells
G1; Control	48.1a	54.05a	29.06ab	8.93b
G2; 20µM	46.27a	28.94b	14.22c	10.58c
G3; 40µM	30.25b	40.16a	17.93c	11.67c



Figure(1): Percentages of IVF using SMART medium supplied with different

Discussion

In this study, the coenzyme Q10 (CoQ10) with two concentrations (low concentration 20 µM and high concentration 40 µM) were supplemented to SMART medium. An improvement was showed in the percentage of in vitro fertilization as compared to the control group. However, the concentration of CoQ10 (40µM) achieved best improvement in the outcomes ofin vitro fertilization (P<0.05) as compared to the other treated group and control group.

Definitely, in vitro fertilization was enhanced through activity of several factors including presence of mitochondria with adequate numbers and normal activity (11), integrity of plasma membrane (12), regulation of tubulin synthesis and aggregation (13), normal and adequate gene expression for protein synthesis (14), reduction level of ROS (15), presence of ATP (16); presence of CoQ10 (17), and contents and environment of culture media (18).

In the present work, CoQ10 was used due to special properties. CoQ10 is an essential component of the plasma membrane ion transporter (PMIT) system and of the electron transport chain in the inner mitochondrial membrane. Because of its intrinsic functions in cell growth and energy metabolism (ATP synthesis), and its protective effects against

oxidative stress, CoQ10 is a good candidate for supporting growth of cells in culture (16). Moreover, several advantages are reported for CoQ10, as a membrane stabilizer and a regulator of mitochondrial permeability transition, in addition to an antioxidant, an energy promoting agent pores (19). Adequate amounts of CoQ10 are necessary for cellular functions and ATP production, due to its involvement in ATP synthesis(20), and the mitochondrial respiratorychain (electron transport chain, ETC)(18). Actually, the normal plasma membrane for both sperm and oocyte is very important for normal fertilization at molecular level and subsequently normal embryonic cleavage and development (21). It is known that the lipid is the component of plasma membrane and negatively affected by oxidative stress and/or presence of ROS (22). Accordingly, the CoQ10 has antioxidant properties,protecting membrane lipids and proteins and mitochondrial deoxyribonucleic acid (mtDNA) against oxidative damage (18).Therefore, the improvement in the percentages of IVF for the treated groups may be as a result of direct and/or indirect effects of CoQ10. CoQ10 also functions as an intercellular antioxidant at the mitochondrial level, perhaps accounting for its benefit in neurodegenerative diseases (20), and male infertility (23), Furthermore, most CoQ10 in sperm cells is concentrated in the mitochondria of the mid piece and energy dependent processes in the sperm cell depend on the availability of CoQ10 (24).

In this work, the results of IVF may be affected bymaternal inheritance.As compared to the sperm, it was known that the large volume of cytoplasm of oocyte, high number of mitochondria and mtDNA, therefore maternal inheritance has a major role in complete meiosis(25), pronuclear formation(26), fertilization (27), and zygote production(28). In addition to events during embryonic and fetal development (25). Furthermore, the paternal effects are affects fertilization pre-, during and post-fertilization with second degree to maternal effects. It is known that the tail and midpiece structures can be traced for several division cycles (25). The typical mammalian sperm midpiece contains approximately 50-75 mitochondria with one copy of mtDNA in each. In contrast, the mammalian oocyte contains around (105-108) mitochondria (29), and the human oocyte in particular is estimated to contain (105) copies of mtDNA(30). Thus the oocyte's mtDNA copy number exceeds that of the sperm by a factor of at least (103) (30).
In the present study, enhancement of IVF was combined with presence of CoQ10 in the treated groups through improvement sperm parameters like sperm viability, motility and grade activity especially. Certainly, CoQ10 in the seminal fluid shows a direct correlation with semen parameters (31).Mancini and his-coworkers demonstrated high levels of CoQ10 in human seminal fluid that correlate positively with sperm count and motility (32).In a clinical study, exogenous administrationof CoQ10 was effective for improving sperm kinetic features in patients with idiopathic asthenozoospermia(33).
In the present study, there is an improvement in the embryonic development for treated groups of Co Q10 enriched to SMART medium, but non significant difference was observed as compared to the control group. The results show that the addition of CoQ10 (40 µM) has no effect on 1-cell embryo stage and negatively affect 4-cells embryo stage. Same CoQ10 concentration (40 µM) improved the growth of 2- and 8-cells embryo stages.
In general, the rate of early cleavage of embryos (6- to 8-cell stages) was evaluated66 h post insemination, and was highest in medium supplemented with 30 or 100 µM CoQ10, and lowest in 10 µM CoQ10 (16).
Most early studies assumed that the sperm mitochondria participated fully in early embryonic development. However, in 1965, Szollosi(34), reported the fate of sperm midpiece mitochondria in rats, observed that they remain associated with axonemal structures and did not distribute evenly between blastomeres. It is found that these mitochondria swelled and appeared to disintegrate by the eight-cell stage (35).
In the present work, SMART medium was used for IVF and embryonic development in mice. However, same medium was used as medium for in vitro sperm activation for infertile patients (36), and sheep (37). Same medium was used also for sperm preparation, IVM, IVF, and early embryonic development in sheep (38). Additionally, CoQ10 is a promising candidate for supporting thedevelopment of in vitro-produced (IVP) embryos when added to the culture medium (39). Effects of various concentrations of CoQ10 on early cleavage, were investigated in a chemically defined culture system (40). Some of the failures of culture systems that are often overlooked are those leading to increase intracellular pH (41), and disturbed function of the plasma membrane ion transporter (PMIT) system (42). The PMIT system serves as a backup system for the mitochondrial respiratory chain regulating cellular redox homeostasis. Twelve or more carriers are grouped into four multiprotein intramembranous complexes (43).
From the results of the present study it was concluded that the coenzyme Q10 (40µM) enriched to the culture medium improved percentage of in vitro fertilization and no effect on embryonic development.

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