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Effects of Coenzyme Q10 Supplemented to Culture Medium on Morphology of Mouse Early Embryonic Development as a Model for Human Being

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Abstract---

Background: *Zygote produce from once a sperm fertilizes an egg cell. Then, the zygote will begin chain of cellular cleavages to produce multicellular mass, its embryo, than differentiation to different tissues and organism. 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 improve early embryonic development (ED) in mice as a model for human being.*

Materials and Methods: *Superovulation program was preformed to mature healthy female mice with age 10-12 weeks and weight 24-26 gm. After sacrificing superovulated female, oocytes were collected and incubated within CO_2 incubator for less than 1 hour. Also, sperm were collected from vas deference of males. Then sperm parameters were assessed after 30 min. of incubation. Mature oocytes were divided to three groups according toconcentrations of CoQ10 including G1 (control group; SMART medium only), G2 (treated group; SMART medium enriched with 20 μM CoQ10) and G3 (treated group; SMART medium enriched with 40 μM CoQ10). IVF technique was performed for all groups, and assessment of IVF (%), embryonic development stage (%) and abnormal embryo morphology (%) for each embryo stage.*

Results: *Results of the present study appeared non significant differences ($P>0.05$) in the 8-cells stage were assessed among control and treated groups. Within same stage, percentages of abnormal morphology were significantly decreased ($P<0.05$) for both treated groups as compared to the control group. However, non significant differences in the percentages of abnormal embryos morphology were reported between both treated groups of CoQ10.*

Conclusion: *From the results of the present study it wasconcluded that the coenzyme Q10 no effect on embryonic morphology. Further biochemical study is recommended to investigate the effects of CoQ10 on placental formation and functions of pregnant mice.*

Keywords--- *Effects of Coenzyme, Embryonic Development, IVF.*

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I. 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 acidtyrosine is a multistage process requiring at least eight vitamins and several trace elements.Coenzymes are cofactors upon which the comparatively large and complex enzymesabsolutely depend for their function [(2)].Coenzyme Q10 (CoQ10) is a component of the mitochondrialrespiratory chain, play role both in energy metabolismand as antioxidants for cell membranes and lipoproteins [(3)].Coenzyme Q10 biosynthesis is markedly active in testis [(4)], reducedlevels of CoQ10 in seminalplasma and sperm cells of infertile men[(5)].

Different types of culture media were used in the early embryonic development based on animal and human studies. Culture media are classified to two types based on composition. The first one is a simple salt solution formulated with the addition of pyruvate, glucose, lactate and albumin. The second complex media such as SMART with are contain nutrients such as vitamins, amino acids and other metabolites [(6)].

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

II. MATERIALS AND METHODS

2.1. 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.

2.2. 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 7.5 ml of SMART medium to prepare high concentration group (G3; 40 µm). Therefore, each ml contains 40µm of CoQ10.

2.3. 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 injection 15 I.U. of hCG. Sacrificing female and oocytes were recovered (14-16) hours post-hCG by flushing the oviducts.

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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 CO_2 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.

2.4. Sperm Collection and Assessment

Spermatozoa were collected and assessment as mention in details by Fakhrildin[(7)].

2.5. 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 [(8)]. The first visible sign of fertilization, the extrusion of 2nd polr body and then appearance of 2nd pronuclear in the cytoplasm of the oocyte, can be observed 18-22 hours after insemination *in vitro*[(9)].

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.

2.6. Experimental Design

Superovulation program by injecting female mice with PMSG and hCG, sacrificing female and Oocytes were recovered From the oviductal tube**,** then incubation oocytes for one hour, after these sacrificing male and collection sperms from vas deferens and incubating for 30 min, and then IVF was done for three groups; group 1: control culture media only without CoQ10, group2: low concentration of CoQ10(20µm), group3: high concentration of CoQ10(40µm). Then assessment percentages of embryonic development.

III. RESULTS

In this study, 40 male and 110 female mice were used. The number of oocytes were collected from super ovulated 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 (7.46%).

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-

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cell stage and 2-cells stage, also for 4-cells and 8-cells stages.Significant 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 high concentration (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, significant 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: Percentages of Early Embryonic Development Classified According Concentration of CoQ10 Supplemented of SMART Medium

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

IV. DISCUSSION

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 showed 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 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 [(10)]. 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 [(11)]. Adequate amounts of CoQ10 are necessary for cellular functions and ATP production, due to its involvement in ATP synthesis[(12)], and the mitochondrial respiratorychain (electron transport chain, ETC) [(13)].

Actually, the normal plasma membrane for both sperm and oocyte is very important for normal fertilization at molecular level and subsequently for normal embryonic cleavage and development [(14)]. It is known that the lipid is the component of plasma membrane and negatively affected by oxidative stress and/or presence of ROS [(15)]. Accordingly, the CoQ10 has antioxidant properties,protecting membrane lipids and proteins and mitochondrial deoxyribonucleic acid (mtDNA) against oxidative damage [(13)].CoQ10 also functions as an intercellular antioxidant at the mitochondrial level, perhaps accounting for its benefit in neurodegenerative diseases [(12)], and male infertility [(16)], 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 [(17)].

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In general, the rate of early cleavage of bovine 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 [(10)].

Most early studies assumed that the sperm mitochondria participated fully in early embryonic development. However, in 1965, Szollosi [(18)], 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 [(19)].

In this study, results of abnormal embryonic development appeared that the addition of CoQ10 has no effect on percentage abnormal embryonic development at 2-cells and 4-cells embryo stage, while significant decrease in the percentage of abnormal embryonic development at 8-cells stage as compared to the control group.Non significant differenceswere assessed among three groups in the normal morphology of early stages of mice embryos. In the present work, SMART medium was used for embryonic development in mice. However, same medium was used as medium for *in vitro* sperm activation for infertile patients [(20)], and sheep [(21)]. Same medium was used also for sperm preparation, IVM, IVF, and early embryonic development in sheep [(22)]. Additionally, CoQ10 is a promising candidate for supporting thedevelopment of *in vitro*-produced (IVP) embryos when added to the culture medium [(23)]. Effects of various concentrations of CoQ10 on early cleavage, were investigated in a chemically defined culture system [(24)]. Some of the failures of culture systems that are often overlooked are those leading to increase intracellular pH [(25)], and disturbed function of the plasma membrane ion transporter (PMIT) system [(26)]. 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 [(27)].

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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