Vitamin D supplementation in vitamin deficient women undergoing ICSI cycles: Does it affect the fertility outcome? A randomized controlled trial

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Synopsis: Vitamin D supplementation in deficient females improves the clinical pregnancy rate vitamin D deficient females undergoing ICSI cycles

Randomized clinical trial

Abstract

Objective : to determine the effect of vitamin D (VD) supplementation on ICSI outcome in vitamin D deficient females.

Methods: A randomized controlled trial was done in IVF unit of Cairo University from July 2017 to Mach 2021. Level of VD was measured (females with level below 30 ng/ml were eligible). 400 VD deficient (or insufficient) females randomly allocated to 2 groups; VD supplementation (group 1) and non-supplemented group (group 2). Outcome data were analyzed for 187 participants in group 1 and for 186 in group 2. Regression analysis was done to calculate the Odds ratio (OR) for the primary outcome (clinical pregnancy rate, CPR) adjusting for confounders (age, BMI, type and cause of infertility). The study secondary outcome were the fertilization and the implantation rates.

Results: Group 1 had higher fertilization (86% vs 64%, difference of 18%; 95%CI; 14%, 21%, p<0.01) and implantation rates (27% vs 17%, difference 10%; 95%CI 4%, 16%, p<0.01). CPR was higher in group1 (83/187, 44% vs 63/186, 34%, difference of 10%, 95%CI; 1%, 20%; p=0.03). After adjustment, the Odds ratio for pregnancy in group 1 was 2.1 (95%CI: 2.1, 3.3, p=0.01), Conclusion: VD supplementation increases the clinical pregnancy, fertilization and implantation rates in ICSI cycles.

ClinicalTrial.gov Registration number: NCT03209856.

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Introduction

In the last decade, a large body of evidence highlighted the importance of vitamin D (VD) in female reproduction. VD has been suggested to have important roles in fertility and throughout pregnancy (1-3). However, the interest for

its possible effect on the IVF/ICSI outcome was recent (4,5).

Vitamin D receptors (VDR) mRNA was shown to be expressed not only in the calcium regulating tissues, but also in the ovary, uterus and pituitary (6). Also, 1 α hydroxylase (responsible for peripheral tissue activation of VD) was identified in the ovary and endometrium (7). This distribution of VDR and 1 α hydroxylase suggests an important role of VD in female reproduction (8). Calcitriol also enhances the release of human chorionic gonadotropin (HCG) in the syncytiotrophoblast and the expression of HOXA10 (important in endometrial growth and development essential for endometrial receptivity) (9,10).

A relation between VD deficiency and some fertility problems was suggested in the past few years. That deficiency was related to insulin resistance in polycystic ovarian syndrome (PCOS) (11). Moreover, an association between higher body mass index (BMI) and VD deficiency was shown by some investigators, (12). Therapeutic efficacy of VD supplementation was found improve insulin resistance in females with PCOS,(13).

Some recent studies have found a correlation between VD deficiency and poor IVF/ICSI outcomes (5,14). However, randomized controlled trials (RCTs) for assessment of the effect of VD supplementation on fertility outcomes in assisted reproductive techniques (ART) cycles are scarce (15).

Therefore, we aim in this study to evaluate the effect of treatment of VD deficiency (through VD supplementation for 8 weeks before the start of ICSI cycles) on fertility outcome after ICSI trials. To the best of our knowledge, this is the first big RCT addressing this issue.

Material and methods

The study was conducted in the IVF unit of department of Obstetrics and Gynecology, Cairo University from July 2017 to March 2021. Approval of the local ethical committee was taken. Informed consents were taken from participants and the ethical standards of Declaration of Helsinki were followed. The trial was registered in ClinicalTrial. gov Registry (NCT03209856) and reporting of the study conformed to CONSORT guidelines (16).

The study participants were VD deficient females undergoing ICSI trial in the age of 20 to 35 years. Serum 25 hydroxy VD (25 (OH) VD) was assessed by ELISA test (25-OH Vitamin D3/D2, ORGENTEC Diagnostika GmbH, Mainz, Germany). Accordingly if they are deficient or insufficient in VD ((25 (OH) VD < 30 ng/ml), (17)), they were eligible for the study. Patients with poor prognostic factors namely; endometriosis or previous repeated failure were excluded from the study.

The serum 25 (OH) VD was chosen for assessment of VD deficiency as the activation of VD occurs peripherally thus the serum active form (calcitriol; 1,25 dihydroxy VD) does not represent the actual VD status (18).

400 VD deficient women were randomly allocated to either one of two groups. Blocked randomization (block size of 4 and 6) was chosen with a ratio of 1:1 between the 2 groups. The decision for the participant to be in group 1 or 2 was written in sealed envelopes which were opened when the patient is eligible to participate in the study (i.e. shown to be VD deficient by serum level). The first group (Group 1; VD supplemented group) took 50,000 IU VD orally/week (17) (10,000 IU daily VD for 5 days a week) for 8 weeks before the start of ICSI cycle (Vitamin D , Solaray, Park city, UT, USA). The second group did not take VD supplementation.

Ovarian stimulation was accomplished using antagonist protocol. Follicular stimulation started on the second or third day of the cycle by daily SC 150 to 300 IU HMG (Merional, IBSA, Lugano, Switzerland). The choice

of the HMG dose was according to female age, BMI, antral follicular count (AFC) and previous response to stimulation (if present). Daily SC 0.25 mg cetrorelix (Cetrotide, Merck Serono, Darmstadt, Germany) started on the fifth day of stimulation. The first folliculometry visit was after 5 days from the start of stimulation then the visits were every other day. Ovulation trigger was done when at least 2 to 3 follicles ≥18 mm were present. Ovum pick up (OPU) was done 35-36 hours after the trigger injection. Embryo transfer was done on day 3 from OPU. If there were 4 or more top quality embryos on the third day the transfer was delayed to the fifth day (as per our IVF unit protocol). Progesterone (Cyclogest, Actavis, Barnstaple, UK) in the form 400 vaginal suppositories were taken twice daily from the evening of the day of OPU for two weeks and were continued throughout the first 10 weeks of pregnancy (if it occurred). Serum quantitative B-HCG was done 15 days from the day of ovum pick up. Clinical pregnancy was confirmed if a gestational sac was evident by transvaginal ultrasound done 4 weeks after embryo transfer.

Fertilization rate was calculated by dividing the number of 2 pronuclear fertilized oocytes by the total number of sperm injected mature ova (MII oocytes), while implantation rate is the number of gestational sacs divided by the total number of transferred embryos.

Clinical pregnancy rate is considered the primary outcome while the fertilization rate and the implantation rate are the study secondary outcome.

Sample size calculation was based on comparison of the clinical pregnancy rate (CPR) as the primary outcome between group 1 (vitamin D deficient group who took vitamin D supplementation) and group 2 (deficient group who will not take the supplementation). Previous data (19) suggested that a pregnancy rate of 41% in vitamin D deficient women following IVF/ICSI cycles. We set the clinical significant difference at 15% the alpha error at 0.05, power at 80% and dropout rate at 10% (cancelled cycles or lost for follow up). Thus we needed 200 participants in each arm. Sample size calculation was done using IBM SPSS SamplePower software, release 3.0.1 (IBM Corp., Armonk, NY, USA).

Statistical analysis: description of data was in the form of mean (SD), or count (%) according to the data type. Comparison of data between groups was done by t-test, Chi-Square and Fisher test. A logistic regression analysis was done to assess the effect of Vitamin D supplementation on CPR after adjustment for possible confounders; namely; age, BMI, type of infertility and cause of infertility. Statistical analysis was done as an intention to treat analysis. Nevertheless, it was the same as per-protocol analysis (all participants followed the allocated group). Statistical analysis was done using SPSS software, version 23 (IBM Corp., Armonk, NY, USA).

Results

Figure 1 shows the Consort flow chart of the study population. 701 females referred to IVF clinic were screened for eligibility for the study. 80 cases were excluded before testing for vitamin D status due to presence of endometriosis or repeated IVF failure. Another 176 cases were excluded after assessment of serum vitamin D for being VD sufficient. 45 females declined to participate in the study. 200 VD deficient participants were allocated to each arm of the study; group 1 (VD supplemented) and group 2 (VD non-supplemented). 13 and 14 cases were excluded in data analysis from group 1 and group 2 respectively due to cycle cancellation or patients being lost in follow up after embryo transfer (Figure 1).

The two study groups had similar clinical characteristics; namely; age, BMI, type of infertility (primary or secondary), duration of infertility and cause of infertility. They

also have similar VD level (before starting VD supplementation to group 1), (Table 1).

Regarding the ICSI cycle outcomes, group 1 had improved fertilization, implantation and pregnancy outcomes in comparison to group 2. Although, the number of retrieved oocytes were similar, the number of mature ones were higher in group 1 (6.4 ± 3.3 vs 5.6 ± 1.9, difference of 0.8, 95%CI; 0.2, 1.4, p<0.01). Similarly, group 1 had higher number of fertilized oocytes (5.3 ± 3.1 vs 3.5 ± 1.4, difference of 1.8; 95%CI; 1.3, 2.3, p<0.01) and higher fertilization rate (86% vs 64%, difference of 18%; 95%CI; 14%, 21%, p<0.01). The number of top quality embryos were also higher in group 1 (3.8 ± 2 vs 3.4 ±1.4, difference of 0.4; 95%CI; 0.1, 0.8, p=0.01). Group 1 had higher chance of day 5 embryo transfer (55% vs 40%) due to higher number of good quality embryos. In most participants of both study groups; 2 embryos were transferred (94% vs 95%). Single embryo transfer was done to the remaining participants (as they had only one transferable embryo). This is because the local protocol of the IVF unit implies 2 embryo transfer whenever possible. The implantation rate was also higher in the VD supplemented group (27% vs 17%, difference 10%; 95%CI 4%, 16%, p<0.01). (Table 2)

Clinical pregnancy rate (the study primary outcome) was higher in VD supplemented group (83/187, 44% vs 63/186, 34%, difference of 10%, 95%CI; 1%, 20%; p=0.03), (Table 2). After adjustment for possible confounders in regression analysis, the Odds ratio for pregnancy in group 1 was 2.1 (95%CI: 2.1, 3.3, p=0.01), (Table 3)

Discussion

In the present study, we evaluated the effect of VD supplementation on ICSI outcome in VD deficient females. Recently, the was increasing interest in finding correlation between VD deficiency and various infertility causes and poor pregnancy outcomes after assisted reproductive techniques (4, 14, 20). However, little is known about the effect of vitamin D supplementation on ICSI outcome (13).

In the present randomized controlled trial, clinical pregnancy rate was significantly higher in VD supplemented group. This result may be due to the positive effect of VD on various aspects of fertility at the pituitary, ovary and endometrial receptivity level (4). These effects probably explains the higher number of fertilized oocytes, higher fertilization and implantation rates.

A recent study has found similar higher pregnancy rates in PCOS females undergoing IVF cycles (after vitamin D supplementation in deficient cases) (13). To the best of our knowledge till now, this is the first big randomized controlled trial assessing the effect of VD supplementation on pregnancy outcome in females undergoing ICSI cycles.

Nevertheless, this study has its limitation. We could not assess the livebirth rate in the study groups which is the best tool for evaluation of pregnancy outcome in ICSI cycles. This limitation arise from the fact that our pregnant patients are hard to follow up. Moreover, most of our patients are from far areas. That is why if we assess the livebirth rate, the expected high dropout rate will affect the accuracy of results. So, we preferred the clinical pregnancy rate to be the primary outcome of the study.

In conclusion; VD supplementation in deficient females undergoing ICSI cycles increases the clinical pregnancy rate, fertilization rate and implantation rate. And in the view of this conclusion, Vitamin D supplementation is recommended in deficient cases to improve ICSI outcome.

Author contributions

EFO: Study design, data analysis and revision of the manuscript

SHG: Study design, conduct and revision of the manuscript

AS: Study design, conduct and revision of the manuscript

MSA: Study design, conduct and revision of the manuscript

MG: Study design, conduct, and manuscript revision

EE: Data analysis and revision of the manuscript

MFS: Study design, conduct and revision of the manuscript

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Conflict of interest

The authors report no conflicts of interest in this research.

References

1. Voulgaris N, Papanastasiou L, Piaditis G, Angelousi A, Kaltsas G, Mastorakos G, et al. Vitamin D and aspects of female fertility. Hormones (Athens). 2017;16(1):5-21.
2. Lerchbaum E, Obermayer-Pietsch B. Vitamin D and fertility: a systematic review. European journal of endocrinology. 2012;166(5):765-78.
3. Aghajafari F, Nagulesapillai T, Ronksley PE, Tough SC, O'Beirne M, Rabi DM. Association between maternal serum 25-hydroxyvitamin D level and pregnancy and neonatal outcomes: systematic review and meta-analysis of observational studies. BMJ (Clinical research ed). 2013;346:f1169.
4. Fichera M, Török P, Tesarik J, Della Corte L, Rizzo G, Garzon S, et al. Vitamin D, reproductive disorders and assisted reproduction: evidences and perspectives. Int J Food Sci Nutr. 2020;71(3):276-85.
5. Zhao J, Huang X, Xu B, Yan Y, Zhang Q, Li Y. Whether vitamin D was associated with clinical outcome after IVF/ICSI: a systematic review and meta-analysis. Reproductive biology and endocrinology : RB&E. 2018;16(1):13.
6. Parikh G, Varadinova M, Suwandhi P, Araki T, Rosenwaks Z, Poretsky L, et al. Vitamin D regulates steroidogenesis and insulin-like growth factor binding protein-1 (IGFBP-1) production in human ovarian cells. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme. 2010;42(10):754-7.
7. Agic A, Xu H, Altgassen C, Noack F, Wolfler MM, Diedrich K, et al. Relative expression of 1,25-dihydroxyvitamin D3 receptor, vitamin D 1 alpha-hydroxylase, vitamin D 24-hydroxylase, and vitamin D 25-hydroxylase in endometriosis and gynecologic cancers. Reproductive sciences (Thousand Oaks, Calif). 2007;14(5):486-97.
8. Irani M, Merhi Z. Role of vitamin D in ovarian physiology and its implication in reproduction: a systematic review. Fertility and sterility. 2014;102(2):460-8.e3.
9. Barrera D, Avila E, Hernandez G, Mendez I, Gonzalez L, Halhali A, et al. Calcitriol affects hCG gene transcription in cultured human syncytiotrophoblasts. Reproductive biology and endocrinology : RB&E. 2008;6:3.
10. Daftary GS, Taylor HS. Pleiotropic effects of Hoxa10 on the functional development of peri-implantation endometrium. Mol Reprod Dev. 2004;67(1):8-14.

11. Wehr E, Pilz S, Schweighofer N, Giuliani A, Kopera D, Pieber TR, et al. Association of hypovitaminosis D with metabolic disturbances in polycystic ovary syndrome. European journal of endocrinology. 2009;161(4):575-82.

12. Bosdou JK, Konstantinidou E, Anagnostis P, Kolibianakis EM, Goulis DG. Vitamin D and Obesity: Two Interacting Players in the Field of Infertility. Nutrients. 2019;11(7).

13. Zhao J, Liu S, Wang Y, Wang P, Qu D, Liu M, et al. Vitamin D improves in-vitro fertilization outcomes in infertile women with polycystic ovary syndrome and insulin resistance. Minerva Med. 2019;110(3):199-208.

14. Chu J, Gallos I, Tobias A, Tan B, Eapen A, Coomarasamy A. Vitamin D and assisted reproductive treatment outcome: a systematic review and meta-analysis. Human reproduction (Oxford, England). 2018;33(1):65-80.

15. Aflatoonian A, Arabjahvani F, Eftekhar M, Sayadi M. Effect of vitamin D insufficiency treatment on fertility outcomes in frozen-thawed embryo transfer cycles: A randomized clinical trial. Iranian journal of reproductive medicine. 2014;12(9):595-600.

16. Moher D. David Moher, MSc The CONSORT Guidelines: improving the quality of research. Consolidated Standards of Reporting Trials. Interview by Bonnie Horrigan. Altern Ther Health Med. 2002;8(3):103-8.

17. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. The Journal of clinical endocrinology and metabolism. 2011;96(7):1911-30.

18. Jones G, Horst R, Carter G, Makin H. Contemporary diagnosis and treatment of vitamin D-related disorders. J Bone Miner Res. 2007;22 Suppl 2:V11-5.

19. Polyzos NP, Anckaert E, Guzman L, Schiettecatte J, Van Landuyt L, Camus M, et al. Vitamin D deficiency and pregnancy rates in women undergoing single embryo, blastocyst stage, transfer (SET) for IVF/ICSI. Human reproduction (Oxford, England). 2014;29(9):2032-40.

20. Moridi I, Chen A, Tal O, Tal R. The Association between Vitamin D and Anti-Müllerian Hormone: A Systematic Review and Meta-Analysis. Nutrients. 2020;12(6).

Table (1): Clinical and laboratory characteristics of the study groups.

Characteristic	Group 1(Vitamin D supplemented) n=187	Group 2 (non-supplemented with Vitamin D) n=186	Difference between groups (95%CI)	P-value
Age (years)	30.1 ± 4.2	29.6 ± 3.5	0.4 (-1.3, 1.2)	0.3 [a]
Body mass index	29.2 ± 5	29.6 ± 3.6	-0.4 (-1.3, 0.4)	0.3 [a]
Type of infertility			NS	0.1[b]
Primary	132 (71%)	117 (63%)		
Secondary	55 (29%)	69 (37%)		
Duration of infertility (years)	5.2 ± 2.5	4.8 ± 2.3	-0.4 (-0.1, 0.8)	0.2 [a]
Cause of infertility			NS	0.8[b]
Male	45(24%)	44 (23%)		
Ovulatory	46 (24%)	42 (22%)		
Combined factors	43 (22%)	48 (25%)		
Tubal factor	37 (20%)	35 (19%)		
Unexplained	16 (9%)	17 (9%)		
Vitamin D level (ng/ml)	13.8 ± 6	14.7 ± 6	-0.9 (-2.2, 0.4)	0.2 [a]

Values are in the form of mean ± SD, or count (percent). NS, non-significant. [a] , T-test. [b], Chi-Square test. CI; confidence interval

Table (2): ICSI cycle outcome in the study groups.

Characteristic	Group 1(Vitamin D supplemented) n=187	Group 2 (non-supplemented with Vitamin D) n=186	Difference between groups (95%CI)	P-value
Retrieved oocytes	8.7 ± 3.8	8.1 ± 1.8	0.6 (-0.01, 1.2)	0.05
Number of mature oocytes	6.4 ± 3.3	5.6 ± 1.9	0.8 (0.2, 1.4)	<0.01*
Fertilized oocytes	5.3 ± 3.1	3.5 ± 1.4	1.8 (1.3, 2.3)	<0.01*
Fertilization rate[a]	83%	64%	18% (14%, 21%)	<0.01*
Number of top quality embryos (1 and 2)	3.8 ± 2	3.4 ±1.4	0.4 (0.1, 0.8)	0.01*
Day of transfer (after ovum pickup)				
Day 5	103 (55%)	74 (40%)	15% (5%, 25%)[b]	0.01*
Day 3	84 (45%)	112 (60%)		
Embryo transfer				
2 embryos	178 (95%)	178 (95%)	NS	0.8
Single embryo	9 (5%)	8 (5%)		
Clinical pregnancy rate	83 (44%)	63 (34%)	10.5% (1%, 20.3%)	0.03*
Implantation rate[c]	27%(97/365)	17% (63/364)	10% (4%, 16%)	<0.01

Values are in the form of mean ± SD, or count (percent). NS, non-significant. CI; confidence interval. a ,fertilization rate was calculated by dividing the number of fertilized oocytes by the total number of sperm injected mature ova. b , difference was calculated in percentage of females who had day 5 transfer. c , Implantation rate is the number of gestational sacs divided by the total number of transferred embryos.

Table (3): Logistic regression for testing the effect of vitamin D supplementation on clinical pregnancy rate adjusting for possible confounders (age, BMI, type of infertility; primary or secondary and cause of infertility).

Study group	Odds ratio (95%CI)[a]	P-value
Vitamin D supplemented group versus Vitamin D non-supplemented group	2.1 (1.3 to 3.3)	0.01*

[a], the odds of having versus non-having pregnancy after ICSI cycle. * ; indicates significance. CI; confidence interval

Figures

Figure 1 Consort flow chart of the study population