Coenzyme Q10 in the Follicular Fluid and Its Relation to Oocyte Maturity, Fertilization Rate, Embryo Grading, and Pregnancy Rate

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Abstract

Background: Infertility was define as; a disease characterized by the failure to establish a clinical pregnancy after 12 months of regular unprotected sexual intercourse, or due to an impairment of a person's capacity to reproduce, either as an individual or with his/her partner. According to the latest definition by the international glossary on infertility and fertility care, regular sexual intercourse is an important determinant for the occurrence of pregnancy.

Aim of the study: To assess the relation between Coenzyme Q10 level in the follicular fluid with oocyte maturity, fertilization rate, embryo grading, and pregnancy rate

Patients and method: A prospective cross sectional study conducted at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies / Al-Nahrain University and in fertility center at Al-Sadr general hospital during the period from December 2019 to August 2020. Sixty infertile couples were enrolled in this study; all underwent ICSI cycles

Results: Mean level of Co Q10 in Grade III and IV (0.387 ± 0.54) than that in Grade I and II (0.539 ± 0.65), CoQ10 total in pregnant were 0.79 ± 0.63 and in non-pregnant were 0.381 ± 0.2, A threshold of 0.27 of CoQ10 had a sensitivity of 80.0% and specificity of 67.0 %, PPV was (88.0%), NPV (45%) and accuracy was (74%). ROC curve of CoQ10 for pregnancy prediction

Conclusion: Significant decrease of Co Q10 between in Grade III and IV than that in Grade I and II. CoQ10 total were increase in pregnant women than non-pregnant.

Keyword: *CoQ10, pregnant rate, oocyte maturity, fertilization rate, embryo grading.*

Introduction

Infertility was define as; a disease characterized by the failure to establish a clinical pregnancy after 12 months of regular unprotected sexual intercourse, or due to an impairment of a person's capacity to reproduce, either as an individual or with his/her partner. According to the latest definition by the international glossary on infertility and fertility care, regular sexual intercourse is an important determinant for the occurrence of pregnancy. (1)

In addition, infertility defined as a disease, which generates disability as an impairment of function based on the latest definition by the WHO. (2) Both males

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and females are equally responsible for the causes of infertility, and most of the infertile couples have one of these three major causes including a male factor, ovulatory dysfunction, or tubal peritoneal disease. (3)

If the female partner is 35 year of age or older, evaluation should be initiated after 6 months of unprotected intercourse. Fecundability, or the ability to achieve pregnancy in one menstrual cycle, is a more accurate measurement to evaluate fertility potential. The fecundity rate in a normal couple who has had unprotected intercourse is approximately 20% to 25% for the first 3 months, followed by 15% during the next 9 months. (4)

Assisted Reproductive Technology (ART) is defined as a fertility treatment in which eggs and sperm are handled for the purpose of establishing a pregnancy. (5) Assisted Reproductive Technology (ART) now enables several treatment options, including ovulation induction followed by in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). (6)

Coenzyme Q10 (CoQ10), also known as ubiquinone or ubidecarenone, is a vitamin-like nutrient and lipid-soluble compound. As its name implies, it is ubiquitous and present in all human cells. It is primarily located in the mitochondria and also found in cell membranes and lipoproteins. The primary function of CoQ10 is its role in cellular energy production, where, along the inner mitochondrial membrane, the electron transport chain (ETC) uses CoQ10 as a component in oxidative phosphorylation con- verting products of metabolism (carbohydrates, fats, and proteins) into energy as ATP.(7)

Oocyte quality and coenzyme Q10

In in vitro fertilization cycles, the most important issue was the quality of oocyte. The main factors affecting the oocyte quality are the age of the women and the status of ovarian reserve. The inappropriately age-related decrease in oocyte quality is a main difficulty in the treatment of older patients. The main reason for this decrease was related to an accumulation of point mutations and deletions of mitochondrial deoxyribonucleic ac-id (DNA). (8,9)

Aim of the Study

To assess the relation between Coenzyme Q10 level in the follicular fluid with oocyte maturity, fertilization rate, embryo grading, and pregnancy rate

Patients and Method

A prospective cross sectional study conducted at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies / Al-Nahrain University and in fertility center at Al-Sadr general hospital during the period from December 2019 to August 2020. The study was approved by the local Medical Ethical Committee of the Arab Board council/ Obstetrics and Gynecological department. Sixty infertile couples were enrolle in this study; all underwent ICSI cycles. All selected patient was subjecte to -

· The basic fertility workup of the fertility center that consists of history- taking, physical examination, ovulation detection, evaluation of tubal patency and uterine cavity, and semen analysis.

· Assessment of follicular fluid Coenzyme Q10 level in the day of ova pick up.

Inclusion criteria:

· Age 18-40 years old.

· Early follicular phase FSH, LH, cycle day 2 E2, TSH and prolactin (PRL) hormonal level which was done as part of the work up must be within normal.

· Unexplained infertility or tubal function infertility

· Fresh transfer

· Informed consent

· Regular Menstrual cycle

· Normal ovulatory function

· Normal BP, not smoking, not taking any supplements

Exclusion Criteria:

· Age >40 years old.

· Abnormal uterine cavity due to polyp myoma, or congenital anomalies, endometriosis.

· Uncontrolled systemic disease as diabetes

mellitus, HT, or uncontrolled endocrinological disorder.

- Women with empty follicle syndrome.
- Women with no fertilized oocyte.
- Couples with semen collection failure at oocyte retrieval day
- Male factor infertility
- Diminished ovarian reserves as assessed by AFC < 5 and AMH < 1.2 ng/dl.

Statistical Analysis

Collected data were analyzed using SPSS version 23.0 for windows (SPSS Statistics, IBM, USA) and the results were expressed as mean ± standard deviation (SD).Differences of means within groups were examined by paired sample t-test. P values < 0.05 were considered as statistically significant

Results

Pregnancy were happened in 33/60 patients (55%) and 27/60 (45%) were not pregnant (fig 1)

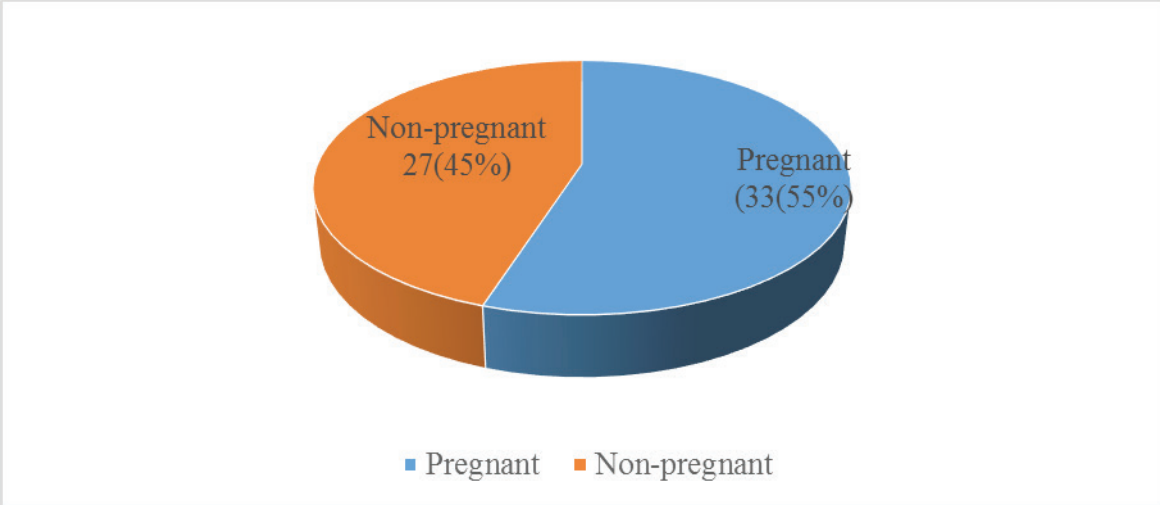

Fig 1: Pregnancy outcome in the studied group

Oocytes characteristics in pregnant and non-pregnant ladies

Table 1 , demonstrated the oocyte characteristics of pregnant and non-pregnant ladies. The mean total number of retrieved oocytes in pregnant ladies was 9.90 ±6.34; and that of non-pregnant women was 8.61 ± 3.88 the difference was statistically not significant (P=0.37). However, mean of number of abnormal and ruptured oocytes was significantly lower in women who succeeded to get pregnant when compared to that of women who failed to get pregnant, 1.02 ±0.51 versus 1.48 ±1.06, respectivel (P=0.04). There was also no significant difference in mean germinal vesicle oocyte number between both groups, 1.18 ±1.02 versus 1.10 ±0.92, respectively (P=0.7). The difference in mean MI and MII oocyte numbers between both groups were also not significant, 1.41 ±1.34 versus 1.32±1.06 and 6.49 ±3.51 versus 6.01 ±2.38, respectively (P > 0.05). In addition, there was no significant difference in mean number of injected oocytes between pregnant and non-pregnant women, 7.22±4.43 versus 6.51±2.46 (P = 0.47).

Table 1: Oocyte characteristics in the studied group

GV	1.18 ±1.02	1.10 ±0.92	0.7 NS
M I	1.41 ±1.34	1.32 ±1.06	0.82 NS
M II	6.49 ±3.51	6.01 ±2.38	0.5 NS
Number of injected oocyte	7.22 ±4.43	6.51 ±2.46	0.47 NS

n: number of cases; †: Independent samples t-test; NS: not significant at P > 0.05; S: significant at P ≤ 0.05; GV: germinal vesicle; MI: metaphase I oocytes; MII: metaphase II oocytes

n: number of cases; †: Independent samples t-test; NS: not significant at P > 0.05; S: significant at P ≤ 0.05; GV: germinal vesicle; MI: metaphase I oocytes; MII: metaphase II oocytes

Fertilization rate and embryo characteristics in pregnant and non-pregnant women

These characteristics were shown in table (2). The difference in mean fertilization rate between pregnant and non-pregnant women was statistically not significant, 74.01 ±21.38 versus 68.91±15.67, respectively (p=0.2). In addition, there was significant difference in mean number of grade I and II embryos between pregnant and non-pregnant groups, 3.87±1.03 versus 2.01 ±1.09, respectively (P= <0.001). Moreover, there was significant difference in mean number of grade III and IV embryos between pregnant and non-pregnant groups, 0.85±0.61 versus 1.92±0.89, respectively (P= <0.001).

Table 2: Fertilization rate and embryo quality

Characteristics	**Pregnant (n=33)**	**Non pregnant (n=27)**	**P value †**
Fertilization rate (%)	74.01 ±21.38	68.91 ±15.67	0.2 NS
Number of GI and GII embryos	3.87 ±1.03	2.01 ±1.09	< 0.001 S
Number of GIII and GIV embryos	0.85 ±0.61	1.92 ±0.89	< 0.001S

n: number of cases; †: Independent samples t-test; S: significant at P ≤ 0.05

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Coenzyme Q 10 levels in follicular fluid and with pregnancy result

The mean concentration of coenzyme Q 10 in the grade I and II was 0.539 ± 0.65 ng/ml while for grade III and IV was 0.387 ± 0.54. Significant decrease in CoQ10 in grade III and IV than that in grade I and II (P=0.03)(Table 3).

Table 3: The association between coenzyme Q 10 levels in follicular fluid with embryo quality

		CoQ10 (ng/ml)	P value
Embryo quality	Grade I and II	0.539 ± 0.65	0.03
	Grade III and IV	0.387 ± 0.54	

Coenzyme Q 10 levels in follicular fluid with pregnancy results

The mean level of Coenzyme Q 10 in pregnant women was 0.79 ± 0.63 and in non-pregnant women was 0.381 ± 0.21 with significant increase in pregnant women (P=0.002) (Table 4)

The mean level of Coenzyme Q 10 in women within the age < 35 years old was 0.66± 0.23 and in women with age ≥ 35 years was 0.521 ± 0.21 with significant difference were found (P=0.04) (Table 5)

Table 4: The differences between coenzyme Q 10 levels in follicular fluid with pregnancy results of the studied group

	Pregnant (n=33)	Non-pregnant (n=27)	P value
CoQ10 (ng/ml)	0.79 ± 0.63	0.381 ± 0.21	0.002

Table 5: The differences between coenzyme Q 10 levels in follicular fluid with age of the studied group

	<35 years (n=45)	≥ 35 years (n=15)	P value
CoQ10 (ng/ml)	0.66± 0.23	0.521 ± 0.21	0.04

The area under curve (AUC) of serum levels of CoQ10 for expectation of pregnancy was (0.80) (p = 0.002, 95% confidence interval 0.63–0.91). A threshold of 0.27 of CoQ10 had a sensitivity of 80.0% and specificity of 67.0 %, PPV was (88.0%), NPV (45%) and accuracy was (74%). ROC curve of CoQ10 for pregnancy prediction is shown in Fig. 2.

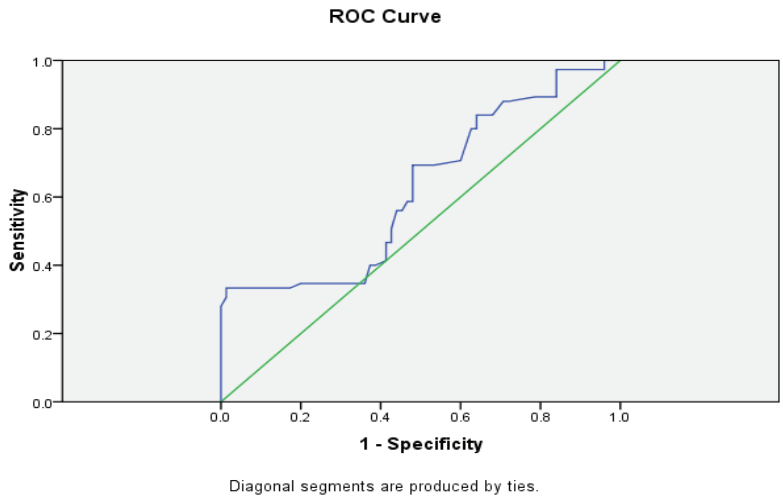

Fig 2: ROC curve for serum Coenzyme Q10 for prediction of pregnancy (AUC=0.8).

Discussion

As we show in the current study, there was no significant difference in the mean dose of gonadotropin and duration of stimulation between pregnant or non-pregnant, which is in agreement with many other previous studies and these findings confirm the findings of other studies. (10-12)

However, it is not agreed with other studies that mentioned prolonged duration of gonadotropin stimulation is an independent negative predictor of ART success and obese women with BMI >30 kg/m2 required a higher dose of gonadotropin for stimulation and they face a lower likelihood of pregnancy after ICSI. (13,14)

As for oocytes characteristics in pregnant and non-pregnant ladies in the present study we found that there is a significant association were found between mean of number of abnormal and ruptured oocytes was in women who failed to get pregnant when compared to that of women who succeed to get pregnant. This is similar to the results were obtained by several other studies. (15, 16). But it is in contrast to another studies showed that the dark zona pellucida (DZP) does not affect the fertilization, embryo quality, or pregnancy rate. (17, 18)

In the present study, we found that there is significant difference in mean number of grade I and II embryos between pregnant and non-pregnant groups. Moreover, there was significant difference in mean number of grade III and IV embryos between pregnant and non-pregnant groups. which is similar to that found in may previous studies that have found better quality embryo and the number of embryos were statistically significant predictors of clinical pregnancy. (19,20)

The current study shows that the mean level of Coenzyme Q 10 in pregnant women was significantly increase than that in non-pregnant women. Turi A, et al, 2012 found that Protein levels of CoQ10/ concluded significantly in mature versus dysmorphic oocytes. Similarly, CoQ10/Cholesterol was significantly increase in grading I–II against grading III–IV embryos. (21) And total levels of CoQ10 were higher in follicular fluids related with mature oocyte and high-grade embryos, telling a possible correlation to the mechanisms of control and growth in follicular ambient. As reported in sperimental in vitro cultures of myocardial cells, the CoQ10 stimulated the formation of ATP that in reproductive biology could accelerate formation of the blastocoels cavity and consequently the hatching process (22, 23), second the existence of CoQ10 may precise ionic imbalance that happens in cultures of embryos. (24)

Giannubilo SR et al, 2018 concluded that CoQ10 of oral supplementation might improve follicular fluid oxidative metabolism and oocyte quality, especially in over 35-year-old women. (25)

Conclusion

Significant decrease of Co Q10 between in Grade III and IV than that in Grade I and II**,** CoQ10 total were increased in pregnant women than non-pregnant

Conflicts of Interest: No

Source of Funding: Self

Ethical Clearance: was taken from the scientific committee of the Iraqi Ministry of health

References

1. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, et al. The international glossary on infertility and fertility care, 2017. Human reproduction. 2017 Sep 1;32(9):1786-801.
2. Vander Borght M, Wyns C. Fertility and infertility: Definition and epidemiology. Clinical biochemistry. 2018 Dec 1;62:2-10.
3. Anwar S, Anwar A. Infertility: a review on causes, treatment and management. Women's Health Gynecol. 2016; 5:2.
4. Callahan T, Caughey AB. Blueprints obstetrics and gynecology. Lippincott Williams & Wilkins; 2013 Jan 28. 20:346-55.
5. Sunderam S, Kissin DM, Crawford SB, Folger SG, Jamieson DJ, Warner L, Barfield WD. Assisted reproductive technology surveillance—United States, 2012. Morbidity and Mortality Weekly Report: Surveillance Summaries. 2015 Aug 14;64(6):1-29.
6. Estill MS, Bolnick JM, Waterland RA, Bolnick AD, Diamond MP, Krawetz SA. Assisted reproductive

technology alters deoxyribonucleic acid methylation profiles in bloodspots of newborn infants. Fertility and sterility. 2016 Sep 1;106(3):629-39.

7. Rodick TC, Seibels DR, Babu JR, Huggins KW, Ren G, Mathews ST. Potential role of coenzyme Q 10 in health and disease conditions. Nutrition and Dietary Supplements. 2018 Feb 14;10:1-1.
8. Bentov Y, Casper RF. The aging oocyte—can mitochondrial func-tion be improved? Fertil Steril. 2013;99(1):18–22.
9. Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, RovioAT, Bruder CE, et al. Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature. 2004;429:417–23.
10. Purandare, N., Emerson, G., Kirkham, C., Harrity, C., Walsh, D. and Mocanu, E., (2017). The duration of gonadotropin stimulation does not alter the clinical pregnancy rate in IVF or ICSI cycles. Irish Journal of Medical Science, (1971-), 186(3), pp.653-657.
11. Khalid, S., Kelly, J., Sutton, F., Harrity, C., Purandare, N., Deignan, K., Coughlan, C. and Mocanu, E., (2014). In GnRH antagonist treatment does gonadotropin dose reduction during stimulation affect clinical pregnancy rate?. Fertility and Sterility, 102(3), pp.e219-e220.
12. Barash, O.O., Hinckley, M.D., Rosenbluth, E.M., Ivani, K.A. and Weckstein, L.N., (2017). High gonadotropin dosage does not affect euploidy and pregnancy References 83 rates in IVF PGS cycles with single embryo transfer. Human Reproduction, 32(11), pp.2209-2217.
13. Chuang M, Zapantis A, Taylor M, Jindal SK, Neal-Perry GS, Lieman HJ, Polotsky AJ. Prolonged gonadotropin stimulation is associated with decreased ART success. Journal of assisted reproduction and genetics. 2010 Dec 1;27(12):711-7.
14. Ozgun MT, Uludag S, Oner G, Batukan C, Aygen EM, Sahin Y. The influence of body mass index on FSH dose and pregnancy rate in women undergoing ICSI-embryo transfer. Journal of the Turkish German Gynecology Association. 2009 Mar 1;10(1):1-5.
15. Shi W, Xu B, Wu LM, Jin RT, Luan HB, Luo LH. Oocytes with a dark zona pellucida demonstrate lower fertilization, implantation and clinical pregnancy rates in IVF/ICSI cycles. PloS one. 2014;9(2).
16. Rienzi L, Ubaldi FM, Iacobelli M, Minasi MG, Romano S, Ferrero S, et al. Significance of metaphase II human oocyte morphology on ICSI outcome. Fertility and sterility. 2008 Nov 1; 90(5):1692-700.
17. Balaban B, Ata B, Isiklar A, Yakin K, Urman B. Severe cytoplasmic abnormalities of the oocyte decrease cryosurvival and subsequent embryonic development of cryopreserved embryos. Human reproduction. 2008 Aug 1;23(8):1778-85.
18. Hafiz P, Nematollahi M, Boostani R, Jahromi BN. Predicting implantation outcome of in vitro fertilization and intracytoplasmic sperm injection using data mining techniques. International journal of fertility & sterility. 2017 Oct;11(3):184.
19. Karabulut S, Yilmaz E, Korkmaz O, Keskin I. Effects of Early Embryo Cleavage on Embryo Quality and Pregnancy Outcome. Ann Infert Rep Endocrin. 2018; 1 (2).;1010.
20. Shen S, Khabani A, Klein N, Battaglia D. Statistical analysis of factors affecting fertilization rates and clinical outcome associated with intracytoplasmic sperm injection. Fertility and sterility. 2003 Feb 1;79(2):355-60.
21. Turi A, Giannubilo SR, Brugè F, Principi F, Battistoni S, Santoni F, Tranquilli AL, Littarru G, Tiano L. Coenzyme Q10 content in follicular fluid and its relationship with oocyte fertilization and embryo grading. Archives of gynecology and obstetrics. 2012 Apr 1;285(4):1173-6.
22. Kishi T, Okamoto T, Takahashi T, Goshima K, Yamagami T. Cardiostimulatory action of coenzyme Q homologues on cultured myocardial cells and their biochemical mechanisms. The clinical investigator. 1993 Aug 1;71(8):S71-5.
23. Soom AV, Boerjan ML, Bols PE, Vanroose G, Lein A, Coryn M, Kruif AD. Timing of compaction and inner cell allocation in bovine embryos produced in vivo after superovulation. Biology of reproduction. 1997 Nov 1;57(5):1041-9.
24. Dumoulin JC, van Wissen LC, Menheere PP, Michiels AH, Geraedts JP, Evers JL. Taurine acts as an osmolyte in human and mouse oocytes

and embryos. Biology of reproduction. 1997 Mar 1;56(3):739-44.

25. Giannubilo SR, Orlando P, Silvestri S, Cirilli I, Marcheggiani F, Ciavattini A, Tiano L. CoQ10 supplementation in patients undergoing IVF-ET: the relationship with follicular fluid content and oocyte maturity. Antioxidants. 2018 Oct;7(10):141.