Impact of Vitamin D Deficiency on ICSI cycle Outcome

Original Article

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ABSTRACT

Background: Emerging data identifies critical roles for vitamin D and many areas of medicine are involved: regulation of cellular growth, immunity, central nervous system, cardiovascular diseases, and reproduction.
Objective: To determine the impact of serum vitamin D level on ICSI cycle outcome.
Design: A prospective cohort study.
Methods: Patients who will undergo ICSI cycle with age ≤ 38 years , adequate ovarian reserve were included in this study., women with poor ovarian reserve and patients with freeze all cycles were excluded. Vitamin D Serum samples were obtained On the day of the ovum pick-up, participants were divided into 2 groups :Deficiency: (serum vitamin D ≤30ng/ml), Sufficiency: (serum vitamin D≥30ng/ml).Quantitative B-hCG was done 14 days after emberyo transfer
Result : A total of 135 women were included in the study after undergoing ICSI , women were divided into two groups as following :Group A:(n = 68) with serum vitamin D level ≥30 ng/ml and Group B:(n = 67) with serum vitamin D level < 30 ng/ml at day of ovum pick-up.. pregnancy was significantly higher in group A (76.5 %) compared to group B (58.2 %) with P-value < 0.05. With regards to pregnancy continuation there was significant increase in ongoing pregnancy among group A (88.5 %) compared to group B (61.5 %) and significant decrease in the number of aborted cases among group A (11.5 %) compared to group B (38.5 %) with *P-value* < 0.05.
Conclusions: Vitamin D serum level may play a role in ICSI cycle out come . and it seems to valuable to adjust its level in patients with deficiency before starting their ICSI cycle.

Key Words: Abortion rate, ICSI, infertility, pregnancy rate, vitamin D.

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INTRODUCTION

The role of vitamin D in calcium-phosphate homeostasis and bone metabolism is well-known. Recently the knowledge about the importance of vitamin D has grown and its physiology has made considerable progress[1].

Emerging data identifies critical roles for vitamin D in many areas of medicine e.g regulation of cellular growth, immunity and autoimmunity, central nervous system, cardiovascular diseases, infections, malignancies and reproduction[2].

Vitamin D levels are usually determined by measuring the serum 25-hydroxy vitamin D concentration. Severe deficiency defined when 25-hydroxy vitamin D serum levels are <10 ng/mL, deficiency when levels are 10 to <20 ng/mL, and insufficiency when levels are 20–30 ng/mL and when its level is >30 ng/mL it is defined as sufficient[3].

In addition, it has been suggested that vitamin D plays an important role in metabolism, and that human health will be adversely affected when vitamin D levels are below 30 ng/mL[4].

Several studies have demonstrated that vitamin D played an important role in the reproductive system due to the expression of vitamin D receptors and vitamin D metabolizing enzymes in many female reproductive tissues, including the pituitary, ovary, uterus, and placenta. More ever Vitamin D has a role in ovarian steroidogenesis and involved in the pathophysiology of some disorders in women in child bearing age[5].

In patients who underwent single embryo transfer at blastocyst stage, vitamin D deficiency (<20 ng/mL) emerged as an independent predictor of lower clinical pregnancy rates as compared with non- deficient women[6].

Finally, women with sufficient 25-hydroxy vitamin D concentration had significantly higher chance of obtaining top quality embryos, thus generally supporting a favorable effect of vitamin D at both ovarian and endometrial level[7].

AIM OF THE WORK

To assess the relation between serum level of vitamin D and ICSI outcome in infertile women.

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PATIENTS AND METHODS

A prospective cohort study was conducted at Rahem fertility center, in Zagazig, Egypt and Zagazig University Hospital. One hundred thity five infertile women undergoing Intracytoplasmic Sperm Injection (ICSI) were included in the study between November 2020 and November 2021.

This study was approved by the ethical committee and all women had signed a written and informed consents.

Patients scheduled for ICSI with age ≤ 38 years and adequate ovarian reserve ; Antral Follicle Count (AFC) ≥7, and Anti Mullerian Hormone (AMH) ≥1.1 according to Bologna criteria were included in the study.

Severe male factor, endometriosis, women with poor ovarian reserve Bologna criteria (AFC<7 , AMH<1.1) and patients with freeze all cycles were excluded[8].

Vitamin D was measured on the day of ovum pick-up (OPU). The serum was separated by centrifugation, and the samples were frozen at -20°C until assayed. Vitamin D was assayed by ELISA 25-OH Vitamin D total, (ORG 570 25-OHVitaminD3/D2) DRG Diagnostics, according to the manufacturer's protocol. According to the quantitative assessment of serum vitamin D levels participants were divided into two groups:

- Deficiency: (serum vitamin D ≤30ng/ml)
- Sufficiency: (serum vitamin D≥30ng/ml

Quantitative β-HCG was done 14 days after transfer. Clinical pregnancy was confirmed by transvaginal ultrasound done 15 days after positive pregnancy test with at least one gestational sac in the uterine cavity.

The parameters analyzed and compared between both groups were: Clinical parameters e.g male and female ages, infertility etiology, infertility duration, body mass index (BMI) and the evaluation of the ovarian reserve. ICSI parameters e.g the total oocytes retrieved and the number of mature oocytes, the fertilization rate, the cleavage rate, the number and status of the embryos, the pregnancy rate, and the implantation rate.

Measurement outcomes

Primary outcome

Both groups were investigated for pregnancy 2 weeks after embryo transfer by serum β-HCG and TVS 15 days later

Secondary outcomes

Number of mature oocytes, fertilization rate, number of good quality embryos and abortion rate.

Statistical analysis

The data were analyzed using the SPSS version 20.0. The continuous variables were determined as the mean ± standard deviation or median values, and categorical data were reported as percentage (%). The Chi-square test and Fisher's exact test were used to compare the qualitative variables. Univariate analyses determined the association between the serum Vitamin D levels with patients and cycle parameters (Student's t test, Mann-Whitney U test as appropriate. The sample size was calculated based on an expected clinical pregnancy per embryo transfer in patients with sufficient vitamin D levels of 40% and stating a 60% reduction in women with insufficient vitamin D levels. With 80% power and a significant level of 5%, the calculated number of patients to be recruited was at least 102. This assumption was based on our institution's pregnancy rate and previous vitamin D IVF studies. A one-year recruitment period was planned.

RESULTS

A total of 135 women were included the study. Cases were divided into two groups as following:

Group A: (n = 68) with vitamin D level ≥30 ng/ml. at day of OPU

Group B: (n = 67) with vitamin D level < 30 ng/ml. at day of OPU

The two groups show no statistically significant difference with regard age (*P-value* > 0.05) however Group A was significantly associated with high BMI. With regard causes and types of infertility there was no significant difference between both groups (*P-value* > 0.05)

The mean duration of infertility was significantly longer in group B (8.56±2.85y) than group A (4.99±2.11y) while there was no statistically significant difference regarding Mean (AFC) and mean (AMH) level between both groups. (Table 1)

Hormonal panel including TSH, PRL, FSH and LH was done for each group; results showed no difference in both groups except for LH which was significantly high in B group.

The difference between both groups regarding the mean dose and duration of human menopausal gonadotropin hormone were insignificant more ever the mean number of oocytes retrieved was comparable in both groups but The mature oocytes number, fertilization rate and number of good quality embryos were significantly higher in group A. (Table 2)

Additionally, the clinical pregnancy rate was significantly higher in group A (76.5 %) than group B (58.2 %) (P-value < 0.05). The ongoing pregnancy rate was significantly higher in group A (88.5 %) compared to group B (61.5 %) with significant decrease in the number of aborted cases in group A (11.5 %) compared to group B (38.5 %) with *P-value* < 0.05. (Table 3)

A significant positive correlation was found between vit D serum level and mature oocyte (r = 0.628, *P* <0.001), fertilization rate (r =0.513, P <0.001) and number of good quality embryos (r =0.568, P <0.001). (Table 4)

Table 1: Clinical characteristics between both studied groups:

Variable			Group A (n=68)	Group B (n=67)	t/ χ^2	*P*
Infertility	Primary	N	56	53	0.23	0.63 NS
		%	82.4%	79.1%		
	Secondary	N	12	14		
		%	17.6%	20.9%		
Causes Of Infertility	Tubal factor	N	6	13	3.12	0.08 NS
		%	8.8%	19.4%		
	Male factor	N	1	13	2.85	0.09 NS
		%	1.5%	19.4%		
	PCOs	N	1	4	1.92	0.17 NS
		%	1.5%	6.0%		
	Sex Selection	N	56	52	0.47	0.49 NS
		%	82.4%	77.6%		
	Unexplained	N	2	6	2.19	0.14 NS
		%	3.0%	9.0%		
Duration of infertility (year)	Mean ± SD		4.99±2.11	8.56±2.85	8.28	<0.001**
AFC	Mean ± SD		13.28±4.11	12.2±4.12	1.52	0.13 NS
AMH (ng/ml)	Mean ± SD		2.32±0.88	2.51 ±0.91	1.23	0.22 NS
Age (year)	Mean±SD		32.29±3.73	32.02±3.97	0.41	0.68 NS
BMI (Kg/m^2)	Mean±SD		35.36±4.58	33.12±6.36	2.35	0.02*

PCOs: poly cystic ovarian syndrome. AFC: Antral follicle count. AMH: anti mullerian hormone.

SD: Standard deviation t: Independent t test χ^2: Chi square test NS: Non significant ($P > 0.05$) *: Significant (< 0.05) **: Highly significant ($P < 0.001$)

Table 2: ICSI cycle characteristic distribution between studied groups:

Variable	Group A (n=68) Median (range)	Group B (n=67) Median (range)	MW	*P*
NO of Oocyte	15 (6-20)	12 (1-38)	1.883	0.06 NS
NO of Mature oocyte	13 (1-18)	10 (1-20)	2.772	0.007*
NO of fertilization rate	15 (7 – 21)	13 (2 – 16)	6.75	<0.001**
NO of good Embryo	10 (1-15)	3 (1-12)	4.305	<0.001**

MW: Mann Whitney test

NS: Non significant ($P > 0.05$) *: Significant (< 0.05) **: Highly significant ($P < 0.001$)

Table 3: primary outcome distribution between studied groups

Variable			Group A (n=68)	Group B (n=67)	χ^2	*P*
Pregnancy	-VE	N	16	28	5.12	0.02*
		%	23.5%	41.8%		
	+VE	N	52	39		
		%	76.5%	58.2%		
Variable			(n=52)	(n=39)	χ^2	P
Pregnancy continuation	Ongoing	N	46	24	9.58	0.002*
		%	88.5%	61.5%		
	Abortion	N	6	15		
		%	11.5%	38.5%		

χ^2:Chi square test NS: Non significant ($P > 0.05$) *: Significant (< 0.05)

Table 4: Correlation between vit D level and mature oocyte, fertilization rate and number of embryos

Variable	Vit D level (ng/mL)	
	r	*P value*
Mature oocyte	0.63	<0.001**
Fertilization rate	0.51	<0.001**
Number of embryos	0.57	<0.001**

r: Pearson's correlation coefficient **: highly Significant *(p value* <0.001)

DISCUSSION

Vitamin D receptors (VDR) have been found in reproductive organs ; the uterus, endometrium, ovaries and placenta, indicating that vitamin D has a favorable physiological function in women's reproductive health[9].

Paffoni et al reported that The effect of 25 hydroxy vitamin D levels on ART results may be mediated by endometrial receptivity rather than ovarian stimulation or embryo characteristics[10].

Vitamin D is described as "the sun vitamin"[11]. Actually, the role of vitamin D in ovarian steroidogenesis is now well established[12]. Despite the luxury of sunshine exposure in North Africa allowing vitamin D synthesis all over the year, in this study, tiny levels of vitamin D have been recorded. It may be explained by limited sun exposure and/or sunblock using, lack of government regulation for vitamin D supplementation in food and female commitment to religious clothes recommendations.

The aim of this study was to assess the relation between serum level of vitamin D and ICSI outcome in infertile women.

It has been suggested that vitamin D deficiency contributes to metabolic disorders and may be found in the metabolic syndromes, namely obesity and insulin resistance[13] our data revealed a positive correlation between serum vitamin D levels and BMI, which was higher among the group of women with vitamin D sufficiency as reported by Polyzos et al[6].

It should be noted that vitamin D deficiency and high BMI are still among the rare modifiable risk factors before starting an IVF, which may hold beneficial therapeutic implications[14].

Importantly, our results showed no difference between both groups regarding mean number of oocyte retrieved; while mean number of mature oocytes (13 vs. 10), had a positive correlation with vitamin D level ($r = 0.628$, $P < 0.001$) as it was significantly higher among patients in group A with $p = 0.007$, 0.00 and 0.038 respectively.

Gebriel et al agreed with our results as they stated that number of mature oocytes was significantly higher among group with normal vitamin D (mean 10.623 ± SD5.712) than among deficient group (mean 8.928 ± SD 3.083) respectively with (*P-value* 0.027)[15].

However, Polyzos et al stated that no significant differences were observed between vitamin-D deficient women and those with vitamin D levels ≥ 20 ng/ml regarding the number of mature oocyte and percentage of top quality embryos. These differences might be due to different cut off level defining vitamin D deficiency[6].

Additionally, we found a significant high fertilization rate and good quality embryos in patients with sufficient serum level of vitamin D , however It is still unclear why the vitamin D deficiency group had lower comparable results . In agreement with our results Liu et al stated that lower serum vitamin D level are associated with a lower fertilization rate in IVF[16].

Finally, we reported higher clinical and ongoing pregnancy rate among patients with sufficient serum vitamin D level going with the results reported by Mnallah et al. Who reported that women with 25 OH vitamin D ≥ 30 ng/ml had a significantly higher clinical pregnancy rate compared to those with deficient 25 hydroxy vitamin D serum level[14].

additionally, Polyzos et al reported that positive hCG, clinical pregnancy and live birth rates were significantly lower in vitamin-D deficient women compared with those with 25-OH vitamin D values exceeding 20 ng/ml[6].

On the other hand Banker et al and Ha et al stated that vitamin D deficiency leads to lower reproductive outcomes, though not statistically significant and, thereby, does not have a negative influence on in-vitro fertilization–intracytoplasmic sperm injection outcomes, it also could be explained by different cut off of vitamin D distribution[17,18].

Recently, Ko et al reported that the live birth rate of the first IVF cycle in the vitamin D-deficient group was significantly lower compared to the non deficient groups[19].

The result of our study is to some extent sensitive, accurate and powerful as being a prospective cohort study with less liability to include bias. Every effort was made to ascertain that all follow-up data were documented, and only complete information was included in data analysis, and all clinical assessment of study outcomes were done by the same team.

A Relatively small sample size and time of the study that going with COVID 19 pandemic is considered the most prominent study limitations.

CONCLUSIONS

Vitamin D serum level may play a role in improvement of ICSI cycles outcome , more ever plenty of studies including larger sample size still needed to proof the intense need of vitamin D correction in infertile woman with low serum level before starting an ICSI cycle.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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