RESEARCH ARTICLE

# The Effect of L-carnitine on Apoptotic Markers (Annexin V and Clusterin) in Polycystic Ovarian Syndrome Women undergoing ICSI

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***Received: 10th October, 2022; Revised: 30th October, 2022; Accepted: 20th November, 2022; Available Online: 25th December, 2022***

## ABSTRACT

**Background:** Despite abnormal apoptosis of granulosa cells is believed to have a role in the pathophysiology of polycystic ovarian syndrome (PCOS), the cellular and molecular processes behind this are unknown. Atresia occurs in developing PCOS follicles, likely facilitated by increased androgen levels. Atretic follicles are eliminated in the absence of tissue injury or inflammation, suggesting that programmed cell death may be the mechanism behind this process.

**Aim of the Study:** The current study evaluated the protective effect of L-carnitine (LC) against programmed cell death and, eventually, endometrial receptivity.

**Patients and Methods:** This prospective case-control research was performed at the Al-Nahrain University's high institute for infertility diagnosis and assisted reproductive technologies and the Al-Farah Specialist Fertility Center. Sixty women diagnosed with the polycystic ovarian syndrome were recruited in this research and began their IVF/ICSI cycle; clusterin and annexin V were measured as apoptotic markers in the early follicular phase of the cycle CD2-in the serum and in the follicular fluid on the day of ova pickup. Endometrial thickness was assessed by ultrasound examination.

**Results:** Both serum annexin V and clusterin were decreased significantly after treatment ($p< 0.05$). The mean endometrial thickness in all enrolled women was 5.30 ± 0.70 mm. There was no significant difference in mean endometrial thickness between the study and placebo groups, 5.43 ± 0.63 *versus* 5.37 ± 0.66 mm, respectively ($p = 0.441$). There was no significant difference in mean serum annexin V ($p = 0.101$), but follicular fluid annexin V and serum and follicular fluid clusterin were lower in the study group than in the placebo group in a significant manner ($p< 0.05$).

**Conclusion:** The treatment of patients with PCOS resulted in lowering serum and follicular fluid annexin V and clusterin, indicating a significant reduction in oxidative stress and apoptosis, leading to better quality oocytes, better quality embryos and improved endometrial receptivity and embryo implantation.

**Keywords:** Annexin V, Clusterin, ICSI, L-carnitine, Polycystic ovarian syndrome

International Journal of Drug Delivery Technology (2022); DOI: 10.25258/ijddt.12.4.33

**How to cite this article:** Alhussien ZA, Mossa HAL, Abood MS. The Effect of L-carnitine on Apoptotic Markers (Annexin V and Clusterin) in Polycystic Ovarian Syndrome Women undergoing ICSI. International Journal of Drug Delivery Technology. 2022;12(4):1682-1686.

**Source of support:** Nil.

**Conflict of interest:** None

## INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a complex medical issue with the following principal characteristics: menstrual irregularities, elevated androgen levels, and/or small cysts in one or both ovaries. The disorder can be predominantly biochemical (hyperandrogenemia) or morphological[1,2] (polycystic ovaries). PCOS is a heterogeneous disease that affects at least 7% of adult women.[3] PCOS is a common cause of infertility in women.[4,5]

Although abnormal apoptosis of granulosa cells is believed to have a role in the pathophysiology of the polycystic ovarian syndrome (PCOS), the cellular and molecular processes behind this are unknown.[6] Atresia occurs in developing PCOS follicles, likely facilitated by increased androgen levels. Atretic follicles are eliminated in the absence of tissue injury or inflammation, suggesting that programmed cell death may be the mechanism behind this process.[7]

L-carnitine is a tiny, water-soluble molecule that is critical for fat metabolism. It is a quaternary ammonium complex that may be produced from the amino acids lysine and methionine.[8] It is required for proper mitochondrial fatty acid oxidation and acyl-CoA ester excretion and influences adenosine triphosphate (ATP) levels. L-carnitine has been shown to

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**Table 1:** A summarization of the demographic features of infertile women who participated in the current research.

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

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

maintain mitochondrial membranes, enhance the organelle's energy supply, and protect the cell against apoptosis.[9]

Therefore, the current study aimed to evaluate the protective effect of L-carnitine (LC) against programmed cell death and, eventually, endometrial receptivity.

## PATIENTS AND METHODS

The high institute for infertility diagnosis and assisted reproductive Technologies, AL-Nahrain University and Al-Farah Specialist Fertility Center collaborated in this prospective case-control research. Sixty infertile women with the PCOS were recruited in this research and began their IVF/ICSI cycle; written informed permission consent were obtained. All patients underwent a thorough history taking, a comprehensive general and gynecological examination, and comprehensive infertility investigations, which included the analysis of the husband's seminal fluid, a hormonal assay, a transvaginal ultrasound, saline, hysterosonography, and/or hysterosalpingography to assess the uterine cavity and tubal patency. Enrolled women underwent controlled ovarian hyperstimulation (COH) for IVF/ ICSI cycle. They were grouped into treatment groups (n = 30) who were treated with L-carnitine® tablet for two months (VERO UNIVERSAL and NOW) in addition to the folic acid tablet one tablet 400 Mcg at the same time (treatment group that was undergone a flexible antagonist protocol for ICSI).

Second group, thirty (30) PCOS infertile women have had been treated only with folic acid 400 Mcg as the placebo group.

All participating women underwent ICSI using a flexible antagonist protocol. A polycystic ovarian syndrome is diagnosed when at least two of three criteria established by the Rotterdam consensus workshop group are met. Women with evidence of endocrine abnormalities were excluded from the study as well as women with tubal blockage.

An early follicular phase follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were essential in all patients, anti-mullerian hormone (AMH) was measured, in addition, serum prolactin, free testosterone, progesterone and thyroid function test Triiodothyronine (T3) thyroxine (T4) thyroid-stimulating hormone (TSH) were performed on days 2-3 of the cycle (CD2) for assessment of the hypothalamus-pituitary function, serum E2 (on CD2-3).

Clusterin and annexin V were measured in the early follicular phase of the cycle CD2 in the serum and in the follicular fluid on the day of ovarian pickup. Endometrial thickness was assessed by ultrasound examination.

Data were analyzed using SPSS version 23 and Microsoft Office Excel 2010. Categorical variables were presented as number and percentages, 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. The chi-square test and yates correction tests were used to compare the proportion between study groups. The bi-serial Pearson correlation test was used to study

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

| Characteristic | Total<br>*n* = 60 | Study group<br>*n* = 30 | Placebo group<br>*n* = 30 | *p-value* |
|---|---|---|---|---|
| LH (mIU/mL) | | | | |
| Mean ± SD | 7.41 ± 2.49 | 7.62 ± 2.11 | 7.20 ± 2.84 | 0.520 I<br>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<br>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<br>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<br>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<br>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<br>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 $p > 0.05$

the correlation of pregnancy outcome to other variables. The level of significance was set at $\leq p$ 0.05.

## 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, the study group ($n = 30$) and the placebo group ($n = 30$). The mean age of all enrolled infertile women were 29.45 ± 3.51 years and there was no significant difference in mean age, mean BMI, mean onset of menarche, frequency distribution according to the type of infertility, mean infertility duration and frequency of previous ICSI trials between study group and placebo group ($p > 0.05$).

Table 2 summarizes the serum hormonal levels of infertile women participating in the current research. Between the study and placebo groups, there was no significant change in mean serum LH, FHS, estradiol ($E_2$), AMH, prolactin, and TSH levels ($p > 0.05$).

Comparison of serum annexin V and clusterin of study group before and after treatment is shown in table 3. Both serum annexin V and clusterin were decreased significantly after treatment ($p < 0.05$).

Table 4 compares serum (post-treatment) and follicular fluid annexin V levels between the study and control groups.

**Table 3:**Comparison of serum Annexin V and Clusterin of study group before and after treatment

| Characteristic | After<br>*n* = 30 | Before<br>*n* = 30 | *p-value* |
|---|---|---|---|
| Serum Annexin V after treatment | | | |
| Mean ± SD | 3.02 ± 0.79 | 3.59 ± 0.97 | 0.001 P<br>HS |
| Range | 1.9 -4.5 | 1.9 -5.1 | |
| Serum Clusterin after treatment | | | |
| Mean ± SD | 6.08 ± 3.04 | 7.51 ± 3.13 | 0.034 P<br>S |
| Range | 1.7 -11.5 | 3.1 -14.5 | |

***n***: number of cases; **SD**: standard deviation; **P**: paired *t*-test; **S**: significant at $p \leq 0.05$; **HS**: highly significant at $p \leq 0.01$

There was no significant change in mean serum Annexin V (p = 0.101), however, there was a significant difference in follicular fluid Annexin V and serum and follicular fluid Clusterin in the study group compared to the placebo group (p 0.05).

The comparison of mean endometrial thickness between the study group and placebo group is shown in Table 5. The mean endometrial thickness in all enrolled women were 5.30 ± 0.70 mm and there was no significant difference in mean endometrial thickness between study and placebo groups, 5.43 ± 0.63 versus 5.37 ± 0.66 mm, respectively ($p = 0.441$).

**Table 4:** Serum Comparison (after treatment) and follicular fluid annexin V between study group and control group

| *Characteristic* | *Total n = 60* | *Study group n = 30* | *Placebo group n = 30* | *p-value* |
|---|---|---|---|---|
| Serum annexin V | | | | |
| Mean ± SD | 3.58 ± 2.63 | 3.02 ± 0.79 | 4.14 ± 3.58 | 0.101 I NS |
| Range | 1.9 -18 | 1.9 -4.5 | 2.1 -18 | |
| Serum clusterin | | | | |
| Mean ± SD | 7.80 ± 4.19 | 6.08 ± 3.04 | 9.52 ± 4.50 | 0.001 I HS |
| Range | 1.6 -22 | 1.7 -11.5 | 1.6 -22 | |
| Follicular fluid annexin V | | | | |
| Mean ± SD | 5.41 ± 2.80 | 4.47 ± 3.45 | 6.35 ± 1.52 | 0.008 I HS |
| Range | 2.8 -18 | 2.8 -18 | 4.2 -8.8 | |
| Follicular fluid clusterin | | | | |
| Mean ± SD | 10.32 ± 3.89 | 8.39 ± 4.14 | 12.24 ± 2.44 | < 0.001 I HS |
| Range | 4.5 -27.3 | 4.5 -27.3 | 8.8 -16.9 | |

***n***: number of cases; **SD**: standard deviation; **I**: independent samples *t*-test; **NS**: not significant at $p > 0.0$; **S**: significant at $p \leq 0.05$; **HS**: highly significant at $p \leq 0.01$

**Table 5:**Comparison of mean endometrial thickness between study group and placebo group

| *Characteristic* | *Total n = 60* | *Study group n = 30* | *Placebo group n = 30* | *p-value* |
|---|---|---|---|---|
| Endometrial thickness (mm) | | | | |
| Mean ± SD | 5.30 ± 0.70 | 5.43 ± 0.63 | 5.37 ± 0.66 | 0.441 I NS |
| Range | 4 -6 | 4 -6 | 4 -6 | |

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

## DISCUSSION

In this study and concerning serum hormonal levels, there was no significant difference in mean serum LH, FSH, E2, AMH, prolactin and TSH, between the 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 on the day of hCG trigger has shown a significant rise in the study group and this finding is going to be discussed in the section on stimulation characteristic.

In the current study, there was no significant difference in mean endometrial thickness between the L-carnitine group and placebo groups; this indicates that treatment with L-carnitine has no significant impact on endometrial thickness in women with PCOS. It has been documented that endometrial thickness can significantly affect pregnancy outcomes in women with PCOS,[10,11] but, the insignificant variation in endometrial thickness between the L-carnitine group and placebo group will raise the possibility of another determinant of successful pregnancy outcome in women with PCOS treated with L-carnitine. Ismail *et al.*[12] in 2014 had stated that L-carnitine treatment significantly improved endometrial thickness in PCOS women; however, it disagrees with Ismail *et al* in this regard. On the other hand, current study results agreement with Sheida *et al.*[13] (2021) who stated that adding L-carnitine to stimulation protocol in PCOS women undergoing ART resulted in no significant improvement in endometrial thickness. Therefore, it appears that the effect of L-carnitine on endometrial thickness in women with PCOS is controversial and needs further research to be clarified.

In the current study, L-carnitine resulted in a significant reduction in both serum annexin V and serum clusterin in PCOS women. After treatment, serum annexin V in the L-carnitine group was lower than that of the control group, but the difference was statistically insignificant. Nevertheless, serum clusterin of the L-carnitine group was significantly lower than that of the control group. Moreover, follicular fluid annexin V and clusterin levels were significantly lower in the L-carnitine group than in the control group. Therefore, L-carnitine substantially reduced serum and follicular fluid annexin V and clusterin in women with PCOS.

Indeed, the serum level of annexin V is an indicator of apoptosis, programmed cell death, because of its ability to bind and externalize cell membrane phosphatidylserine, an important step in programmed cell death.[14] The association between apoptosis and oxidative stress has been observed by previous authors and anti oxidants have been shown to reduce apoptosis.[15-17] The use of L-carnitine as anti oxidant supplementation has been demonstrated in a variety of medical disciplines.[18,19] Additionally, investigations have shown decreased L-carnitine levels in people with PCOS and strong connections between lower L-carnitine levels and an increased risk of developing hyperinsulinemia in patients with PCOS.[20-23] Moreover, experimental studies have shown that adding L-carnitine can improve ovarian dysfunction in PCOS, which is linked to its antioxidant activity. Based on these data, one

can suggest, to explain the improved biochemical and clinical pregnancy rates in the current study, that L-carnitine, through its anti oxidative activity, has resulted in the prevention of apoptosis and this may improve the ovarian environment and oocyte quality in addition to improved endometrial environment and process of implantation; however, the exact molecular mechanisms needs further experimental research work to be elucidated.

Clusterin, an extracellular chaperone, has also been shown to induce apoptosis[24] by probably an oxidative stress molecular basis.[25] Therefore, it can be suggested that the anti oxidant role of L-carnitine has resulted in decreased serum and the follicular fluid level of clusterin by reducing apoptosis and this led to improved ovarian as well as the endometrial environment, making better quality oocytes and a better chance of endometrial implantation. To document such a suggestion, experimental work is needed to determine the exact molecular mechanisms.

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