GYNECOLOGY

Single oral dose of vitamin D_3 supplementation prior to in vitro fertilization and embryo transfer in normal weight women: the SUNDRO randomized controlled trial

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BACKGROUND: Improving in vitro fertilization success is an unmet need. Observational studies have suggested that women with deficient or insufficient vitamin D have lower chances of in vitro fertilization success, but whether supplementation improves clinical pregnancy rate remains unclear.

OBJECTIVE: This study aimed to determine whether oral vitamin D_3 supplementation improves clinical pregnancy in women undergoing an in vitro fertilization cycle.

STUDY DESIGN: The "supplementation of vitamin D and reproductive outcome" trial is a 2-center randomized superiority double-blind placebo-controlled trial. Subjects were recruited between October 2016 and January 2019. Participants were women aged 18 to 39 years with low vitamin D (peripheral 25-hydroxyvitamin D of $<$30 ng/mL), serum calcium of $\geq$10.6 mg/dL, body mass index of 18 to 25 kg/m^2, and antimüllerian hormone levels of $>$0.5 ng/mL and starting their first, second, or third treatment cycle of conventional in vitro fertilization or intracytoplasmic sperm injection. The primary outcome was the cumulative clinical pregnancy rate per cycle. Pregnancies obtained with both fresh or frozen embryo transfers were included. Clinical pregnancy was defined as an intrauterine gestational sac with a viable fetus. The primary analysis was performed according to the intention-to-treat principle and could also include natural conceptions. Secondary outcomes included total dose of gonadotropins used, embryologic variables (number of oocytes retrieved, number of suitable oocytes retrieved, fertilization rate, and rate of top-quality embryos), and clinical outcomes (miscarriage rate and live birth rate).

RESULTS: Overall, 630 women were randomized 2 to 12 weeks before the initiation of the in vitro fertilization cycle to receive either a single dose of 600,000 IU of vitamin D_3 (n=308) or placebo (n=322). Interestingly, 113 (37%) and 130 (40%) women achieved a clinical pregnancy in the treatment and placebo groups, respectively (P=.37). The risk ratio of clinical pregnancy in women receiving vitamin D_3 was 0.91 (95% confidence interval, 0.75–1.11). Compared with the placebo, vitamin D_3 supplementation did not improve the rate of clinical pregnancy. Exploratory subgroup analyses for body mass index, age, indication to in vitro fertilization, ovarian reserve, interval between drug administration and initiation of the cycle, and basal levels of 25-hydroxyvitamin D failed to highlight any clinical situation that could benefit from the supplementation.

CONCLUSION: In women with normal weight with preserved ovarian reserve and low vitamin D levels undergoing in vitro fertilization cycles, a single oral dose of 600,000 IU of vitamin D_3 did not improve the rate of clinical pregnancy. Although the findings do not support the use of vitamin D_3 supplementation to improve in vitro fertilization success rates, further studies are required to rule out milder but potentially interesting benefits and explore the effectiveness of alternative modalities of supplementation.

Key words: cholecalciferol, infertility, in vitro fertilization, vitamin D

Introduction

Worldwide, more than 8 million infants have been born after in vitro fertilization (IVF) procedures. However, despite the recent refinements in ovarian stimulation protocols and the introduction of new technologies in the laboratory, the live birth rate per initiated cycle remains between 19% and 22% and does not exceed 50% even in couples with optimal prognosis.[1] Improving the effectiveness of IVF is an unmet need.

Several observational studies have correlated low serum 25-hydroxyvitamin D (vitamin D) levels to a reduction of both natural fertility and in vitro fertilization (IVF) success.[2,3] Such findings may be explained by the targeted action elicited by vitamin D in reproductive physiology and mediated by the binding of the active form to its receptor identified in the ovary, uterus, endometrium, and placenta.[2,4,5] The enzyme 1-alpha-hydroxylase, which converts the inactive form of 1,25-dihydroxyvitamin D to its active form, is expressed in the ovary and endometrium, supporting a local paracrine role of the vitamin. Epidemiologic evidence further fueled this hypothesis of the hormone as a regulator of human reproductive function. Even if evidence is not univocal, low vitamin D has been associated with endometriosis, polycystic ovary syndrome, uterine fibroids, and adverse obstetrics outcomes, including preeclampsia, gestational diabetes mellitus, low birthweight and preterm birth.[2,5–8]

A recent meta-analysis assessed the reproductive outcomes of 3711 women undergoing IVF cycles and found favorable outcomes correlated with vitamin D replete status. Women with sufficient vitamin D levels had significantly higher clinical pregnancy rates in autologous oocyte IVF cycles with an odds ratio (OR) of 1.47 (95% confidence interval [CI], 1.20–1.69).[3] A second more selective meta-analysis confirmed

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AJOG at a Glance

Why was this study conducted?

Observational studies have suggested that women with deficient or insufficient serum vitamin D undergoing in vitro fertilization (IVF) have lower chances of pregnancy success, but whether vitamin supplementation improves clinical pregnancy rate remains unclear.

Key findings

In this randomized clinical trial that included 630 women with deficient or insufficient peripheral levels of vitamin D, a single oral dose of 600,000 IU of vitamin D_3 before the initiation of IVF did not improve the chances of pregnancy.

What does this add to what is known?

In women undergoing IVF, preliminary administration of a single high oral dose of vitamin D_3 did not improve the rate of clinical pregnancy.

this finding, showing an OR of 1.47 (95% CI, 1.02−2.08) for women with peripheral levels of ≥30 ng/mL.[9] However, these authors failed to highlight any effect when focusing on the threshold of 20 ng/mL. Moreover, causality of these observations has only been tested in few underpowered interventional studies investigating the possible beneficial effects of vitamin D_3 supplementation in improving the success rate of IVF; however, the results were inconsistent.[10−14] In addition, it is worth pointing out that the cutoff points advocated for vitamin D insufficiency (20−29.9 ng/mL) or deficiency (<20 ng/mL) are based on studies investigating bone health. None of the guidelines for vitamin D were validated for fertility outcomes.[9] In fact, in a recent large cohort of women (N=2569) undergoing embryo transfer, Cai et al[15] failed to identify any cutoff point associated with lower chances of success.

Motivated by the evidence suggesting a possible effect of vitamin D on IVF success and by the appealing profile of vitamin D_3 supplementation in terms of costs and safety, the primary hypothesis for this randomized controlled trial (RCT) was that oral supplementation with a high dose of vitamin D_3 would have improved clinical pregnancy rates in women with low peripheral vitamin D undergoing IVF. In addition, we assessed the effect on gonadotropin doses needed to induce the ovarian follicular growth and the embryologic variables that were prespecified secondary outcomes.

Materials and Methods

Trial design

This pragmatic 2-center randomized superiority double-blind placebo-controlled clinical trial evaluated the efficacy of a single dose of oral administration of vitamin D_3 (cholecalciferol 600,000 IU) in improving the rates of clinical pregnancy in women undergoing IVF. The protocol of the study has been previously reported in detail and was herein succinctly described.[16] The study was approved by the institutional review boards of the 2 participating centers. Patients provided a written informed consent to participate. The EudraCT registration number is 2015-004233-27. The trial was initially registered with Agenzia Italiana del Farmaco on March 8, 2016, and the date of initial participant enrollment was October 10, 2016. Trial registration in EudraCT was delayed because of administrative issues and occurred on November 17, 2017.

The data were collected by trial site personnel and stored in an electronic data capture database. The statistician of the coordinating center analyzed the data. All the authors vouched for the accuracy and completeness of the data and the fidelity of the trial to the protocol. All of them contributed to the interpretation of the results and the preparation of the manuscript. No pharmacologic manufacturer contributed to the planning, design, or conduct of the trial. Trial pills were manufactured by the hospital pharmacologic service of the coordinating center. None of the authors or the participating units received any financial support from the manufacturers with economic interests in vitamin D products during the last 5 years.

Participants

Women undergoing IVF with or without intracytoplasmic sperm injection (ICSI) at the infertility units of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico and IRCCS San Raffaele Scientific Institute between October 2016 and January 2019 were considered for study entry. Recruitment was consecutive but was interrupted during the summer period (June to September) to prevent a potentially relevant confounding effect of transient intense sun exposure during summertime. Only women scheduled for controlled ovarian stimulation aimed at embryo transfer were included, whereas those who were scheduled for frozen oocytes or embryos and those undergoing oocyte retrieval for fertility preservation were excluded. Additional inclusion criteria were as follows: age of 18 to 39 years, body mass index (BMI) of 18 to 25 kg/m^2, no more than 2 previous completed IVF cycles (ie, no more than 2 oocyte retrievals regardless of the number of embryo transfers), preserved ovarian reserve as documented by a level of serum antimüllerian hormone (AMH) of >0.5 ng/mL, and availability of ejaculated semen (couples requiring surgical retrieval of the spermatozoa were excluded). Women who were already taking vitamin D_3 and those with contraindications to vitamin D_3 (hypercalcemia or conditions leading to high peripheral calcium levels, such as sarcoidosis, tuberculosis, or parathyroid diseases) were excluded. Women that, for any reason, could not initiate the IVF cycle within the following 12 weeks were not included but were invited to refer again for inclusion at a later time when they were about to initiate IVF.

Eligible women who agreed to participate underwent a peripheral

blood test to assess serum concentration of vitamin D and calcium. Women were excluded and not randomized if vitamin D levels exceeded 30 ng/mL or calcium was below 10.6 mg/dL. Eligibility for these 2 dosages was checked by an external researcher not involved in patients' management. Researchers, caregivers, and participants were unaware of the results of these dosages.

Intervention and procedures

Supplementation was given as a single administration of oral 600,000 IU of vitamin D_3 to ensure maximal adherence. This modality is expected to properly maintain peripheral levels of vitamin D above 30 ng/mL for 3 months,[17] that is, a period that in most cases properly covers a complete IVF cycle (including both fresh and frozen cycles). Generally, infertile women embarking in IVF are overburden by complex treatments, and ensuring simplicity was considered a priority at the time of study design.

Recruited subjects were planned to initiate IVF cycle within 2 to 12 weeks after administration of the drug. Eligible women were randomized 1:1 into 2 arms. The treatment group received a single oral administration of 600,000 IU of vitamin D_3 diluted in olive oil. The control group received a placebo (only the olive oil preparation). The 2 drugs (vitamin D_3 and placebo) were visually indistinguishable. Randomized patients were given the drug at the time of randomization by researchers in the hospital. They consumed the drug in front of the researcher. During the trial, research staff, caregivers (both physicians and embryologists), and participants were unaware of treatment allocation. Randomization was centralized to the pharmaceutical service of the hospital of the organizing center. The allocation sequence was computer generated and unrevealed to participants, caregivers, and embryologists. The randomization list was stratified for the 2 participating centers.

To maximize the ability of the trial to observe a treatment effect, participants were invited to refrain from using supplements with vitamin D_3 outside of the trial context. In addition, the participants were invited to initiate the cycle within the scheduled period (2−12 weeks) and to promptly perform the subsequent transfers in case of cryopreservation of supernumerary embryos.

An internal validation of the treatment was done on the day of oocyte retrieval by testing the peripheral levels of vitamin D. For women who had their ovarian stimulation cycle interrupted, this assessment was made on the day the cycle was stopped.

Women underwent their IVF cycle according to the clinical standards of the participating centers. The policies used in these centers were recently described in detail elsewhere.[18,19] They include the possibility of embryo transfer cancellation because of elevated risk of developing ovarian hyperstimulation syndrome or premature raise of serum progesterone (documented by the presence of peripheral progesterone above 1.5 ng/mL on the day of ovulation trigger) and the adoption of elective single-embryo transfer in most cases. Apart from the abovementioned assessment of peripheral vitamin D on the day of ovulation trigger or cycle interruption, no additional or particular intervention was introduced.

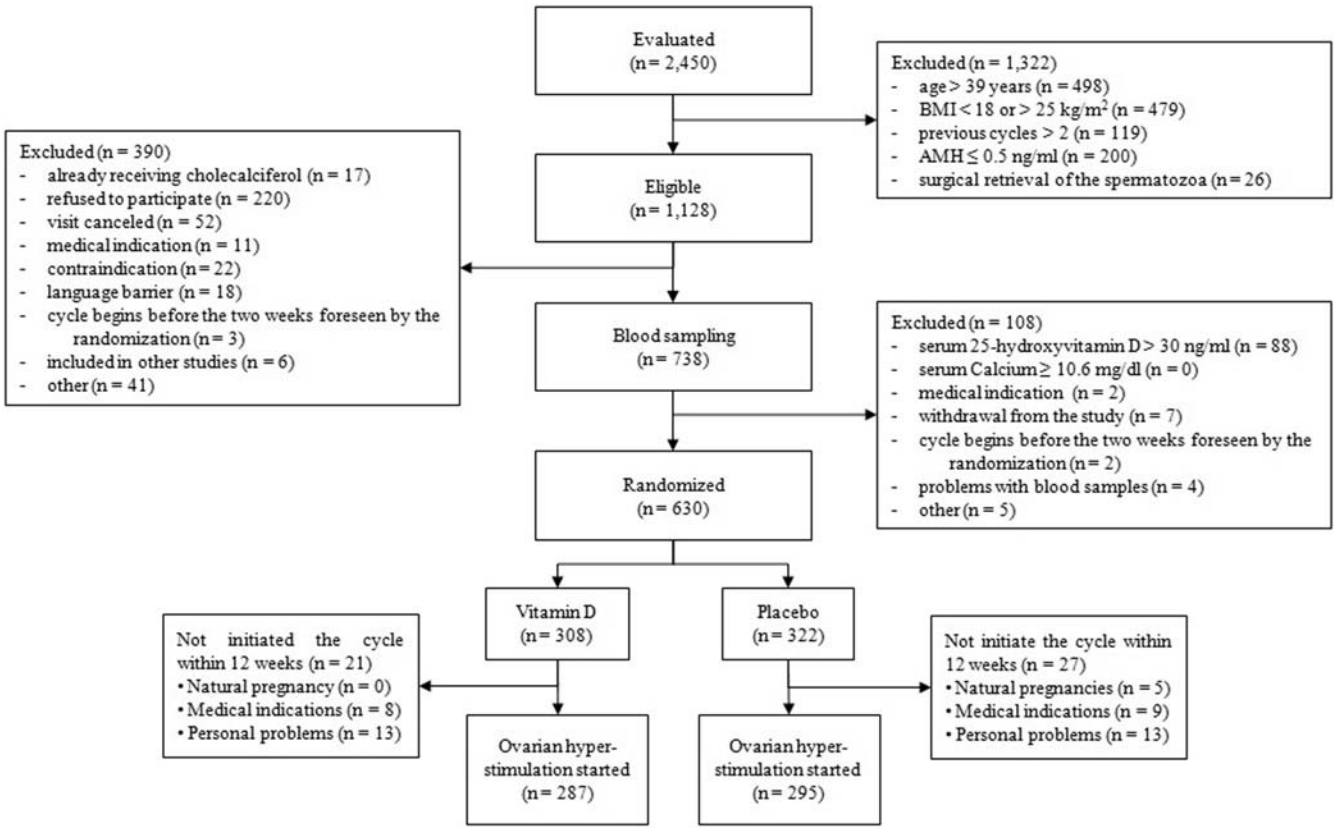
Outcome measures

The primary outcome was the cumulative clinical pregnancy rate per randomized woman. Clinical pregnancies obtained with the transfer of supernumerary frozen embryos were included in the outcome. The study was designed as a pragmatic intention-to-treat trial, and therefore, natural pregnancies recorded before oocytes retrieval were included in the primary outcome. Clinical pregnancy was defined as the presence of at least 1 intrauterine gestational sac with a viable fetus.

Clinical pregnancy rate rather than live birth rate was chosen as the primary outcome because the single administration of a high dose of vitamin D_3 allowed to increase peripheral levels of vitamin D for about 3 months. This time frame is generally sufficient to complete a full IVF cycle (including fresh and frozen transfers) but cannot efficiently cover the whole duration of pregnancy. However, for women achieving pregnancy, the follow-up was prolonged up to the time of delivery or pregnancy termination, and data on live birth rate were presented. More specifically, secondary outcomes included total dose of gonadotropins used, embryologic variables (number of oocytes retrieved, number of suitable oocytes retrieved, fertilization rate, and rate of top-quality embryos), and clinical outcomes (time to pregnancy, miscarriage rate, termination of pregnancy, stillbirth, live birth, gestational age, birthweight, and neonatal health). In addition, data were presented according to the recently implemented core outcomes set for infertility research.[20,21] Natural pregnancies occurring after the oocyte retrieval were not included. For women achieving clinical pregnancies, additional pregnancies that could be subsequently obtained with frozen transfers (transfers after a miscarriage or live birth) were not included in the analysis. Recruitment was stopped once the scheduled sample size was reached. Follow-up was prolonged up to the end of August 2020.

Given the well-known pharmacologic and safety profile of vitamin D_3,[17,22] we did not deem necessary a strict monitoring of the included women, and only severe adverse events were recorded (death, life-threatening conditions, new or prolonged hospitalization, persistent or relevant disability, or congenital anomalies). Only maternal events occurring within 3 months from randomization were considered because a single dose of 600,000 IU is expected to raise peripheral levels for no more than 3 months.[17] However, for congenital anomalies, all pregnancies obtained using the oocytes retrieved during the index cycle could be considered independently from the time passed from randomization because the origin of these defects may take place early in pregnancy. Conversely, we did not make efforts to identify cases of neonatal hypercalcemia[23] because, given the months interval from vitamin administration, relatedness of such events to pre-IVF

FIGURE 1
Flowchart of the study



BMI, body mass index.

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supplementation would have been implausible.

Sample size calculation and statistical analyses

The sample size was calculated on the basis of the following assumptions: (1) expected cumulative clinical pregnancy rate in women with insufficient vitamin D levels (20%); (2) improvement of the absolute rate of cumulative clinical pregnancy rate in supplemented women (10%) (success rate thus raising from 20% to 30%); (3) type I and II errors set at 0.05 and 0.20; and (4) expected proportion of women with insufficient vitamin D (90%). These assumptions were made on the basis of evidence from previous study contributions from our groups.[24,25] Specifically, we observed a clinical pregnancy rate in women with peripheral values of vitamin D_3 of <20 and ≥20 ng/mL of 20% and 31%, respectively.[24] Excluding the summer season, the rate of women with insufficient levels varied between 8% and 27%.[25] On these bases, we predicted an 18-month period of recruitment and a total number of women to be recruited of 700. Taking into consideration the expected rate of excluded women of 10% because of serum vitamin D levels of ≥30 ng/mL, this scheduled sample size could allow us to randomize at least 300 women per arm.

Statistical analysis was made per intention to treat. No adjustment for the primary outcome was foreseen, and the result was presented as crude relative risk (RR) and 95% CI. Data were reported as mean±standard deviation, median (interquartile range [IQR]), and number (percentage) and were compared using the Student *t* test, nonparametric Mann-Whitney test, chi-square test, or Fisher exact test, as appropriate. The Shapiro-Wilk test was preliminary performed to assess the consistency of the data with normal distribution. *P* values below .05 were considered statistically significant. Exploratory subgroup analyses were done for basal levels of vitamin D (<20 ng/mL vs 20−29.9 ng/mL), race (White vs others), indication (unexplained infertility vs others), ovarian reserve, BMI, interval between drug administration and initiation of the cycle, and age. For the last 4 variables, subjects were divided into 2 groups based on the median values of the whole cohort of randomized women.

Results

The flowchart of the study is shown in Figure 1. Overall, 738 women were initially eligible; however, 20 women

subsequently declined to be recruited before randomization, and 88 women were excluded because peripheral levels of vitamin D and calcium did not fulfill our selection criteria. Of the women, 630 were randomized, 308 received cholecalciferol 600,000 IU, and 322 were allocated to placebo. Baseline characteristics of the study groups are shown in Table 1. Median (IQR) serum levels of vitamin D at study entry were 20.0 (15.5−23.6) and 19.9 (14.6−23.9) ng/mL (P=.66), respectively, for treated and control subjects. In addition, 23 women in the treatment arm (7%) and 34 women in the control group (11%) did not perform oocyte retrieval (P=.21). Reasons for dropout are depicted in Figure 1 and did not significantly differ between the study groups.

IVF outcomes for women who underwent oocyte retrieval (285 and 288 women) are shown in Table 2. No significant difference emerged. The dose of gonadotropins needed, the total number of developed follicles, the number of oocytes retrieved, the number of suitable oocytes, the fertilization rate, the total number of embryos, and the total number of top-quality embryos were similar in both treatment groups. Serum levels of vitamin D at the time of oocyte retrieval or cycle cancellation were 52.2 (41.1−64.8) and 19.8 (14.1−24.6) ng/mL in women who did and did not receive vitamin D_3, respectively (P<.001).

IVF outcome in the subgroups of women performing fresh embryo transfer (169+159 women) is summarized in Table 3. None of the studied variables were found to significantly differ.

The cumulative clinical pregnancy rate per oocyte retrieval, the primary outcome of the study, did not differ between the study groups. Interestingly, 113 (37%) and 130 (40%) women achieved a clinical pregnancy in the treatment and placebo groups, respectively (P=.37). The RR of clinical pregnancy in women receiving vitamin D_3 was 0.91 (95% CI, 0.75−1.11). The core clinical outcomes are shown in Table 4.

Severe adverse events were observed in 4 cases. They were all amenable to the group of congenital anomalies. They included 2 pregnancy terminations (1 for Down syndrome in the cholecalciferol arm and 1 for hydrocephalus in the placebo arm) and major congenital abnormalities at birth (1 Down syndrome and 1 Prader-Willi syndrome, both belonging to the placebo arm).

TABLE 1
Baseline clinical characteristics of the study groups

Characteristics	Cholecalciferol n=308	Placebo n=322
Age (y)	35.0 (32.0−37.0)	35.0 (33.0−37.0)
BMI (kg/m^2)	20.8 (19.5−22.5)	21.1 (19.7−22.9)
Previous pregnancies	85 (28)	91 (28)
Previous deliveries	41 (13)	41 (13)
FSH (IU/mL)	6.9 (5.7−8.5)	7.2 (5.9−9.0)
AMH (ng/mL)	2.2 (1.2−4.5)	2.2 (1.3−3.8)
AFC	12.0 (8.0−17.0)	12.0 (8.0−16.0)
Duration of infertility (y)	3.0 (2.0−4.0)	2.5 (2.0−4.0)
Previous IVF cycles	80 (26)	71 (22)
Indication to IVF		
Unexplained	112 (37)	103 (32)
Endometriosis	38 (12)	45 (14)
Tubal factor	26 (8)	23 (7)
Male factor	93 (30)	114 (35)
Genetic (PGT)	15 (5)	15 (5)
Mixed	24 (8)	22 (7)
Basal 25-hydroxyvitamin D (ng/mL)	20.0 (15.5−23.6)	19.9 (14.6−23.9)

Data are presented as median (interquartile range) or number (percentage).

AFC, antral follicle count; *AMH*, antimüllerian hormone; *BMI*, body mass index; *FSH*, follicle-stimulating hormone; *IVF*, in vitro fertilization; *PGT*, preimplantation genetic testing.

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Finally, we performed exploratory subgroup analyses for the primary outcome to investigate whether vitamin D_3 supplementation could be of some benefit in specific situations. We focused on BMI, age, indications to IVF, biomarkers of ovarian reserve, interval between drug administration and initiation of the cycle, and basal levels of vitamin D (Figure 2). We failed to identify any subgroup that could benefit from the supplementation.

Comment

Principal findings

In this RCT involving reproductive age women with deficient or insufficient levels of vitamin D undergoing IVF cycles, supplementation with a single oral dose of 600,000 IU of vitamin D_3 compared with placebo did not result in an increased rate of clinical pregnancy. In addition, surrogate markers of IVF success, such as ovarian responsiveness or embryo number and quality were not improved. Subgroup analyses failed to identify any condition that might benefit from supplementation. Although secondary, the surrogate findings and the subgroups analyses tend to reinforce the general conclusion emerging from the trial, that is, that oral vitamin D_3 supplementation is ineffective.

Results

To our knowledge, 5 RCTs investigated the possible benefits of vitamin D_3 supplementation before IVF.[10−14] In fact, 3 of the studies showed some benefits, but results were difficult to interpret because vitamin D_3 was part of a multiple intervention. Vitamin D_3 was administered

TABLE 2
IVF cycle outcome in the 2 study groups

Characteristics	Cholecalciferol n=285	Placebo n=288	*P* value
Total number of follicles≥11 mm	11 (7–16)	11 (7–15)	.50
Total dose of gonadotropins (IU)	2075 (1550–2763)	2100 (1500–2769)	.98
Duration of stimulation (d)	9 (8–11)	9 (8–11)	.91
Number of oocytes retrieved	8 (5–12)	7 (4–12)	.22
IU of gonadotropins for retrieved oocyte	283 (150–517)	276 (150–550)	.55
Number suitable oocytes[a]	6 (4–9)	6 (3–9)	.13
Number of women without suitable oocytes	3 (1)	10 (3)	.09
Technique[b]			.34
Classical IVF	82 (29)	70 (25)	
ICSI	200 (71)	208 (75)	
Fertilization rate (%)[b]	71 (69–73)	73 (71–75)	.33
Number cleavage embryos[b]	4 (2–6)	4 (2–6)	.48
Number top-quality embryos[b]	1 (0–3)	1 (0–2)	.07
Fresh embryo transfer not performed[b]	113 (40)	119 (43)	.76
High risk of OHSS[c]	75 (66)	76 (64)	
No viable cleavage embryos[c]	20 (18)	24 (20)	
PGT[c]	14 (12)	12 (10)	
Other[c]	4 (4)	7 (6)	
Total number of transfers performed			.43
0	34 (12)	48 (17)	
1	162 (57)	156 (54)	
2	55 (19)	54 (19)	
≥3	34 (12)	30 (10)	
Total number of embryos transferred			.37
0	34 (12)	48 (17)	
1	137 (48)	124 (43)	
2	70 (25)	74 (26)	
≥3	44 (15)	42 (14)	

Data are presented as median (interquartile range) or number (percentage), unless otherwise indicated.

IVF, in vitro fertilization; *ICSI*, intracytoplasmatic sperm injection; *OHSS*, ovarian hyperstimulation syndrome; *PGT*, preimplantation genetic testing.

[a] Suitable oocytes refer to metaphase II oocytes and type 1 cumulus-oocyte complex according to the European Society for Human Reproduction and Embryology Istanbul Consensus Conference (2011); [b] Data refer to subjects retrieving at least 1 suitable oocyte (282 and 278 for treated and untreated women, respectively); [c] The percentages refer to the number of women who did not perform embryo transfer despite retrieving oocytes (113 and 119).

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together with vitamin E in the first RCT[11]; with folic acid, alpha-lactalbumin, myo-inositol, and melatonin in the second one[14]; and with omega-3 and olive oil in the third.[13] Conversely, 2 studies investigated the exclusive effects of vitamin D_3. Aflatoonian et al[10] investigated the effectiveness of 50,000 IU of vitamin D_3 weekly for 6 to 8 weeks in a specific group of women undergoing frozen embryo transfer but failed to show any effect. In a previous study by Abedi et al,[12] the same scheme of treatment, that is, vitamin D_3 50,000 IU weekly for 6 to 8 weeks, was associated with a significantly higher clinical pregnancy rate in women undergoing a complete ICSI cycle. Regardless of the inconsistencies and the differences in study designs, it is noteworthy that all 5 studies were underpowered for robust conclusions; the total number of included women varied between 85 and 114.

Our study was designed to detect a 10% absolute increase in the success rate of the procedure. This target was chosen on the basis of previous epidemiologic evidence of our group showing an 11% absolute difference between women with peripheral vitamin D of <20 and ≥20 ng/mL.[24] However, the current trial cannot rule out milder but still interesting benefits from a clinical and economic point of view. Future studies and meta-analyses are warranted. In addition, it must be underlined that the idea that vitamin D_3 supplementation before IVF may be valuable regardless of the possible benefits on the success of the procedure because of its favorable effects on pregnancy and perinatal outcomes is also not supported. We failed to observe any difference in obstetrics outcome in our study. Furthermore, recent systematic reviews of studies testing the potential effects of vitamin D supplementation in pregnancy failed to show a significant benefit.[26,27] Overall, even if our study did not document any adverse effect of vitamin D_3 supplementation thus confirming the excellent safety profile of this treatment, it did not provide encouraging evidence for recommending vitamin D assessment and vitamin D_3 treatment before the initiation of IVF cycles.

TABLE 3
Outcome of fresh embryo transfers in the 2 study groups

Characteristics	Vitamin D n=169	Placebo n=159	*P* value
Number of embryos transferred			.33
1	140 (83)	128 (81)	
2	29 (17)	29 (18)	
3	0 (0)	2 (1)	
Stage of embryos transferred			.36
48 h	7 (4)	12 (7)	
72 h	118 (70)	103 (65)	
Blastocyst	44 (26)	44 (28)	
Viable intrauterine pregnancy	54 (32)	52 (33)	.91
Lost at follow-up	0 (0)	1 (2)	.49
Miscarriage	6 (11)	3 (6)	.49
Therapeutic abortion[a]	1 (2)	0 (0)	1.00
Early stillbirth	1 (2)	0 (0)	1.00
Live birth	46 (85)	48 (92)	.36

Data are presented as number (percentage), unless otherwise indicated.

[a] Pregnancy termination was decided because of Down syndrome.

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Research implications

Typically, 2 main explanations can be postulated to explain the inconsistency between molecular, epidemiologic, and interventional findings. Firstly, vitamin D deficiency or insufficiency may be associated with other undetected confounders, thus not providing an essential role per se. Vitamin D status may be a surrogate marker of the general state of health. For example, outdoor physical activity, adiposity, smoking, ethnicity, inflammation status, general nutritional conditions, and other unknown factors may produce spurious protective associations.[28–30] In addition, we would like to call attention to the current definitions of vitamin D insufficiency and deficiency, which are based on peripheral levels of vitamin D and are derived from endocrine studies on bone and calcium metabolism. However, no evidence supports the idea that peripheral levels of vitamin D necessary for bone and calcium homeostasis overlap those needed to ensure optimal functioning in the uterus and ovaries. As aforementioned, a large cohort study aimed at identifying a cutoff point for infertility problems failed to provide any meaningful information.[9] To note, vitamin D seems to have a paracrine action in these districts, being locally transformed into the active form. We cannot exclude that vitamin D function may be adequately ensured in these districts even with extremely low levels of peripheral vitamin D. Unfortunately, we could not assess whether or not vitamin D_3 supplementation could be effective only in women with extremely low peripheral levels (<10 ng/mL) because of an insufficient number of these cases (we observed 13 clinical pregnancies of 30 among women allocated to vitamin D_3 group and 9 of 24 among controls; P=.78).

A second explanation for the lack of effectiveness of vitamin D_3 in our trial may be related to the modality of supplementation.[31] We opted for a single oral high dose of cholecalciferol (600,000 IU) as the simplest and most friendly modality of administration. This choice has guaranteed full adherence to the protocol of treatment (all randomized women received the drug) and had previously shown to result in sufficient peripheral levels of vitamin D for up to 3 months, the duration of time needed to cover the oocyte retrieval and up to 2 embryo transfers. Notably, if the benefit had been exerted on oocyte and embryo quality, the impact on the success of the procedure would have lasted beyond the time frame of peripheral vitamin D sufficiency (frozen embryos would have benefited from supplementation even if transferred several months later). However, we cannot exclude that opting for a daily (2000 IU) or weekly (50,000 IU) administration or starting supplementation earlier would have resulted in better outcomes. For example, initiating 6 months before the IVF cycle rather than 2 to 12 weeks would have allowed the whole process of folliculogenesis (lasting about 6 months)

TABLE 4
Clinical reproductive outcomes in the 2 study groups

Outcome	Cholecalciferol n=308	Placebo n=322	*P* value
All pregnancies	124 (40)	135 (42)	.69
Ectopic pregnancy	2 (2)	0 (0)	.14
Early pregnancy loss[a]	9 (7)	5 (4)	.20
Clinical pregnancies	113 (37)	130 (40)	.37
Twins	7 (6)	7 (5)	.79
Time to clinical pregnancy (d)[b]	74 (34–161)	81 (36–129)	.63
Outcome of clinical pregnancies			
Lost at follow-up[c]	0 (0)	2 (2)	.19
Miscarriage	13 (12)	16 (12)	.85
Pregnancy termination[d]	1 (1)	1 (1)	.92
Stillbirth	1 (1)	0 (0)	.28
Cumulative live births	98 (32)	110 (34)	.55
Gestational age	39+1 (38+0–40+1)	39+1 (38+3–40+3)	.57
Birthweight (g)	3190 (2949–3485)	3175 (2835–3370)	.55
Neonatal mortality	0 (0)	0 (0)	1.00
Major congenital abnormalities[e]	0 (0)	2 (2)	.50

Data are presented as median (interquartile range) or number (percentage), unless otherwise indicated.

[a] Early pregnancy loss refer to women who were diagnosed with an intrauterine gestational sac but without a viable embryo; [b] The data refer only to women who achieved a clinical pregnancy. The duration was calculated as the time between randomization and transfer of the embryo that led to clinical pregnancy; [c] Women lost to follow-up were excluded from the count of live births; [d] Pregnancy terminations were decided because of Down syndrome (cholecalciferol) and hydrocephalus (controls); [e] Data represent 1 child with Down syndrome and 1 child with Prader-Willi syndrome.

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to take place in a vitamin D sufficient milieu. Our decision was taken on pragmatic bases, taking into consideration the burden of treatments that women embarking in IVF have to face. Demonstrating that a single 600,000 IU dose of cholecalciferol few weeks before the initiation of IVF was effective would have facilitated prompt adoption of this modality into everyday clinical practice. Importantly, dose, modality, and duration of assumption of cholecalciferol may not be the unique reasons for failure. Generally, in several clinical and research settings, we may have excessively simplified our vision of vitamin D metabolism. The vitamin D production in subcutaneous tissues (which represents 90% of our reserve) is accompanied by the production of several other metabolites whose importance may have been neglected.[32]

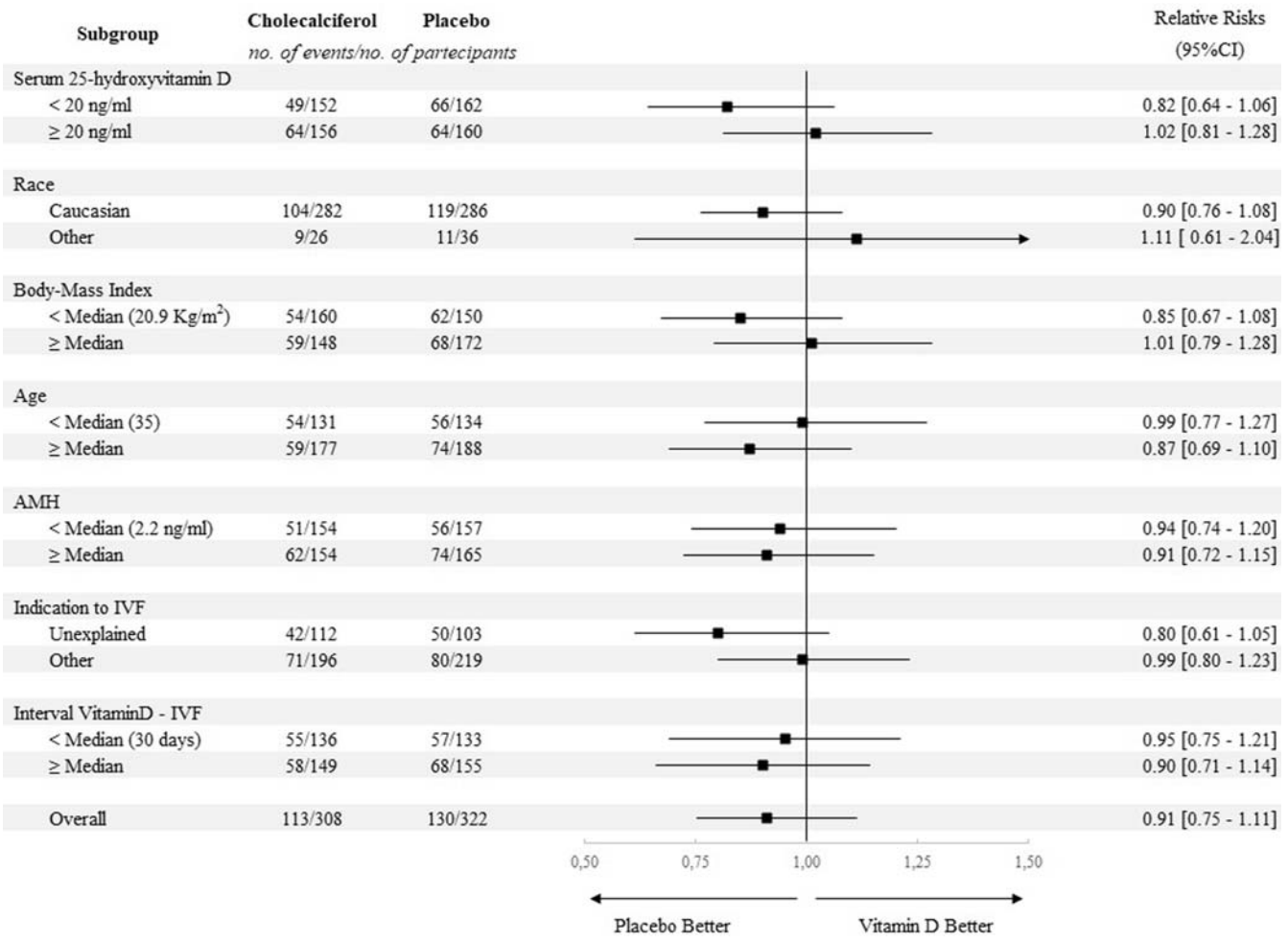
In this regard, it has also to be clarified that we measured peripheral levels of vitamin D in randomized women at the time of oocytes retrieval showing remarkably higher levels in women allocated to the active treatment compared with placebo (52.2 [41.1–64.8] vs 19.8 [14.1–24.6] ng/mL). However, measurement of total vitamin D after stimulation may be less reliable because, as a consequence of the high estrogen levels, the synthesis of vitamin D binding protein increases and the bound vitamin D is not biologically active.[33] A similar confounding effect was demonstrated in pregnancy.[34] Measuring free vitamin D would have provided more robust evidence in support of our strategy of supplementation.

Strengths and limitations

Strengths included the pragmatic approach, large sample size, completeness of data, and availability of information for all core outcomes recently advocated for studies in infertility. An additional interesting strength of the study was the scant diffusion among the studied population of over the counter vitamin D_3 supplementation, which is conversely very common in the United States where the recent RCTs on the preventive effects of vitamin D for type 2 diabetes mellitus, cardiovascular diseases, and cancer were run.[29,35] In contrast to the United States, peripheral assessment of vitamin D and subsequent tailored supplementation is infrequently done in Italy, allowing us to study a practically naive population. Accordingly, basal average levels of vitamin D in our population were 19 to 20 ng/mL, thus markedly lower than the averages of 28 to 31 ng/mL observed in the 2 RCTs carried out in the United States.[29,35]

Limitations include the selection criteria that excluded women who were older, overweight or with a compromised ovarian reserve. One may postulate that vitamin D_3 could be particularly efficacious in these groups of subjects. Exclusion of older women and those with AMH levels of <0.5 ng/mL was decided to enhance the power of the study, whereas exclusion of women with obesity was decided to overcome the complex and still unclear confounder role of adiposity on vitamin D bioavailability.[28] Inferences on our findings should take into consideration this limitation. Furthermore, although exploratory and of limited value, the subgroup analyses performed based on age (younger and older than 35 years), BMI (higher and lower than 20.9 kg/m^2), and AMH levels (higher and lower than 2.2 ng/mL) did not provide support to this concern. A second limitation was the choice of the primary outcome. We opted for clinical pregnancy rate rather than live birth rate. The choice of this outcome was based on the selected modality of supplementation ensuring optimal levels of vitamin D for 3 months and not for the entire duration of pregnancy. However, data on live birth was available and reported in the study. In addition, there was an important discrepancy between the basal assumption of 20% success rate in the nonsupplemental arm used to calculate the sample size and the results

FIGURE 2
Exploratory subgroup analyses



The reported relative risks refer to the chances of achieving clinical pregnancy in women randomized to cholecalciferol in every subgroup of women. The number of subjects was lower for the analysis on the interval between drug administration and IVF because those who did not initiate the cycle were excluded.

AMH, antimüllerian hormone; *CI*, confidence interval; *IVF*, invitro fertilization.

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observed in the trial (40%, in the non-supplemented group). The progressive technological improvements of the procedure in the participating centers since the time of the study design (2012) may have caused a meaningful change in the success rate. Nonetheless, the upper limit of the 95% CI of the RR for clinical pregnancy (1.11) allowed us to rule out an absolute benefit of more than 4.4% (0.40×0.11). Lastly, we limited our safety evaluation to the record of severe adverse events. We observed 2 severe adverse events in the cholecalciferol arm (1 Down syndrome and 1 stillbirth) and 3 in the control arm (1 hydrocephalus, 1 Down syndrome, and 1 with Prader-Willi syndrome). Relatedness of the 2 adverse events in the active arm was deemed unlikely. Even if the studied mode of vitamin D administration (600,000 IU in a single dose) is well established and considered safe, the significance of our study would have been improved by the performance of more accurate safety evaluations, such as serial measurements of calcium, phosphate, parathyroid hormone, and estimated glomerular filtration rate.

Conclusions

We failed to document a benefit of cholecalciferol supplementation with a single oral administration of 600,000 IU of vitamin D on IVF-mediated clinical pregnancy rate. Further studies are required to rule out milder but potentially interesting benefits and explore the effectiveness of alternative modalities of supplementation.

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