The Correlation of Coenzyme Q10 Level in Follicular Fluid and Serum on Oocyte Retrieval and Embryo Transfer Days with Age and BMI of Women Undergo ICSI Cycle

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Abstract

Background: Patients with decreased ovarian reserve and advanced age present the most challenging circumstances for IVF treatments. Oxidative stress was suggested as one of the major contributors of poor ovarian reserve .However the basic causes are still unknown ,but it was suggested that with aging and an imbalance in redox activity, the reserve decreases along with follicular consumption. ***Purpose***: This study seeks to evaluate the correlation between Co Q 10 level and the age. ***Patients and methods***: From October 2021 to February 2021, a study was conducted on 45 infertile women who were undergoing ICSI. Patients ranged in age from 20 to 42 years old. Infertility were with a time span of 1 to 20 years. For each patient, CoQ10 level of the follicular fluid and serum were measured by ELISA kits. The morphology of the oocytes and the quality of the resulting embryo were evaluated. The protocol of all infertile females was the antagonist protocol. All females had their serum levels of AMH, Luteinizing hormone (LH), Follicle-stimulating hormone (FSH), and Oestradiol (E2) measured on the second or third day of their cycle. The serum oestradiol (E2) level was re-measured on the day of the hCG injection .***Results :*** According to general characteristics, study participants are distributed. With a mean age of 32.6 years and a standard deviation (SD) of 5.3 years, the study participants' ages ranged from 23 to 42 years. The bulk of the study's participants were younger than 35. (71.8 %). Negative correlations between age and serum and follicular CoQ10 levels were shown to be statistically significant ($P< 0.05$). Follicular fluid and serum CoQ10 levels both demonstrated statistically significant, moderately positive correlations with AMH. Statistically significant moderate negative associations ($P> 0.05$) between the levels of FSH and CoQ10 in the serum and follicular fluid were found. comparisons between two groups, one of which was made up of people over 35, and the other of which was made up of people under 35. The results demonstrated that younger age groups had higher levels of AMH and estrogen as well as thicker endometrial thickness than older groups did. LH and FSH levels were, however, lower in young women than in older women. ***Conclusion:*** In conclusion, the findings revealed a negative relationship between age and the CoQ10 concentrations in blood and follicular fluid.

Keywords: CoQ10, ICSI, Age-related disorder and BMI.

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Introduction:

The most difficult situations in IVF procedures are patients with diminished ovarian reserve and advanced age (Gat et al., 2016).Female reproductive capacity drastically decreases in the fourth decade of life as oocyte quality and quantity fall with age .Aging process is a physiological changes include diminished mitochondrial function, decreased anti-oxidant activity, and an increase in oxidative stress, which is indicated by an increase in the formation of reactive oxygen species (ROS). Damage from oxidative processes likely leads to an increase in ROS generation, which causes more oxidative damage to macromolecules like DNA and electron transport proteins (Awad et al., 2018). An oxidative imbalance between the production of reactive oxygen species (H_2O_2, superoxide, O_2•, and hydroxyl radical, or HO•) and antioxidant mechanisms like superoxide dismutase, catalase, glutathione peroxidase, ascorbic acid, tocopherol, glutathione, and CoQ10, leading to a state of oxidative stress, is a common theory to explain some of the pathophysiology of aging (Wallace et al., 2010). Coenzyme Q10, also known as CoQ10 or ubiquinone, is an isoprenoid side-chained tiny lipophilic structure that is present in all cell membranes (Awad et al., 2018). In humans, synthesis is carried out by a group of enzymes called complex Q that is located in the mitochondrial matrix membrane. All cell membranes contain CoQ10, which is a crucial part of the electron transport chain (ETC) that carries electrons between complexes I/II and III (Alcazar-Fabra et al,. 2016). CoQ10 plays a crucial anti-oxidant role in maintaining the plasma membrane and other intracellular membranes and preventing the peroxidation of membrane phospholipids(Gutierrez-Mariscal et al., 2018). Age-related declines in CoQ10 levels are likely a factor in the harm caused to membranes by peroxidation (Blatt et al., 2011). Because CoQ10 from the food has a very poor bioavailability, it is thought that the majority of tissues get their CoQ10 from endogenous synthesis (Ben-Meir et al.2015). This research aims to assess the relationship between age and Co Q 10 level.

Patients and methods:

From October 2021 to February 2021, a study was conducted on 50 infertile women who were undergoing ICSI at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/ Al-Nahrain University/ Baghdad/ Iraq, regardless of whether they had previously undergone ICSI. The morphology of the oocytes and the quality of the generated embryo were assessed in the same institute's laboratory. The age of patients was between 20 and 42. Primary and secondary infertility were also present, with a time span of 1 to 20 years. All couples underwent a basic reproductive assessment, which included a history, physical examination, hormone measurement, and the exclusion of uncontrolled endocrine problems, as well as sperm analysis for their partner. The protocol of all infertile females was the antagonist protocol. On the second or third day of their cycle, all females had their serum levels of AMH, Luteinizing hormone (LH), Follicle-stimulating hormone (FSH), and oestradiol (E2) tested. On the day after the hCG injection, the serum oestradiol (E2) level was re-measured. Co Q10 levels in serum and follicular fluid were measured in day of oocyte retrieval and re-measured in serum in day of embryo transfer. I**nclusion criteria**:- Female age between 20 and 42.,the BMI of the female within normal and under 30, the partner with adequate normal sperms that could be used for ICSI., fresh ICSI cycle. **Exclusion criteria :** Females with uncontrolled endocrine or systemic diseases, Male with severe oligoasthenospermia, azospermia or underwent testicular biopsy. **History questions includes: Medical history** (thyroid illness symptoms, diabetes, hirsutism, weight increase or loss, nipple discharge, lower abdomen or pelvic pain, mumps history, and etc.). **Past surgical history** like cesarean section, appendectomy, diagnostic or therapeutic curettage, thyroid surgery, any abdominal surgery, etc.. **Medicine history**, including medication allergies, oral contraceptive pill use, steroid, chemotherapy, radiation, and non-steroidal anti-inflammatory medications, etc. **Menstrual history** includes (age of menarche, length of cycle, character of cycle, presence of dysmenorrhea). **Past gynecological**: history of vaginal discharge and if present character of the discharge, pelvic pain, previous exposure to pelvic inflammatory disease, history of PAP smear, and Past obstetrical history (gravidity, parity, history of pregnancy induced hypertension, gestational diabetes, miscarriage and if present at which trimester, vaginal bleeding, previous history of cervical cerclage, history of birth of an abnormal child, post-partum hemorrhage, etc.). **Family history of infertility**: premature ovarian failure, diabetes mellitus, thyroid diseases, congenital abnormalities, etc. **History of marriage**: number, date of marriage, coital timing and frequency, methods of contraception if present, etc. **Fertility history**: type, duration, cause if known, any previous investigations and treatment including IVF-ICSI trials and their outcome. **Social history**: occupation, smoking, alcohol, address, animal contacts. **Examination had been done for both partners including: Body mass index BMI:** is categorized into four groups according to the conventional WHO classification: underweight (18.5 kg/m2), normal weight (18.5–24.9 kg/m2), overweight (25–29.9 kg/m2), and obese (30 kg/m2) (Lim et al.,2017) by measuring height (ht.) in meters and weight (wt.) in kilograms and applying the equation (BMI= wt./ ht2). **General examinations**: cardiovascular, Respiratory, abdominal, central nervous system, thyroid examination, breast, hirsutism. **Gynecological examination**: vaginal and cervical abnormalities, discharge, erosions, uterine size, position and mobility, adnexal and Cul- de sac mass or enderness. **Hormonal Assay**: The ovarian reserve was assessed using FSH, LH, and AMH, and the hypothalamus-pituitary-ovarian (HPO) axis function was evaluated using serum prolactin, progesterone, and 17-estradiol (E2).

Statistical analysis: The data analyzed using Statistical Package for Social Sciences (SPSS) version 26. The data presented as mean, standard deviation and ranges. Categorical data presented by frequencies and percentages. Independent t-test (two tailed) was used to compare the continuous variables accordingly. Receiver operating characteristic (ROC) curve analysis was used for CoQ10 levels at oocyte retrieval and at embryo transfer days as predictors for poor response. Pearson's correlation test (r) was used to assess correlation between continuous variables accordingly. A level of P – value less than 0.05 was considered significant.

Results:

The distribution of study patients by general characteristics is shown in Figure 1, and table 1, Study patients' age was ranging from 23 to 42 years with a mean of 32.6 years and a standard deviation (SD) of ± 5.3 years. The majority of research participants were under the age of 35. (57.8 percent). In this study, 60% of study participants had normal BMI levels; 64.4 percent of them reported having primary infertility complaints, and the majority of them had been experiencing infertility for less than 10 years (82.2 percent).

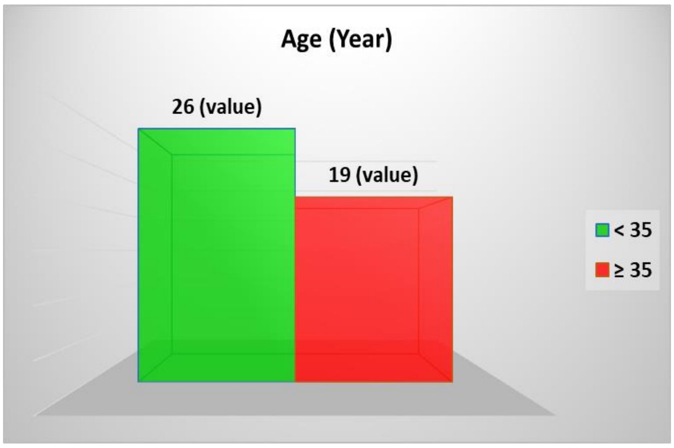

Figure 1: Distribution of study patients by age

Table 1: Distribution of study patients by general characteristics

Variable	No. (n= 45)	Percentage (%)
BMI level		
Normal	27	60.0
Overweight	18	40.0
Type of infertility		
Primary	29	64.4
Secondary	16	35.6
Duration of infertility (Year)		
< 10	37	82.2
≥ 10	8	17.8

Statistically significant negative correlations (P < 0.05) were detected between age and serum CoQ10 level at oocyte retrieval day, follicular fluid CoQ10 level at oocyte retrieval day, and of serum CoQ10 level at embryo transfer day. Statistically significant moderate positive correlations (P < 0.05) were detected between BMI and serum CoQ10 level at oocyte retrieval day, follicular fluid CoQ10 level at oocyte retrieval day, and of serum CoQ10 level at embryo transfer day. No significant correlations between duration of infertility with all of serum CoQ10 level at oocyte retrieval day, follicular fluid CoQ10 level at oocyte retrieval day, and of serum CoQ10 level at embryo transfer day (Table 2).

Table 2: Correlation between CoQ10 levels with general characteristics

Parameters \ CoQ10 levels (µg/ml)		Serum at oocyte retrieval day	Follicular F. at oocyte retrieval day	Serum at embryo transfer day
Age (Year)	**r**	- 0.326	- 0.363	- 0.309
	P - Value	**0.029**	**0.014**	**0.039**
BMI (kg/m²)	**r**	0.379	0.413	0.33
	P - Value	**0.01**	**0.005**	**0.027**
Duration of infertility (Year)	**r**	0.273	0.138	0.278
	P - Value	**0.07**	**0.366**	**0.065**

Serum CoQ10 level at oocyte retrieval day, follicular fluid CoQ10 level at oocyte retrieval day, and serum CoQ10 level at embryo transfer day all showed statistically significant moderate positive relationships with AMH (P< 0.05). Statistically significant moderate negative correlations (P < 0.05) were detected between FSH level with serum CoQ10 level at oocyte retrieval day, follicular fluid CoQ10 level at oocyte retrieval day, and of serum CoQ10 level at embryo transfer day. There was no link between the levels of LH, prolactin, progesterone, and endometrial thickness and the levels of CoQ10 in the blood, follicular fluid, or serum on the day of ovulation or the day of embryo transfer (Table 3).

Table 3 : Correlation between CoQ10 levels with general characteristics

Hormones \ CoQ10 levels (µg/ml)		Serum at oocyte retrieval day	Follicular F. at oocyte retrieval day	Serum at embryo transfer day
AMH (ng/ml)	**r**	0.604	0.611	0.554
	P - Value	**0.001**	**0.001**	**0.001**
Estradiol (pg/ml)	**r**	0.522	0.532	0.483
	P - Value	**0.001**	**0.001**	**0.001**

FSH (IU/L)	**r**	- 0.538	- 0.525	- 0.527
	P - Value	**0.001**	**0.001**	**0.001**
LH (IU/L)	**r**	0.089	0.06	0.046
	P - Value	**0.56**	**0.694**	**0.763**
Prolactin (ng/ml)	**r**	0.129	0.163	0.051
	P - Value	**0.112**	**0.104**	**0.661**
Progesterone (ng/ml)	**r**	0.219	0.198	0.252
	P - Value	**0.091**	**0.122**	**0.088**
Endometrial thickness (mm)	**r**	0.292	0.282	0.265
	P - Value	**0.052**	**0.061**	**0.079**

Discussion:

One of the first physiological processes that aging negatively affects is female fertility (Ben-Meir et al., 2015). Due to the fact that maternal aging is accompanied by a decline in oocyte mitochondrial function (Kujjo et al., 2013). Increased ROS production from oxidative processes is anticipated to enhance oxidative damage to macromolecules like DNA and electron transport proteins in mitochondria (Awad et al., 2018). In this study, mean and a standard deviation (SD) of age was 32.6 ± 5.3 years, ranging from 23 to 42 years, with the highest proportion was aged < 35 years (57.8%). Moreover, 60% of them had normal BMI level; 64.4% complained from primary infertility; and the majority of them had infertility for < 10 years' duration (82.2%). Hasan et al. presented a study in 2020 that involved 65 infertile women who underwent ICSI cycles. With a mean and SD of 30.82± 5.83 years and a range of 19 to 38 years, the highest proportion was seen in patients under the age of 35 (73.8%). Additionally, 40.0% of the patient population was overweight (Hasan et al., 2020). Additionally, 63.2 % of them had normal BMI levels, (Al-Muttalibi et al., 2019). A significant negative connection between age and serum CoQ10 level at oocyte retrieval day, follicular fluid CoQ10 level at oocyte retrieval day, and serum CoQ10 level at embryo transfer day was found in this study, according to the findings ($P < 0.05$). After 30 years of age, some human tissues' CoQ10 concentrations start to decline, which may speed up aging (Miles et al., 2004). A result demonstrated by a previous study (Barcelos et al., 2019). There was disagreement with the Akarsu et al. study from 2017, which revealed no connection between the women's ages on the day of the hCG trigger administration and their CoQ10 levels (Akarsu et al., 2017). CoQ10 concentrations were found to be decreased with advancing age and this decline coincides with a decline in fertility and the increase in embryo aneuploidies (Gat et al., 2016). The administration of CoQ10 improved breeding results, reduced follicle loss, and enhanced mitochondrial energetics in the aged animal model (Bentov et al., 2014). In the previous study showed that CoQ10 supplementation in an elderly animal model prevented the loss of ovarian reserve and enhanced mitochondrial function (Ben-Meir et al., 2015). It is unknown if low CoQ10 is a result of aging, maybe correlating with a decline in mitochondrial electron transport performance, or if it is a factor in the aging process (Barcelos et al., 2019). In a different study, it was discovered that adding CoQ10 to the culture medium might reverse the effects of aging on mouse oocytes that were past their prime (Zhang et al., 2019). The current study found significant moderate positive correlations between BMI and serum CoQ10 levels on the day of oocyte retrieval, follicular fluid CoQ10 levels on the day of oocyte retrieval, and serum CoQ10 levels on the day of embryo transfer (P< 0.05), but no significant correlations between the duration of infertility and any of the three serum CoQ10 levels on the day of oocyte retrieval, follicular fluid CoQ10 levels on the day of embryo . In contrast to what Akarsu and colleagues reported in2017, the current study showed no relationship between CoQ10 levels and the age or BMI of the women and agreed with the current findings that there was no relationship between CoQ10 levels and length of infertility (P>0.05) (Akarsu et al., 2017). Some authors said that there is a correlation between BMI and CoQ10 levels and that obesity causes oxidative stress and has been demonstrated to decrease oocyte mitochondrial function. CoQ10 concentrations are higher in subfertile, obese women's serum but not in their follicular fluid (Luzzo et al., 2012). Concerning the relationship between hormonal and clinical parameters in this study, a significant moderate positive correlation between serum CoQ10 levels on the day of oocyte retrieval, in the follicular fluid on the day of oocyte retrieval, and on the day of embryo transfer was found for both AMH and E2 levels (P< 0.05). In rodents, CoQ10 treatment reversed the cisplatin-induced ovarian damage, leading to greater serum AMH concentrations, more AMH-positive follicles, and fewer atretic follicles. Cisplatin is a chemotherapy medication used to treat ovarian, bladder, head & neck, lung, testicular, and cervical cancer(Özcan et al., 2016). Two groups were compared by Yangying et al, one was given a CoQ10 supplement and the other was given a placebo , Peak E2 serum values in the CoQ10 group were significantly higher (Yangying et al., 2018). An earlier research (Bentov et al.,2014) discovered that after 60 days of treatment with 600 mg CoQ10, there was a higher peak concentration of E2, more high-quality embryos, a trend toward lower aneuploidy, and a higher clinical pregnancy rate(Bentov et al.,2014). Moreover, a significant moderate negative correlations were detected between FSH level with serum CoQ10 level at oocyte retrieval day, follicular fluid at oocyte retrieval day, and at embryo transfer day (P<0.05) , that was in agreement with Yangying, who discovered that in the same group of women receiving CoQ10 supplementation for 60 days, basal day-3 FSH levels were considerably lower than they were before therapy (Yangying et al., 2018). In the current study, there were no significant correlations between the levels of serum CoQ10 on the day of oocyte retrieval, in the follicular fluid on the day of oocyte retrieval, or on the day of embryo transfer with either of the hormones LH, prolactin, progesterone, or endometrial thickness (at the second day). El Refaeey disagreed with current study ,which revealed that the CoQ10 group had considerably greater serum progesterone than the control group (El Refaeey et al., 2014).

Conclusion:

In summary found a negative correlation between serum and follicular fluid CoQ10 concentrations and age. Our results might be supportive of the usage of CoQ10 as a supplement in late reproductive women age undergoing in vitro fertilization. Further studies that will involve a larger, randomized, and controlled group are needed to draw definitive conclusions for the clinical administration of CoQ10 in female infertility.

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