The effect of L-carnitine and vitamin D supplementation on intracytoplasmic sperm injection outcomes in patients with polycystic ovarian syndrome

Samir M El-Sayed
(Department of Obstetrics and Gynecology, Alexandria University)

Abstract

Background: Polycystic ovarian syndrome (PCOS) represents the most common endocrine pathology in women of reproductive age globally. There is a link between vitamin D deficiencies; lower levels of L-carnitine and PCOS. The purpose of this study is to see how L-carnitine and vitamin D supplementation affects intracytoplasmic sperm injection outcomes in patients with polycystic ovarian syndrome.

Patients and methods: The current study included (40) PCO patients who were arranged to perform ICSI owing to resistance to various methods of induction of ovulation. They were divided into two groups; Group (I) involved (20) patients received 3 grams l-carnitine with 20mcg vitamin D3 daily for 3 months before the ICSI cycle and during the ICSI cycle till the time of HCG measurement. Group (II) involved (20) patients who didn't receive L-carnitine and vitamin D3 neither before nor during ICSI cycle. The patients in this group continued receiving traditional metformin.

Results: After treatment, there was a significant decrease in HOMA IR in group I more than group II (p 0.01), and a significant decrease in HOMA IR after treatment in group I compared to group II (p 0.05). There was a significant increase in number of oocytes; the mean number of M2 oocytes and the mean injected number of oocytes in group I more than group II. Furthermore, the high quality of embryos and the pregnancy rate showed a significant increase in group I more than group II.

Conclusion: This study's findings add clinical support to the evidence that vitamin D and L-carnitine may play a role in intracytoplasmic sperm injection success rates in PCOS patients.

Keywords: Intracytoplasmic sperm injection, L-carnitine, polycystic ovarian syndrome, Vitamin D.

Corresponding author:
Samir M El-Sayed
(Department of Obstetrics and Gynecology, Alexandria University)

Introduction

Polycystic ovarian syndrome (PCOS) is a common endocrine disorder in women that causes a variety of signs and consequences.(1,2) It has already been for a long time that the disease has a likelihood of 8-13% in all breeding age categories.(3,4) Polycystic ovarian syndrome (PCOS) is a complicated condition inside which hereditary, hormonal, environmental, and behavioral characteristics all interact to produce a heterogeneous phenotype with reproductive, energy metabolism, and psychological variables that impact women's well-being and life quality throughout their lives.[5,6,7]

According to specialty society recommendations, the existence of at least two of the following three parameters is required for the diagnostic workup: chronic anovulation, hyperandrogenism (clinical or biological), and polycystic ovaries.(6,7) It is an exemption diagnosis, and abnormalities that simulated clinical characteristics of PCOS must always be ruled out. Thyroid issues, hyperprolactinemia, and non-classical congenital adrenal hyperplasia are examples.(8) If clinical manifestations point to another causative agent, some patients may require more comprehensive diagnostic procedures.[8,19]

Insulin resistance and compensating hyperinsulinemia are found in about 80% of obese women with PCOS and 30-40% of lean women, according to published studies.(9,20) There are numerous choices for treatment to decrease the extent of clinical characteristics in PCOS patients. Each doctor should be capable of selecting the most appropriate guidelines for PCOS and the possibility of childbirth.[10]

Vitamin D deficiency was linked to a significant decrease in ovulation frequency, pregnancy rate, and possibility of a live birth in PCOS women receiving ovarian stimulation for fertility problems.(21) An increasing body of research indicated that vitamin D could well be linked to PCOS-related symptoms such as ovulatory dysfunctions, hyperandrogenism, insulin resistance, dyslipidaemia, and metabolism-related risk factors (Muscogiuri et al., 2017).(25) One other study found that taking vitamins D and E together for two months before embryo transfer enhanced the clinical pregnancy rate from 23% to 62% and the live birth rate from 16% to 43% in women with PCOS experiencing IVF (Fatemi et al., 2017).[12]

L-carnitine is essential for glucose metabolism and oxidative stress.(29,30,32) Low serum L-carnitine amounts, also in non-obese women, have been linked to insulin resistance and hyperandrogenism, based on the research (Celik et al., 2017).(5) Researchers have discovered lower concentrations of L-carnitine in PCOS patients, as well as potentially major correlations among reduced ranks of L-carnitine and an increased risk of hyperinsulinemia in PCOS patients (Jamilian et al., 2017 Samimi et al., 2016)[15,28]

The present study aims to determine the impact of L-carnitine and vitamin D supplementation on intracytoplasmic sperm injection outcomes in polycystic ovarian syndrome patients.

Patients and Methods

This is single - blinded randomized placebo-controlled study included (40) PCO patients who were arranged to perform ICSI after unsuccessful ovulation induction using various routines.

- Eligibility criteria of participants were assessed by personal interviews and based on their medical records. The participants' age ranged between 20 to 38 years, BMI ranged between 20-34 kg/m², and serum FSH is lower than 10 m IU/ml in 3rd day of menstrual cycle.
- Exclusion criteria were; heart diseases, liver or kidney deficiencies, known cases of endometriosis (approved histologically), any uterine anomalies, hydrosalpinx,

and severe male factor infertility (sperm count < 5 million per milliliter or total azoospermia, normal morphology <4%). Those consumed vitamin and antioxidant supplementations in the last three months before the trial start date were excluded from the study.

The study's patients were divided into two groups:

- **Group (I):** involved (20) patients received 3 grams l-carnitine with 20mcg vitamin D3 daily for 3 months before the ICSI cycle and during the ICSI cycle till the time of HCG measurement.
- **Group (II):** involved (20) patients did not receive l-carnitine and vitamin D3 neither before nor during ICSI cycle. The patients in this group continued receiving traditional metformin therapy.

The HOMA index (marker of insulin resistance IR) was calculated as [baseline glucose] x [baseline insulin]/22.5 for all included patients in both groups after a complete history was taken, including that of the length of infertility, physical examination, and evaluation of male partners. Early findings in all patients were analyzed and after 3 months of l-carnitine and vitamin D therapies. All women underwent ICSI using either agonist or antagonist protocol with their basic elements according to the case.

- The primary outcomes measures were the quality of oocyte, number of oocytes, quality of embryos and number of embryos transferred.
- Secondary outcomes were clinical pregnancy, rate of miscarriage, multiple pregnancy rate and ectopic pregnancy rate.

The metabolic changes and the outcome of ICSI cycle were recorded and analyzed.

Statistical analysis of the data

- Data were fed to the computer using IBM SPSS software package version 24.0.
- Qualitative data were described using number and percent. Comparison between different groups regarding categorical variables was tested using Chi-square test.
- Quantitative data were described using mean and standard deviation for normally distributed data.
- For normally distributed data, comparison between two independent populations was done using independent t-test.
- Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

Results

Table (1) had shown the basic demographic and hormonal screening test of the two studied groups, with the mean age, BMI, and FSH levels showing insignificant differences (p >0.05).

Table (1): Comparison between two groups as regard to demographic and basic laboratory findings

	Group I	Group II	P value
Age			
Range	23-38	22-37	0.155
Mean	30.1	28.8	N.S.
SD	3.67	4.31	
BMI			
Range	25-30.9	26-31.8	0.064
Mean	27.965	28.82	N.S.
SD	1.709	1.77	
FSH			
Range	6.7-9.33	7.06-9.84	0.074
Mean	8.03	8.45	N.S.
SD	0.905	0.908	

Regarding HOMA IR, it was discovered that the mean HOMA IR in the two groups before treatment was high (>2.5), and there was no significant difference when comparing the two groups before treatment. However, after treatment, there was a significant decrease in HOMA IR in group I more than group II

(p 0.01), and there was also a significant decrease in HOMA IR after treatment in group I when comparing with group II (p 0.05), as shown in table (2). Table (2): Comparison

Table (2): Comparison between the two studied groups regarding pretreatment and posttreatment HOMA IR values.

HOMA IR	Group I	Group II	P1 value
Pretreatment Range Mean SD	2.6-3.8 3.28 0.370	2.9-3.7 3.34 0.246	0.275 N.S.
Post treatment Range Mean SD	0.8-2.4 1.49 0.467	2-2.5 2.25 0.176	0.001*
P2	0.001*	0.061 N.S.	

P1 comparison between group I and II at the same time

P2 comparison between before and after treatment in the same group.

Table (3), showed the number and quantification of oocytes in the two studied groups, it was found that there was a significant increase in the number of oocytes retrieved in group I more than group II (p <0.01), also the mean number of M2 oocytes was significantly higher in group I more than group II, the mean injected number of qualified oocytes was significantly higher in group I more than group II.

Table (3): Comparison between two groups as regard to patient's oocytes number and quantification

Oocyte	Group I	Group II	P value
Number retrieved Range Mean SD	8-14 11.55 1.986	5-8 6.45 1.234	0.001*
M2 oocyte Range Mean SD	6-12 8.7 2.055	3-8 6.25 1.51	0.001*
Number of injected oocytes Range Mean SD	6-10 7.5 1.504	3-5 4.1 0.852	0.001*

Table (4), showed the quality of embryos in the two studied groups, the grade A embryos were significantly higher in group I more than group II (p <0.01), the embryos transferred at day 5 are significantly increase in group I more than group II.

Table (4): Comparison between two groups as regard to patient's embryo

Embryo	Group I		Group II		P value
Grade A embryos Range Mean SD	4-8 6.1 1.483		2-4 3 0.858		0.001*
	No	**%**	**No**	**%**	
Transfer at D3	9	45.0	14	70.0	0.05*
Transfer at D5	11	55.0	6	30.0	0.031*

Table (5), showed the final outcome, it was found that the pregnancy rate in group I was 60.0% and in group II was 30.0%, with a significant increase in group I more than group II, while the incidence of twin pregnancy, ectopic pregnancy and abortion shows insignificant difference between the two groups (p >0.05).

Table (5): Comparison between two groups as regard to patient's outcome

Outcome	Group I		Group II		P value
	No	%	No	%	
Pregnant					
Yes	12	60.0	6	30.0	0.029*
No	8	40.0	14	70.0	
Twin	4	33.3	2	33.3	0.194
Ectopic	0	0.0	0	0.0	-
Abortion	2	16.7	2	33.3	0.500

Discussion

Polycystic ovarian syndrome (PCOS) represents the most common endocrine pathology in women of reproductive age globally. There is a link between vitamin D deficiencies; lower levels of L-carnitine and PCOS. The purpose of this study is to detect the effect of L-carnitine and vitamin D supplementation on intracytoplasmic sperm injection outcomes in patients with polycystic ovarian syndrome.

Our results revealed a significant decrease in HOMA IR in patients received l-carnitine with vitamin D3.

Women with PCOS have indeed inadequate levels of vitamin D, and vitamin D treatment could have a favorable impact on IR in obese women with PCOS (Selimoglu et al., 2010). (29) Several investigators had proposed the correlation among both vitamin D status and metabolic dysfunctions particularly insulin resistance in women with PCOS. Finding from randomized controlled trials indicated that providing PCOS patients with constant smaller concentrations of vitamin D (< 4000 IU/d) or vitamin D as a co-supplement could enhance insulin sensitivity in terms of fasting glucose concentration and HOMA-IR (Łagowska et al., 2018).[18]

Maleki et al., (2019)(22) discovered that carnitine may aid in weight loss, glycemic control, and oxidative stress. Sharkwy and El-Din, (2019)(11) proved that co-treatment with L-carnitine and metformin improved reproduction rate, insulin resistance, and lipid profile in clomiphene citrate-resistant obese PCOS women.

The molecular mechanism underlying the relationship among treatment and PCOS improved performance is uncertain. Even so, according to a previous research, vitamin D3 replacement therapy advanced some biochemical parameters in women with PCOS by raising the amount of soluble receptor for Advanced Glycosylated Ends (AGEs). As a result, vitamin D3 inhibits the progression of inflammation in the pathogenesis of PCOS. Furthermore, vitamin D3 treatment is essential in folliculogenesis because it lowers elevated anti-mullerian hormone levels (Irani et al., 2014).[13]

According to the current study results, the number and quantification of oocyte was significantly improved in patients received l-carnitine and vitamin D3 supplementation.

Recent time, research in (PCOS) rat models was investigated to assess vitamin D effects in the ovary. Dietary vitamin D supplementation increased follicle viability and growth, as well as follicular E2 and P production (Behmanesh et al., 2019).(4) Vitamin D3 injection improved follicle morphology and ultrastructure (e.g., cell junctions, endoplasmic reticulum, and lipid droplets), as well as serum levels of testosterone (Kuyucu et al., 2020).[17]

In prospective studies, serum vitamin D concentration levels strongly associated with the number of mature oocytes retrieved and oocyte fertilization levels in patients undergoing IVF cycles, which was recommended to

be due to anti-inflammatory impacts of vitamin D (Abadia et al. 2016; Liu et al. 2019; Wu et al. 2018).[1]

Considerable lot in vitro studies have noted the pathways by which carnitines promote oocyte and embryo development; underlying mechanisms involve beta-oxidation, antioxidant power, and protection against apoptosis (Placidi et al., 2022).[26]

Dunning and Robker, (2012) (10) stated that L-carnitine impacted oocyte quality since it transports fatty acids and regulates energy production, both of which are crucial in enhancing oocyte maturation. Immature oocytes can cause metabolic and endocrine problems in PCOS (Dumesic and Abbott, 2008). (9) Oral dosing of L-carnitine (5 mg/mL) enhanced the count of oocytes ovulated as well as their competence in contexts of mitochondrial mass and dispersion, as well as oxidative damage in the oocyte and ovary in a mouse model of repetitive ovulation cycles (Miyamoto et al., 2010). [23]

Results of the present study showed significantly embryo transfer increases, pregnancy rate as well as, the grade A embryo significantly increased in patients received l-carnitine and vitamin D3.

Vitamin D could play an essential part in rising ovulation and pregnancy rates in PCOS women (Trummer et al., 2018). (31) Vitamin D supplementation might very well assist pregnant women with PCOS and insulin resistance reinstate normal vitamin D levels in their serum, boosting embryo performance and massively increasing clinical pregnancy rates (Irani et al., 2015). (14) A further study found that obtaining vitamin D six weeks before intracytoplasmic sperm injection improved endometrial quality and clinical pregnancy rate (Polyzos et al., 2014). [27]

In a new analysis, supplements were added to the IVF treatment of women with PCOS. It was discovered that implantation and clinical pregnancy incidence were significantly higher in patients with normal vitamin D levels especially in comparison to those with lowered vitamin D levels (< 20 ng/mL 25(OH)-vitamin D); vitamin D levels correlate strongly with the probability of implantation and clinical childbearing ($p < 0.01$); they enhance embryo quality and the number of high-quality embryos that after vitamin D therapies equals that happening in women with normal vitamin D status (Zhao et al., 2019). [33]

It was found that adding L-carnitine to clomiphene citrate in the follicular till the luteal phase in clomiphene-resistant PCOS cases could contribute significantly in enhancing ovulation performance and clinical pregnancy rate according to Abd-Elfattah et al., (2019) (2) study.

Human embryos exposed to L-carnitine after fertilization had higher implantation rates as well as clinical and continuous childbirth (Kim et al., 2018). (16) The addition of L-carnitine to culture media enhances mitochondrial function in human embryos at the morula stage by raising oxygen consumption rate and ATP production while having no impact on mitochondrial copy numbers (Morimoto et al., 2021). (24)

The current study's findings suggested that taking Vitamin D and L-carnitine supplements could help improve the clinical outcome of ICSI in PCOS patients.

condition

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