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ORIGINAL ARTICLE ***Infertility***

Vitamin D deficiency and pregnancy rates following frozen–thawed embryo transfer: a prospective cohort study

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STUDY QUESTION: What is the effect of vitamin D deficiency on the pregnancy rates following frozen embryo transfer (FET)?.

SUMMARY ANSWER: Vitamin D deficiency does not affect pregnancy rates in FET cycles.

WHAT IS KNOWN ALREADY: Although there is evidence that the potential impact of vitamin D deficiency on reproductive outcome may be mediated through a detrimental effect on oocyte or embryo quality, the rationale of our design was based on evidence derived from basic science, suggesting that vitamin D may have a key role in endometrial receptivity and implantation. Only few retrospective clinical studies have been published to date with conflicting results.

STUDY DESIGN, SIZE, DURATION: This study is the first prospective observational cohort study from the Centre for Reproductive Medicine at the University Hospital of Brussels. The duration of the study was 1 year.

PARTICIPANTS/MATERIALS, SETTING, METHODS: A total of 280 consecutive patients, who had at least one blastocyst frozen and were planned for a FET, were enrolled in the study following detailed information and signing of a written informed consent. Serum analysis of 25-OH vitamin D was measured on the day of embryo transfer, and the impact of vitamin deficiency was investigated on reproductive outcomes.

MAIN RESULTS AND THE ROLE OF CHANCE: Among all patients, 45.3% ($n = 127$) had vitamin D deficiency (<20 ng/ml), and 54.6% ($n = 153$) had vitamin D levels ≥ 20 ng/ml. Positive human chorionic gonadotrophin rates were similar among patients with vitamin D deficiency and women with total serum 25-OH vitamin D levels ≥ 20 ng/ml (40.9 versus 48.3%, $P = 0.2$). Similarly, no difference was found in clinical pregnancy rates in women with vitamin D deficiency [32.2% (41/127)] compared with those with higher vitamin D levels [37.9% (58/153)]; $P = 0.3$. When analyzing the results according to different thresholds, as proposed by the Endocrine Society, clinical pregnancy rates were comparable between vitamin D deficient (<20 ng/ml), vitamin D insufficient (20–30 ng/ml) and vitamin D replete women (≥ 30 ng/ml) [32.3% (41/127) versus 39.5% (36/91) versus 35.5% (22/62), respectively, $P = 0.54$]. Multivariate logistic regression analysis showed that vitamin D status is not related to pregnancy outcome.

LIMITATIONS, REASONS FOR CAUTION: Ethnicity in relation to vitamin D status was not assessed, given that the vast majority of patients included in our study were Caucasian, whereas we did only assess 25-OH vitamin D levels and not bioavailable vitamin D. Furthermore, although we failed to find a difference between vitamin D deficient women and women with vitamin D levels ≥ 20 ng/ml, we need to underscore that our study was powered to detect a difference of 15% in clinical pregnancy rates.

WIDER IMPLICATIONS OF THE FINDINGS: Vitamin D deficiency does not significantly impair pregnancy rates among infertile women undergoing frozen–thawed cycles. The measurement of vitamin D levels in this population should not be routinely recommended.

STUDY FUNDING/COMPETING INTERESTS: No external funding was used for this study. No conflicts of interest are declared.

Key words: frozen embryo transfer / pregnancy rate / vitamin D / blastocyst / implantation

Introduction

Vitamin D is a steroid hormone synthesized mainly by the skin on exposure to ultraviolet light, and vitamin D deficiency is common among reproductive age women. Thus, a role of vitamin D in female reproduction has been suggested, although evidence remains sparse and controversial, with some retrospective studies indicating that vitamin D deficiency is related to lower pregnancy rates (Ozkan *et al.*, 2010; Rudick *et al.*, 2012; Polyzos *et al.*, 2014) and others demonstrating that vitamin D deficiency does not impair reproductive outcome (Anifandis *et al.*, 2010; Aleyasin *et al.*, 2011).

Although the potential impact of vitamin D deficiency on reproductive outcome may be mediated through a detrimental effect on oocyte or embryo quality, evidence derived from basic science suggests that vitamin D may have a key role in endometrial receptivity and implantation (Yoshizawa *et al.*, 1997). Early retrospective studies postulated that vitamin D deficiency may negatively affect pregnancy rates with an effect mediated through the endometrium (Rudick *et al.*, 2012). Nevertheless, the hypothesis of a potential effect of vitamin D deficiency on endometrial receptivity was not confirmed by more recent studies using the oocyte-donor recipient model. Although Rudick *et al.* (2014) has demonstrated that vitamin D deficiency or insufficiency was a detrimental factor for pregnancy rates in oocyte recipients, the most recent report by Fabris *et al.* (2014) failed to identify any association between pregnancy rates and vitamin D status in recipients of donated eggs.

Taking into account the above-mentioned conflicting evidence and the accelerating interest regarding the role of vitamin D in endometrial receptivity, we decided to perform a large prospective cohort study in women undergoing frozen embryo transfer (FET). The scope of the study was to examine the impact of vitamin D levels on clinical pregnancy rates only in an infertile population undergoing embryo transfer of frozen–thawed embryos, in order to evaluate whether a potential negative effect of vitamin D deficiency on pregnancy rates could be mediated through the endometrium.

Materials and Methods

Ethical approval

Institutional review board approval was obtained for the conduction of the study from the Ethical Committee of Universitair Ziekenhuis Brussel (decision number B.U.N 143201317807). All patients gave their informed consent before the date of the embryo transfer to participate in this study.

Patients' eligibility criteria

Overall, 280 consecutive infertile women between 18 and 39 years old, without vitamin D supplementation, who underwent FET cycle with single or double embryo transfer of a blastocyst stage embryo/s (Day 5) were eligible to participate.

The following exclusion criteria were applied:

(1) Women who were planned to have a Day 3 embryo transfer or women who had their cycle cancelled owing to insufficient embryo quality after thawing and warming
(2) Women with uterine abnormalities, endocrine disorders, severe endometriosis, repeated implantation failure and
(3) Patients who underwent embryo transfer (embryo transfer) following *in vitro* maturation.

Serum 25-OH vitamin D measurement

Li-heparin samples from all patients were obtained on the day of the FET and were kept frozen at −20°C until the measurement of 25-OH vitamin D levels. Total plasma 25-OH vitamin D was measured with the Elecsys Vitamin D Total immunoassay on a Cobas6000 immunoanalyzer (Roche Diagnostics, Mannheim, Germany). Total imprecision coefficients of variance were 9.7% at a concentration level of 16.5 ng/ml and 5.9% at 31.7 ng/ml. The analysis was performed the same day or a day after the embryo transfer in the same machine in both groups.

Vitamin D deficiency was defined as serum 25-OH vitamin D levels <20 ng/ml in accordance with the Institute of Medicine (IOM) and the Endocrine Society clinical practice guidelines (Holick *et al.*, 2011; Rosen *et al.*, 2012).

Cryopreservation and thawing–warming procedure

Blastocysts were vitrified by closed vitrification using closed CBS-VIT High Security (HS) straws (Cryo-Bio-System, France) combined with dimethylsulphoxide and ethylene glycol bis (succinimidyl succinate) as the cryoprotectants (Irvine Scientific^{R} Freeze Kit, Ireland). The vitrification and warming procedure was carried out as described by Van Landuyt *et al.* (2015). Fresh blastocyst quality was evaluated on Day 5 and Day 6 using the grading system of Gardner and Schoolcraft (Gardner and Schoolcraft, 1999). Embryos were considered for transfer on Day 5 if they had at least reached the stage of full compaction, early blastocyst (Bl1 or Bl2), full (Bl3), expanded (Bl4) or hatching (Bl5-6) blastocyst. Full, expanded and hatching blastocysts were eligible for fresh transfer if they had at least an inner cell mass (ICM) type C and trophectoderm (TE) quality type B. Supernumerary blastocysts were selected for vitrification on Day 5 or Day 6 if they had reached at least the full blastocyst stage (Bl3) with a good ICM and TE (at least type BB). Top quality embryos at the moment of fresh or vitrified-warmed transfer were considered to have reached at least the full blastocyst stage (Bl3) with an ICM and TE quality type AA or BA. Good quality blastocysts were at least full blastocysts with ICM and TE quality type BB or AB. Moderate quality blastocysts were early blastocysts (Bl1 and Bl2) or at least full blastocysts with ICM and TE type BC, CB, CA, or AC or blastocysts that did not re-expand after warming and which were compacted at the moment of transfer. Embryos were thawed the same day of the embryo transfer, preventing time interval discrepancies between groups.

Preparation of the endometrium

For artificial and natural FET cycles, hormonal serum analysis was performed in Day 1–3 of the cycle to detect a premature estradiol (E2) rise owing to early follicular recruitment or the presence of an ovarian cyst. If circulating E2 levels were more than 80 ng/l, the cycle was cancelled. In principle, normal cycling women in our center are planned for a FET in a natural cycle, while patients with cycle irregularities are planned for FET in an artificially prepared cycle. Nevertheless, the FET regimen remains to the clinicians' discretion and to patient's individual request, as supported by evidence demonstrating that FET with natural or artificial preparation with or without GnRH down-regulation seems to be equally effective (Ghobara and Vandekerckhove, 2008; Glujovsky *et al.*, 2010).

Artificial preparation of the endometrium consisted of 7 days of E2 valerate (Progynova®; Bayer-Schering Pharma AG, Berlin, Germany) at a dose of 2 mg twice daily, followed by 6 days of E2 valerate at a dose of 2 mg three times daily. On Day 13, endometrial thickness was measured by ultrasound scan, and serum E2 and progesterone (P4) levels were analyzed. If serum P4 levels were <1.5 ng/ml and the endometrial thickness was 7 mm or greater, with a triple-line endometrium present, P4 supplementation was started, as described below. If the endometrium was <7 mm in thickness, patients

continued to take oral estradiol until the endometrium thickness was 7 mm or greater, at which point progesterone supplementation was started. If the endometrial thickness remained <7 mm in spite of prolonged E2 priming (with a maximum of 7 days additional estrogen supplementation), the cycle was cancelled.

Natural FET regimen included cycle monitoring through serum E2, P4, LH assessments and serial transvaginal ultrasound examinations. Ovulation triggering was performed either spontaneously by detecting the LH rise or with the administration of 5000 IU of human chorionic gonadotrophin (hCG) as soon as one follicle of 17 mm diameter was observed. All patients with natural FET cycle had at least 7 mm of endometrial thickness the day of the triggering with P4 <1.5 ng/ml, otherwise the cycle was cancelled.

In artificial and natural FET cycles, micronized vaginal progesterone (Utrogestan®; Besins, France) 200 mg three times daily was started as soon as the endometrial thickness measured ≥7 mm on the day of ovulation triggering, respectively. Cryopreserved Day 5 embryos were transferred 7 days after P4 initiation.

Main outcome measures

The primary outcome was clinical pregnancy rates defined as the presence of an intrauterine sac with an embryonic pole demonstrating cardiac activity at 7 weeks of gestation. Our secondary outcomes were positive hCG rates. Serum beta-hCG levels were measured 12 days after embryo transfer. This has been shown as the earliest time point suggested as being useful (Legro *et al.*, 1995; Qasim *et al.*, 1996; Bjercke *et al.*, 1999). A cutoff serum level of >5 IU/l is regarded as a positive beta-hCG. Pregnancy rates were assessed between vitamin-D deficient women (<20 ng/ml) and those with vitamin D levels ≥20 ng/ml. Finally, we also recorded live birth rates for patients included in the study.

Statistical analysis

Continuous variables were analyzed using the independent *t*-test or Mann–Whitney *U*-test depending on the normality of the distribution. Normality was examined by the use of the Shapiro–Wilk test. Categorical variables were analyzed by Pearson's chi-squared test or Fisher's exact test, as appropriate. Seasons were defined prior to data processing according to the calendar definitions of the seasons for Europe with each season lasting 3 months: autumn: September 21–December 20; winter: December 21–March 20; Spring: March 21–June 20; summer: June 21–September 20, as previously described (Wunder *et al.*, 2005). To identify characteristics that may be related with the clinical pregnancy rate, stepwise multivariate logistic regression analysis was performed with the clinical pregnancy as the dependent variable and the vitamin D status as the main independent variable. Univariate regression analyses were performed to identify candidate factors that predict the clinical pregnancy rate. Variables from Table I showing a tendency of association with clinical pregnancy in the univariate analysis were included in the multivariate model. The significance level of the candidate variables to enter the multivariate logistic regression model was set to 0.20, and to stay in the model it was set to 0.05. All candidate variables were simultaneously entered into the logistic regression model. All statistical tests used a two-tailed α of 0.05. Analyses were performed using STATA 13.0 (StataCorp. Stata Statistical Software: Release 13. College Station, TX, USA).

Sample size calculation

Our previous study (Polyzos *et al.*, 2014) has shown that vitamin D-deficient women undergoing fresh embryo transfer have significantly lower pregnancy rates compared with women with vitamin D levels >20 ng/l (41 versus 54%, risk increase of 40%, $P = 0.015$).

Unpublished data from our center have demonstrated 28% of clinical pregnancy rates in women undergoing Day 5 FET. Under the hypothesis that vitamin D-deficient patients will have clinical pregnancy rates of 20% compared with clinical pregnancy rates of 35% in women with vitamin D levels ≥20 ng/l, we calculated that a sample size of 280 women would be essential to have a power of 80% at a level of significance 0.05 to detect a difference in the clinical pregnancy rates between vitamin D-deficient women and the control group.

Table I Characteristics of patients by vitamin D status.

	Vitamin D <20 ng/ml	Vitamin D ≥20 ng/ml	*P* value
Number of patients	127	153	
BMI (kg/m^2)	23.7 (4.3)	22.9 (3.4)	0.2[a]
Age (years)	31.2 (3.7)	31.8 (3.7)	0.15[a]
Infertility type			
Endometriosis	8 (6.3%)	7 (4.6%)	0.8[b]
Genetic	21 (16.5%)	30 (19.6%)	
Idiopathic	27 (21.3%)	30 (19.6%)	
Male	49 (38.6%)	62 (40.5%)	
Ovulation disorder	12 (9.5%)	14 (9.2%)	
Tubal	10 (7.9%)	10 (6.5%)	
Season of FET			
Autumn	43 (33.9%)	52 (34%)	<0.001[b]
Spring	30 (23.6%)	37 (24.2%)	
Summer	9 (7.1%)	36 (23.5%)	
Winter	45 (35.4%)	28 (18.3%)	
Pregnancy in the fresh IVF/ICSI cycle	32 (25.2%)	43 (28.1%)	0.6[b]
Endometrial protocol preparation			
Artificial cycle	21 (16.5%)	28 (18.3%)	0.7[b]
Natural cycle	106 (83.5%)	125 (81.7%)	
Endometrial thickness on day of planning FET	8.3 (2.2)	8.2 (1.7)	0.9[a]
Number of blastocysts transferred			
1	94 (74%)	128 (83.7%)	0.04[b]
2	33 (26%)	25 (16.3%)	
At least one top quality blastocyst in the FET cycle	52 (46%)	61 (54%)	0.8[b]
Blastocyst quality[c]			
N top quality (%)	56 (35.0%)	62 (34.8%)	0.9[b]
N good quality (%)	97 (60.6%)	105 (59.0%)	
N moderate quality (%)	8 (5.0%)	11 (6.2%)	
Positive hCG	52 (40.9%)	74 (48.3%)	0.2[b]
Clinical pregnancy	41 (32.2%)	58 (37.9%)	0.3[b]
Delivery live born[d]	32/118 (27.1%)	51/146 (34.9%)	0.2[b]

BMI, body mass index; FET, frozen embryo transfer.
[a]Two-sample Mann–Whitney test. Values are mean (SD).
[b]Person χ^2 test. Variables are numbers (percentages).
[c]Percentages are calculated per number of embryos transferred (160 in vitamin D < 20 ng/ml group and 178 in vitamin D ≥ 20 ng/ml group).
[d]Data on live births could not be retrieved for nine patients in vitamin D < 20 ng/ml group and seven patients in vitamin D ≥ 20 ng/ml group due to loss of follow-up.

Results

Patients' characteristics

In total, 280 consecutive infertile patients were included in the analysis. Among them, 127 (45.4%) were vitamin D deficient and 153 (54.6%) had levels of vitamin D ≥20 ng/ml.

Patients' baseline characteristics according to vitamin D status are presented in Table I. Comparisons between the two groups did not reveal any significant difference for age, BMI, clinical pregnancy rate in the previous fresh IVF/ICSI cycle, protocol for endometrial preparation, endometrial thickness prior to embryo transfer and the quality of the blastocyst(s) transferred (top or good quality) in the frozen cycle.

The season of the FET and the number of blastocysts transferred (one or two) were the only variables which differed significantly ($P < 0.001$ and $P = 0.04$, respectively) between the two groups.

Main outcomes

Overall, 45% (126/280) of the patients had a positive hCG and 35.3% (99/280) had a clinical pregnancy. As shown in Table I, positive hCG rates did not significantly differ between women with vitamin D deficiency (<20 ng/ml) compared with those with higher vitamin D values [40.9% (52/127) versus 48.7% (74/153), $P = 0.2$]. Similarly, no difference was found in clinical pregnancy rates in women with vitamin D deficiency [32.2% (41/127)] as compared with women with higher vitamin D levels [37.9% (58/153)]; $P = 0.3$.

When analyzing the results according to different thresholds, as proposed by the Endocrine Society (Holick *et al.*, 2011), clinical pregnancy rates were comparable between vitamin D deficient (<20 ng/ml), vitamin D insufficient (20–30 ng/ml) and vitamin D replete women (≥30 ng/ml) [32.3% (41/127) versus 39.5% (36/91) versus 35.5% (22/62), respectively, $P = 0.54$].

Multivariate logistic regression analysis for clinical pregnancy rates

In Table II, the unadjusted odds ratios (ORs) with the standard errors and 95% confidence intervals of the univariate logistic regression analysis between clinical pregnancy rates and age, BMI, clinical pregnancy rate in the previous fresh IVF/ICSI cycle, protocol for endometrial preparation, endometrial thickness prior to embryo transfer and the quality of the blastocyst(s) transferred (top or good quality) are presented. The endometrial preparation ($P = 0.08$) and the clinical pregnancy's outcome in the previous fresh cycle ($P = 0.2$) were included as covariates in the final model. Nevertheless, in multivariable logistic regression analysis, none of the above-mentioned variables managed to stay in the regression model, as the final *P*-values were >0.05.

Table II Univariate logistic regression with ORs for clinical pregnancy rates.

	OR*	SE	95% CI	*P*-value
Vitamin D levels				
<20 ng/ml	1	–	–	0.3
≥20 ng/ml	1.3	0.3	0.8–2.1	
BMI	0.97	0.03	0.9–1.04	0.4
Age	0.9	0.03	0.9–1.04	0.5
Season of FET				
Autumn	1	–	–	0.9
Spring	1.19	0.4	0.6–2.3	
Summer	0.8	0.3	0.4–1.8	
Winter	1.03	0.3	0.3–0.8	
Pregnancy in the fresh IVF/ICSI cycle				
No	1	–	–	0.2
Yes	1.4	0.4	0.8–2.4	
Endometrial protocol preparation				
Natural cycle	1	–	–	0.08
Artificial cycle	0.5	0.2	0.2–1.08	
Endometrial thickness on day of planning FET	1.06	0.06	0.9–1.2	0.3
Number of blastocysts transferred				
1	1	–	–	0.8
2	1.05	0.3	0.6–1.9	
At least 1 top quality blastocyst in the FET cycle				
No	1	–	–	0.3
Yes	1.3	0.3	0.8–2.1	

SE, standard error; CI, confidence interval; FET, frozen embryo transfer; BMI, body mass index.
*Unadjusted OR.

Discussion

To the best of our knowledge, this is the first prospective cohort study investigating the effect of serum vitamin D levels in pregnancy rates of women undergoing FET. According to our results, vitamin D deficiency does not significantly impair pregnancy rates in -patients undergoing FET.

Although there is indeed evidence from basic research suggesting that vitamin D may have a role in reproductive outcome and that its action may be mediated through the endometrium (Vigano *et al.*, 2006; Zarnani *et al.*, 2010), results from clinical studies are conflicting. Our study failed to demonstrate any effect of vitamin D levels on pregnancy rates after FET. This is in line with two recent studies, one in oocyte acceptors (Fabris *et al.*, 2014) and one in infertile women undergoing preimplantation genetic screening (PGS) and embryo transfer of euploid blastocysts, in which no association between vitamin D and pregnancy rates was identified (Franasiak *et al.*, 2015). Nevertheless, our findings are in complete contrast with other studies, either in infertile women undergoing fresh embryo transfer (Rudick *et al.*, 2012) or in oocyte acceptors according to which women with vitamin D levels ≥20 ng/ml consistently had higher pregnancy rates compared with their deficient counterparts (Rudick *et al.*, 2014). Furthermore, our current report is in disagreement with a previous study by our group in infertile patients undergoing elective single embryo transfer (Polyzos *et al.*, 2014), a population in which vitamin D deficiency compromised pregnancy rates by 40%.

In attempt to elucidate the discrepancy between the above-mentioned reports, several explanations may be given. First of all, in spite of the extensive literature with retrospective studies evaluating the role of vitamin D in infertile women, none of the previous studies measured the levels of 25-OH vitamin D on the day of embryo transfer.

In particular, some of the studies did not specify the exact timing of the vitamin D determination, whereas in the vast majority of them stored blood samples were used (Rudick *et al.*, 2012, 2014). The current study, by using a prospective design, evaluated the vitamin D levels of all patients on the exact day of the embryo transfer, ensuring that those serum 25-OH vitamin D levels are robustly reflecting the actual status of the patients.

Second, we cannot exclude that many of the previously published studies might have been prone to selection and information bias, owing to the retrospective study design and the use of previously frozen blood samples. In this regard, and based on our findings, we can claim that vitamin D deficiency is not associated with a decrease in the pregnancy rates following FET.

Finally, apart from the robust methodological approach, a major strength of our study is that based on our selection criteria, we invited and eventually recruited a patient population, which was relatively homogeneous. Only women who reached the blastocyst transfer stage with one or two good/top quality embryo(s) transferred in the FET cycle were included. Thus, we eliminated biases related to embryo quality and we could evaluate whether vitamin D deficiency independently affects clinical pregnancy rates in FET cycles.

On the other hand, we need to highlight several limitations, which need to be taken into consideration when interpreting our results.

First, ethnicity or environmental factors in relation to vitamin D status were not assessed, despite the fact that a previous report suggested a potential relation between ethnicity, vitamin D deficiency and pregnancy rates (Rudick *et al.*, 2012), given that the vast majority of patients included in our study were Caucasian. In the same context, our study was not designed to evaluate bioavailable vitamin D levels, a biomarker which is more robust than 25-OH vitamin D (Powe *et al.*, 2013; Fabris *et al.*, 2014). Nevertheless, it needs to be highlighted that the major advantage of providing bioavailable vitamin D levels is that it may allow us to control for differences in concentrations of vitamin D binding protein between different races, which have been reported between Black and Caucasian races (Powe *et al.*, 2013). Although we cannot provide such data nor reanalyze the samples, it is highly unlikely that this might have affected our results, given that most of the patients included were of Caucasian race.

Second, although the population included was relatively homogenous, several variables, which might have affected the reproductive outcome, differed between vitamin D deficient and women with vitamin D levels $\geq$20 ng/ml. For example, the proportion of SET/DET was significantly higher in the vitamin D-deficient group, whereas vitamin D-deficient women had a lower percentage of top quality embryos compared with women with vitamin D levels $\geq$20 ng/ml. Nevertheless, we need to highlight that although confounding bias is always a threat for the validity of the results of observational studies, differences observed in our study are highly unlikely to have biased our results. The reason behind this assumption is that although several differences were observed in the univariate analysis, results from multivariable logistic regression analysis after adjustment for potential confounders demonstrated that vitamin D deficiency had no impact on clinical pregnancy rates.

Finally, although we failed to find a difference between vitamin D-deficient and non-deficient women, we need to underscore that our study was powered to detect a difference of 15% in clinical pregnancy rates based on previous findings by our group (Polyzos *et al.*, 2014). In this regard, we cannot exclude that smaller differences might exist, which our study might have been underpowered to detect. In addition, although live births are also reported in our study, results should be interpreted with great caution, given that we were unable to monitor the actual vitamin D levels and the use of any supplements during pregnancy.

In conclusion, allowing for the limitations mentioned above, the current study suggests that vitamin D deficiency does not significantly affect pregnancy rates among infertile women undergoing frozen–thawed cycles. Nevertheless, despite that our results failed to demonstrate any effect of low vitamin D levels on clinical outcomes, evidence from basic research supports that vitamin D deficiency may have a detrimental effect on folliculogenesis and embryo implantation. Consequently, evidence from translational research is urgently needed to help us understand whether vitamin D deficiency may affect the expression of genes involved in the folliculogenesis and embryo implantation processes. At this stage, and until such evidence becomes available, vitamin D deficiency should not be considered to have a detrimental effect in the pregnancy rates of patients undergoing frozen–thawed cycles and thus measurement of vitamin D levels in this population should not be routinely recommended. Nevertheless, considering that vitamin D supplementation is a cheap and safe intervention and can be used without basal 25-OH vitamin D measurement, it might be useful to explore smaller benefits in terms of pregnancy rate.

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Authors' roles

N.P.P. was responsible for the concept design. A.V.V and A.V. scrutinized patients' files. P.D. performed the statistical analysis. A.V.V., P.D. and N.P.P. wrote the manuscript. L.V.L, C.B., S.S.-R., A.V. and H.T. contributed to the interpretation of the results and editing of the manuscript.

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Conflict of interest

None declared.

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