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The Effect of L- Carnitine on Oocyte Maturity in Women with Polycystic Ovarian Syndrome Undergoing ICSI

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ABSTRACT

Due to malfunction of the hypothalamus-pituitary-ovary axis and probable dysregulation of ovarian activities, ovary has a large number of small follicles but no developed follicles. L-Carnitine is a tiny, water-soluble chemical that plays a critical role in fat metabolism. It is required for appropriate mitochondrial fatty acid oxidation and acyl-CoA ester excretion, as well as affecting ATP levels. The current study was aiming at evaluating the protective effect of L-Carnitine (LC) against deleterious substances present in serum of patients with PCOS, which may affect the oocyte maturation. 60 infertile women diagnosed with polycystic ovarian syndrome have been enrolled in this study and enter their Intracytoplasmic Sperm Injection (ICSI) cycle; they were informed about the study and signed a written informed consent. They were grouped into treatment group (n=30) who were treated with L-Carnitine® tablet for two months and placebo group (n=30). All enrolled women underwent flexible antagonist protocol for ICSI. Significant increase in mean mature metaphase II (MII) oocyte count in study group than placebo group, oocyte maturity rate was significantly higher in study group in comparison with placebo group. In addition, mean fertilized (2PN) oocyte count was increased significantly in study group in comparison with placebo group. Mean fertilization rate was significantly higher in study group in comparison with placebo group. Treatment with of patients with polycystic ovary syndrome of L-Carnitine resulted better oocyte quality and the improvement in oocyte quality is reflected by mature MII oocytes, maturity rate and fertilized rate with eventual improvement in embryo quality.

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KEYWORDS

L Carnitine, Oocyte Maturity, Polycystic Ovarian Syndrome, ICSI

1. Introduction

Polycystic ovarian syndrome (PCOS) is a diverse endocrine illness that affects roughly one in every 15 women globally. Excessive androgen secretion or activity is the most common endocrine disturbance, and a high percentage of women also have abnormal insulin activity (Carmina et al. [1]). Polycystic ovary syndrome is a common cause of female infertility and menstrual irregularities. Due to malfunction of the hypothalamus- pituitary ovary axis and probable dysregulation of ovarian activities such as follicle selection or follicular atresia, PCOS patients' ovary has a large number of small follicles but no grown follicles (Glintborg and Andersen [2]).

The pathophysiology of polycystic ovarian syndrome is considered to include abnormal granulosa cell death; however, the underlying cellular and molecular pathways are unknown. Developing follicles in PCOS suffer from atresia, which is likely caused by high androgen levels. The removal of atretic follicles happens in the absence of tissue injury or inflammation, implying that programmed cell death is involved in the process (Yin et al and Maciel et al. [4, 5]).

L-Carnitine is a quaternary ammonium compound that may be generated from the two amino acids lysine and methionine. It is a small, water-soluble molecule that plays a critical function in fat metabolism. It impacts adenosine triphosphate (ATP) levels and is required for optimal mitochondrial fatty acid oxidation and excretion of acyl-CoA esters. L-Carnitine has the ability to maintain mitochondrial membranes, enhance energy supply to the organelle, and protect cells from apoptosis (Stibera et al and Zhang et al [6, 7]).

Therefore, the current study was aiming at evaluating the protective effect of L-Carnitine (LC) against deleterious substances present in serum of patients with PCOS, which may affect the oocyte maturation.

2. Materials and Methods

This prospective comparative case control study was conducted in High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University and Al-Farah Specialist Fertility

Center. Sixty infertile women diagnosed with polycystic ovarian syndrome have been enrolled in this study and enter their ICSI cycle; they were informed about the study and signed a written informed consent. All patients were subjected to a full history taking, complete general and gynecological examination and full infertility investigations including: husband's seminal fluid analysis, hormonal assay, trans-vaginal ultrasound, saline, hysterosongraphy and/or hystrosalpingography for evaluation of uterine cavity and tubal patency. Enrolled women underwent controlled ovarian hyper stimulation (COH) for ICSI cycle. They were grouped into treatment group (n = 30) who were treated with L-Carnitine® tablet for two months and placebo group (n = 30). All enrolled women underwent flexible antagonist protocol for ICSI. The diagnosis of PCOS depends on having at least two of three criteria based on the Rotterdam consensus workshop group. Women with evidence of endocrine abnormalities were excluded from study as well as women with tubal blockage.

Data were analyzed using SPSS version 16 and Microsoft Office Excel 2007. Categorical variables were presented as number and percentage while numeric variables were expressed as mean, range and standard deviation. Independent samples *t*-test was used to compare means of numeric variables between study groups. Chi-square test and Yates correction tests were used to compare proportion between study groups. The level of significance was set at $\leq p$ 0.05.

3. Results

The demographic characteristics of infertile women enrolled in the present study are shown in Table 1. The study included 60 PCOS women who were randomly allocated into two groups, study group (n = 30) and placebo group (n = 30). The mean age of all enrolled infertile women was 29.45 ±3.51 years and there was no significant difference in mean age between study group and placebo group (p = 0.071). The mean body mass index (BMI) of all enrolled PCOS women was 27.73 ±3.29 kg/m^2 and there was no significant

difference in mean BMI between study group and placebo group (p = 0.838).

The mean age at time of menarche of all enrolled infertile women was 13.20 ±1.16 years and non-significant difference was noticed in mean age at time of menarche between study group and placebo group (p = 0.270). According to type of infertility, the study included 50 (83.3 %) with primary infertility and 10 (16.7 %) with secondary infertility and there was no significant difference in the frequency distribution of infertile women according to type of infertility between study group and placebo group (p = 0.166). The mean duration of infertility of all enrolled infertile women was 6.77 ±2.82 years and there was no significant difference in mean duration of infertility between study group and placebo group (p = 0.998). According to previous IVF trials, the study included 55 (91.7 %) with previous single trial and 5 (8.3 %) with previous two trials and there was no significant difference in the frequency distribution of infertile women according to previous ICSI trials between study group and placebo group (p = 0.167).

Serum hormonal levels of infertile women enrolled in the present study are shown in table 2. The mean serum LH of all enrolled infertile women was 7.41±2.49 mIU/ml with non-significant difference in mean of serum LH of study group and placebo group (p = 0.520). Mean serum FSH of all enrolled infertile women was 4.97 ±1.28 mIU/ml, non-significant difference was observed in mean of serum FSH between study group and placebo group (p = 0.115). The mean serum estradiol (E_2) of all enrolled infertile women was 50.45 ±34.78 pg/ml with no significant difference in mean of serum E_2 between study group and placebo group (p = 0.804). The mean serum AMH of all enrolled infertile women was 5.72 ±2.42 ng/ml, non-significant difference was also noticed in mean of serum AMH between study group and placebo group (p =0.399).

Table (1): Demographic characteristics of infertile women enrolled in the present study

Characteristic	Total *n* = 60	Study group *n* = 30	Placebo group *n* = 30	*p*
Age (years)				
Mean ±SD	29.45 ±3.51	28.63 ±3.37	30.27 ±3.50	0.071 I
Range	23 -35	23 -34	23 -35	NS
BMI (kg/m^2)				
Mean ±SD	27.73 ±3.29	27.82 ±3.25	27.64 ±3.39	0.838 I
Range	22.01 -37.8	22.8 -34.6	22.01 -37.8	NS
Onset of Menarche (years)				
Mean ±SD	13.20 ±1.16	13.37 ±1.33	13.03 ±0.96	0.270 I
Range	11 -16	11 -16	12 -16	NS
Type of infertility				
Primary, *n* (%)	50 (83.3 %)	27 (90.0 %)	23 (76.7 %)	0.166 C
Secondary, *n* (%)	10 (16.7 %)	3 (10.0 %)	7 (23.3 %)	NS
Duration of infertilit(years)				
Mean ±SD	6.77 ±2.82	6.77 ±2.98	6.77 ±2.71	0.998 I
Range	3 -15	4 -15	3 -13	NS
Previous ICSI trials				
Single, *n* (%)	55 (91.7 %)	26 (86.7 %)	29 (96.7 %)	0.167 Y
Two, *n* (%)	5 (8.3 %)	4 (13.3 %)	1 (3.3 %)	NS

n: number of cases; **SD**: standard deviation; **BMI**: body mass index; **ICSI**: Intracytoplasmic Sperm Injection; **I**: independent samples *t*-test; **C:** chi-square test; **Y**: Yates correction for continuity; **NS**: not significant at $p > 0.05$

The mean serum prolactin of all enrolled infertile women was 12.14 ±5.42 ng/ml and there was no significant difference in mean serum prolactin between study group and placebo group (p = 0.333). The mean serum TSH of all enrolled infertile women was 1.75 ±0.91 mIU/L and there was no significant difference in mean serum TSH between study group and placebo group (p = 0.957). Oocyte characteristics of infertile women enrolled in the present study are shown in Table 3. There was no significant difference in mean total retrieved oocyte count between study group and placebo group (p = 0.839). There was also no significant difference in mean germinal vesicle (GV) oocyte count between study group and placebo group (p = 0.817). In addition, there was also no significant difference in mean immature metaphase I (MI) oocyte count between study group and placebo group (p = 0.807). However, the mean mature metaphase II (MII) oocyte count was significantly higher in study group in comparison with placebo group, 9.17 ±2.63 versus 7.27 ±3.49, respectively (p = 0.021) and the oocyte maturity rate was also significantly higher in study group in comparison with placebo group, 84.52 ±13.65 % versus 62.30 ±16.56 %, respectively (p < 0.001). In addition, the mean fertilized (2PN) oocyte count was significantly higher in study group in comparison with placebo group, 7.93 ±2.63 versus 6.77 ±3.07, respectively (p = 0.042). Moreover, the mean fertilization rate was significantly higher in study group in comparison with placebo group, 66.79 ±14.05 % versus 56.67 ±19.05 %, respectively (p = 0.035).

Table (2): Serum hormonal levels of infertile women enrolled in the present study

Characteristic	Total *n* = 60	Study group *n* = 30	Placebo group *n* = 30	*p*
LH (mIU/ml)				
Mean ±SD	7.41±2.49	7.62 ±2.11	7.20 ±2.84	0.520 I NS
Range	2.06 -12.8	2.8 -11.3	2.06 -12.8	
FSH (mIU/ml)				
Mean ±SD	4.97 ±1.28	5.23 ±1.32	4.71 ±1.20	0.115 I NS
Range	2.10 -8.50	3.40 -8.50	2.10 -7.60	
E_2(pg/ml)				
Mean ±SD	50.45 ±34.78	51.58 ±38.84	49.33 ±30.83	0.804 I NS
Range	21.40 - 195.00	21.40 -159.00	25.00 -195.00	
AMH (ng/ml)				
Mean ±SD	5.72 ±2.42	5.99 ±2.83	5.46 ±1.94	0.399 I NS
Range	2.05 -16	2.8 -16	2.05 -9	
Prolactin (ng/ml)				
Mean ±SD	12.14 ±5.42	12.82 ±6.60	11.45 ±3.91	0.333 I NS
Range	2.00 -30.96	2.00 -30.96	2.70 -23.30	
TSH (mIU/L)				
Mean ±SD	1.75 ±0.91	1.76 ±0.94	1.74 ±0.89	0.957 I NS
Range	0.60 -3.70	0.70 -3.70	0.60 -3.70	

n: number of cases; **SD**: standard deviation; **LH**: luteinizing hormone; **FSH**: follicle stimulating hormone; **E_2**: estradiol; **AMH**: anti-Mullerian hormone; **TSH**: thyroid stimulating hormone; **I**: independent samples *t*-test; **NS**: not significant at $p > 0.05$.

Embryo characteristics of infertilewomen enrolled in the current study are shown in Table 4. There was no significantdifference in mean total count of embryos between study group and placebo group (p = 0.0301). However, good quality embryo (grade I) count was significantly higher in study group than in placebo group, 1.70 ±1.17 versus 1.07 ±0.98, respectively (p = 0.035), as shown in figure 4.5, but there was no significant difference in grade II and grade III embryo mean count between study group and placebo group ($p > 0.05$). There was also no significant difference in transferred embryo mean count and frozen embryo count between study group and placebo group ($p > 0.05$).

Table (3): Oocyte characteristics of infertile women enrolled in the present study

Characteristic	Total *n* = 30	Study group *n* = 30	Placebo group *n* = 30	*p*
Count of retrieved oocytes				
Mean ±SD	11.13 ±3.77	11.03 ±3.17	11.23 ±4.34	0.839 I
Range	4 -20	5 -16	4 -20	NS
GV oocyte count				
Mean ±SD	2.18 ±1.65	2.13 ±1.61	2.23 ±1.72	0.817 I
Range	0 -7	0 - 5	0 -7	NS
Immature metaphase I oocyte count				
Mean ±SD	1.77 ±0.68	1.80 ±0.86	1.75 ±0.56	0.807 NS
Range	0 -4	0 -4	0 -3	
Mature metaphase II oocyte count				
Mean ±SD	8.22 ±3.21	9.17 ±2.63	7.27 ±3.49	0.021 I S
Range	0 -15	5 -14	0 -15	
Oocyte maturity rate %				
Mean ±SD	73.41 ±18.76	84.52 ±13.65	62.30 ±16.56	< 0.001 I HS
Range	0 -100	45.45 -100	0 -83.33	
Fertilized (2PN) oocytes count				
Mean ±SD	7.85 ±2.83	7.93 ±2.63	6.77 ±3.07	0.042 I
Range	0 -12	3 -12	0 -12	NS
FR%				
Mean ±SD	61.73 ±17.09	66.79 ±14.05	56.67 ±19.05	0.035 I S
Range	0 -85.7	27.2 -85.7	0 -81.8	

n: number of cases; **SD**: standard deviation; **GV**: germinal vesicle; **2PN**: 2 pronuclei; **FR**: fertilization rate; **I**: independent samples *t*-test; **NS**: not significant at $p > 0.05$

Table (4): Embryo characteristics of infertile women enrolled in the current study

Characteristic	Total *n* = 30	Study group *n* = 30	Placebo group *n* = 30	*p*
Total count of embryos				
Mean ±SD	4.77 ±2.23	5.07 ±2.18	4.47 ±2.27	0.301 I
Range	0 -9	2 -9	0 -9	NS
Grade 1 embryo count				
Mean ±SD	1.38 ±1.09	1.70 ±1.17	1.07 ±0.98	0.035 I S
Range	0 -3	0 -3	0 -3	
Grade 2 embryo count				
Mean ±SD	2.33 ±1.05	2.50 ±0.90	2.17 ±1.18	0.223 I
Range	0 -5	0 -4	0 -5	NS
Grade 3 embryo count				
Mean ±SD	1.03 ±1.07	1.00 ±1.20	1.07 ±0.94	0.812 I
Range	0 -4	0 -4	0 -4	NS
Transferred embryos count				
Mean ±SD	2.50 ±1.00	2.73 ±0.83	2.27 ±1.11	0.070 I
Range	0 -4	0 -4	0 -3	NS
Frozen embryo count				
Mean ±SD	0.42 ±1.03	0.17 ±0.38	0.67 ±1.37	0.059 I
Range	0 -4	0 -1	0 -4	NS

n: number of cases; **SD**: standard deviation; **I**: independent samples *t*-test; **NS**: not significant at $p > 0.05$

3. Discussion

In In this study and with respect to serum hormonal levels, there was no significant difference in mean serum LH, FSH, E2, AMH, prolactin and TSH, between L-Carnitine group and placebo group. Therefore, it can be concluded that treatment with L-Carnitine did not affect serum hormonal characteristics significantly, but serum estradiol at day of hCG trigger has shown certain improvement or rise in study group.

In the current study, L-Carnitine resulted in significant increase in mature metaphase II oocyte count, oocyte maturity rate, and fertilized oocyte count and fertilization rate. Pregnancy outcome following ICSI is in patients with PCOS and its association with oocyte quality has been discussed by previous authors (Raheem et al. Ludwig et al. [8,9]) and it has been shown that the better the oocyte quality, the higher the pregnancy outcome is.

According to (Sheida et al. [11]), there was no significant improvement in in mature metaphase II oocyte count, oocyte maturity rate, and fertilized oocyte count and fertilization rate in clear discrepancy to our findings; however, according to (Ismail et al. [11]), significant improvement in oocyte quality was observed and this finding supports the findings of the current study. Therefore, according to present study results, we favor the positive impact of L-Carnitine on oocyte quality because this will certainly, at least partially, explain the main important outcome of ICSI cycle. The higher count of good quality embryos was significantly associated with L-Carnitine group, therefore, we can suggest that improvement in oocyte quality has resulted in improvement in embryo quality.

The presence of significant difference in the current study suggests that L-Carnitine treatment has a role in improving oocyte quality of women with

PCOS undergoing ART and this observation is inconsistent with (Sheida et al. [11]) who stated that “the number of retrieved and MII oocytes as well as the number of 2PNs and obtained embryos were similar between groups (p > .05). Moreover, oocyte maturity rate (0.85 ± 0.38 vs. 1.02 ± 0.90), fertilization proportion

(0.62 ± 0.44 vs. 0.80 ± 0.86), fertilization rate (0.70 ± 0.22 vs. 0.76 ± 0.19), were all comparable between L-Carnitine and control groups respectively ($p > .05$)". Nevertheless, our observation is similar observation was of Ismail *et al* in 2014 (Ismail et al. [12]) who described higher number of stimulatedfollicles reaching ≥17 mm diameter and betteroocyte quality and embryo quality as reflected by significantly higher pregnancy rate in L-Carnitine group. Thus, taking current study observation in addition to that of (Ismail et al. [12], Alzaidi, Z et al.,2021[10]), the addition of L-Carnitine to treatment protocol of PCOS women resulted significant improvement in oocyte characteristics and eventually quality of embryos bringing insight to another mechanism for L-Carnitine to improve pregnancy outcome in PCOS women.

5. Conclusions

Treatment of patients with PCOS resulted in improvement of estradiol level at day of hCG trigger reflecting better oocyte quality and the improvement in oocyte quality is reflected by mature MII oocytes, maturity rate and fertilized rate with eventual improvement in embryo quality.

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Conflict of Interest

The authors declare no conflict of interest.

Ethical Clearance

The study was approved by the Ethical Approval Committee.

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