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Research

Effect of vitamin D status on clinical pregnancy rates following in vitro fertilization

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Abstract

Background: Recent studies suggest that vitamin D may play a role in human reproduction. Our goal was to investigate whether vitamin D levels are predictive of implantation and clinical pregnancy rates in infertile women following in vitro fertilization (IVF).

Methods: We prospectively evaluated vitamin D status, as determined by serum 25-hydroxy-vitamin D (25[OH]D) levels, in a cohort of 173 women undergoing IVF at Mount Sinai Hospital, Toronto, Ontario. Serum 25(OH)D samples were collected within 1 week before oocyte retrieval. We classified patients as having sufficient (≥ 75 nmol/L) or insufficient (or deficient; hereafter referred to as "insufficient"; < 75 nmol/L) serum levels of 25(OH)D. We compared patient demographics and IVF cycle parameters between groups. The primary outcome measure was clinical pregnancy (intrauterine sac visible on ultrasound performed 4–5 weeks after embryo transfer).

Results: Of the included women, 54.9% had insufficient 25(OH)D levels and 45.1% had sufficient levels. Women with sufficient levels had significantly higher rates of clinical pregnancy per IVF cycle started (52.5%) compared with women with insufficient levels (34.7%; $p < 0.001$). Implantation rates were also higher in the sufficient 25(OH)D group, but the results were not statistically significant. Multivariable logistic regression analysis (adjusted for age, body mass index and day 5 [v. day 3] embryo transfer) showed that serum 25(OH)D level may be a predictor of clinical pregnancy (adjusted odds ratio 1.01, 95% confidence interval 1.00–1.03).

Interpretation: Our findings suggest that women with sufficient levels of vitamin D are significantly more likely to achieve clinical pregnancy following IVF. Vitamin D supplementation could provide an easy and cost-effective way of improving pregnancy rates; this merits further investigation. Trial registration: ClinicalTrials.gov, no. NCT01348594.

Infertility affects 15% of couples in North America.[1] Recent studies support the role of vitamin D in human reproduction and suggest that vitamin D levels predict reproductive success following in vitro fertilization (IVF).[2,3]

Vitamin D is a prohormone that is acquired exogenously from the diet or produced endogenously in the skin. Vitamin D is metabolized primarily in the liver to 25-hydroxy-vitamin D (25[OH]D), the serum concentration of which can be used as an indicator of vitamin D status. The classification of vitamin D status varies in the literature,[2,4,5] and because the relation between vitamin D and fertility has only recently been investigated, no specific cut-off values have been referenced in the literature. However, a Canadian guideline defined vitamin D deficiency as levels below 25 nmol/L, insufficiency as levels between 25 and 74 nmol/L, and sufficiency as levels of 75 nmol/L and greater.[6] Lower levels of vitamin D (< 75 nmol/L) have also been associated with a higher incidence of certain types of cancer and impaired immune response.[4,5]

People living in countries at higher latitudes, such as the United States and Canada, are more prone to vitamin D insufficiency, especially during the winter months.[7] Vitamin D insufficiency is highly prevalent in women of reproductive age.[2,7] A Canadian study by Veith and colleagues[7] reported that 25.6% of nonwhite and 14.8% of white women of reproductive age (18–35 yr) had insufficient vitamin D levels (defined as < 40 nmol/L). Ozkan and colleagues[2] reported a 36% prevalence of vitamin D insufficiency (50–74 nmol/L) and a 27% prevalence of deficiency (< 50 nmol/L) among women of reproductive age with infertility.[2] Anifandis and colleagues[8] reported that the prevalence of vitamin D insufficiency (20.1–30 ng/mL) or deficiency (< 20 ng/mL) was 79% in a population of women undergoing IVF.

The link between vitamin D status and reproduction has been largely shown in murine models.[9–12] In an early study by Halloran and Deluca,[9] female rats were fed diets that were sufficient or deficient in vitamin D. The rats were then mated

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and their fertility determined. The authors reported that rats fed the diet deficient in vitamin D had 75% reduced fertility and 30% smaller litter sizes than rats fed the sufficient diet.[9] Hickey and colleagues[10] also found that female rats fed a diet lacking vitamin D before mating had significantly smaller litters than rats fed a diet containing vitamin D.

Further evidence supporting an association between vitamin D and reproduction comes from studies of the vitamin D receptor.[12–14] Vitamin D receptors are found in various reproductive tissues, including ovarian and uterine tissue.[12,13,15] Studies by Yoshizawa and colleagues[12] observed normal growth and development before weaning (analogous to human puberty) in mice lacking the vitamin D receptor; however, after weaning, these mice failed to thrive and were infertile. Vitamin D has also been shown to regulate expression of the *HOX* gene in the uterus.[13,14] Vitamin D and the transcription factors produced from the *HOX* genes, specifically HOXA10/11, are hypothesized to function as part of the endocrine signal-transduction pathway, regulating endometrial development in preparation for implantation.[13,14]

Previous studies have examined the effect of vitamin D deficiency on reproductive success following IVF in humans.[2,3,8,16] Ozkan and colleagues[2] performed a prospective cohort study that measured the level of 25(OH)D in the follicular fluid of 84 women undergoing IVF. They found that women with higher levels of vitamin D in their follicular fluid were significantly more likely to achieve implantation and clinical pregnancy following IVF.[2] A subsequent retrospective analysis of vitamin D levels in oocyte donor and recipient cycles (i.e., the recipient receives an embryo derived from an oocyte donor), by Rudick and colleagues[3] also found that vitamin D insufficiency in the oocyte recipients was associated with lower rates of clinical pregnancy. Conversely, a small prospective study by Aleyasin and colleagues[16] did not find a significant difference in biochemical (i.e., very early pregnancy loss) or clinical pregnancy rates across tertiles of follicular fluid 25(OH)D levels.[16] A prospective cohort study by Anifandis and colleagues[8] suggested that higher 25(OH)D levels, combined with decreased glucose levels, in follicular fluid may have a negative impact on the success of IVF. In this study, the rate of clinical pregnancy was 32.3% in the 25(OH)D deficient group (< 20 ng/mL), 32.7% in the insufficient group (20.1–30 ng/mL) and 14.5% in the sufficient group (> 30 ng/mL) (p = 0.047).

In the current study, our goal was to determine whether serum vitamin D levels are predictive of IVF outcomes among infertile women. Our main objective was to investigate whether vitamin D deficiency is associated with lower rates of clinical pregnancy after IVF.

Methods

Study design

We prospectively evaluated vitamin D status, as determined by serum 25(OH)D levels, in a cohort of women undergoing IVF at Mount Sinai Hospital between April 2011 and November 2011. The study was approved by our institution's research ethics board, and informed written consent was obtained from all participants. This study was conducted at an academic hospital-based fertility centre.

Patients

Women undergoing IVF for any indication were eligible for inclusion in this study. Women who met the following criteria were included: aged 18–41 years, follicle-stimulating hormone level 12 IU/L or lower (baseline level, cycle day 3) and able to provide informed consent. We excluded third-party reproduction cycles (i.e., using donor oocytes or a gestational carrier), those with known uncorrected congenital or acquired uterine anomalies, and those who were unable to provide informed consent (e.g., language barrier).

Demographic data and IVF cycle data for each included patient were obtained via chart review.

IVF cycles

The included patients underwent IVF cycles as per standard clinical care. Standard agonist (long luteal and microdose flare) protocols and antagonist IVF protocols were used. The agonist protocols used buserelin acetate (Suprefact, Sanofi Aventis; subcutaneous) starting in the midluteal phase at a dose of 0.5 mg/d for long protocols or at the start of the cycle for microdose flare protocols. The antagonist cycles used cetrolix acetate (Cetrotide, EMD Serono) or ganirelix acetate (Merck), started as per flexible start protocol, when estradiol levels were 2000 pmol/L or higher or the size of the dominant follicle was 14 mm or larger. Recombinant or purified urinary follicle-stimulating hormone, with or without lutenizing hormone or human menopausal gonadotropin, was used for controlled ovarian hyperstimulation; the starting doses were determined before study recruitment based on age, follicle-stimulating hormone level in the early follicular phase, and the number of antral follicles. Doses were increased or decreased on an individual basis. Ovarian response was assessed by serial transvaginal ultrasonograpy and by serum lutenizing hormone and estradiol assays.

Serum 25(OH)D samples were collected before oocyte retrieval. Nuclear maturation was triggered with 10 000 IU of human chorionic gonadotropin (Merck) when 3 or more dominant follicles (≥ 17 mm) were achieved. Transvaginal ultrasound-guided oocyte retrieval was performed 36–38 hours following injection of human chorionic gonadotropin. Ultrasound-guided fresh embryo transfer was performed on day 3 or 5 after fertilization. Vaginal micronized progesterone suppositories were used for luteal-phase support and were continued until fetal cardiac activity was documented.

Outcomes

The primary outcome was the clinical pregnancy rate per IVF cycle started among women with sufficient (≥ 75 nmol/L) or insufficient (< 75 nmol/L) vitamin D levels. Clinical pregnancy was defined as an intrauterine sac visible on ultrasound.

The secondary outcomes were prevalence of vitamin D sufficiency, insufficiency and deficiency and the effect of vita-

min D on IVF cycle parameters (i.e., number of oocytes and implantation rate). Implantation was determined by the presence of a gestational sac, visible by ultrasonography. The implantation rate was calculated as the number of gestational sacs observed by ultrasonography divided by the number of embryos transferred, multiplied by 100.

Statistical analysis

Patients were classified as having sufficient (≥ 75 nmol/L) or insufficient (< 75 nmol/L) 25(OH)D levels. Continuous variables are reported as mean (± standard deviation [SD]), and categorical variables are reported as percentages. We used χ^2 and Student *t* tests or Mann–Whitney U tests to analyze categorical and continuous variables, respectively. We used multivariable logistic regression to evaluate the relation between serum 25(OH)D level and implantation and clinical pregnancy after adjustment for parameters known to influence the IVF success (age, body mass index [BMI], day 5 [v. day 3] embryo transfer). (At our institution, embryo transfer is performed on day 5 if there are at least 5–6 good quality embryos present on day 3.) We calculated sample size (n = 170) using a power of 0.8, a significance level of 0.05, and a 95% confidence interval (CI). We expected the clinical pregnancy rate to be 40% among women with sufficient vitamin D levels and 20% among women with insufficient levels, based on our institution's clinical pregnancy rates and previous vitamin D IVF studies.[2]

All data analyses were performed using IBM SPSS Statistics version 19.0.

Results

We recruited 182 patients, and 173 were included in our analysis. We excluded 9 women because they did not meet our inclusion criteria: 4 women aged greater than 41 years, and 5 women who did not start an IVF cycle. All of the included patients underwent oocyte retrieval, and 162 underwent embryo transfer. Of the 11 patients who did not undergo embryo transfer, 4 women did not have oocytes or embryos of sufficient quality, and 7 had their embryos frozen secondary to ovarian hyperstimulation syndrome (n = 3), fertility preservation (n = 1), hydrosalpinx (n = 1), no sperm available (n = 1) or inappropriate endometrial lining (n = 1). There was no difference in the distribution of these women between insufficient and sufficient groups.

The prevalence of vitamin D deficiency, insufficiency and sufficiency was 1.2%, 53.8% and 45.1%, respectively. In our analyses, we grouped together women with deficient and insufficient 25(OH)D levels (hereafter referred to as "insufficient"). Body mass index was significantly higher among women with insufficient vitamin D levels (mean 24.8 ± SD 4.7) than among women with sufficient levels (mean 23.3 ± SD 3.8; p = 0.02) (Table 1). The remaining patient characteristics did not differ significantly between groups. Table 2 shows the IVF cycle parameters for women in both groups. Those in the sufficient 25(OH)D group were more like to undergo embryo transfer on day 5 (v. day 3; 71.8%) compared to women in the insufficient group (58.9%, p = 0.054) . There was no difference in the number of oocytes retrieved, the percentage of cycles in which intracytoplasmic sperm injection was performed and number of embryos transferred.

We found a higher clinical pregnancy rate per IVF cycle started among women with sufficient 25(OH)D levels (52.5%) than among women with insufficient levels (34.7%; p < 0.001) (Table 2). We also observed a higher clinical pregnancy rate per embryo transfer performed among women in the sufficient group than among women in the insufficient group (54.7% v. 37.9%, p < 0.001). Although the implantation rate was higher in the sufficient group (34.5%) than in the insufficient group (25.6%), this difference was not statistically significant (p = 0.6).

Multivariable logistic regression analysis (adjusted for age, BMI and day 5 [v. day 3] embryo transfer) showed that serum 25(OH)D level was an independent predictor of clinical pregnancy (p = 0.046; Table 3). Increasing clinical pregnancy rates were observed across serum 25(OH)D tertiles (data not shown).

Table 1: Characteristics of 173 women undergoing in vitro fertilization, by vitamin D status

Characteristic	Vitamin D status, mean (± SD)*		*p* value†
	Insufficient or deficient *n* = 95	Sufficient *n* = 78	
25-Hydroxy-vitamin D, nmol/L	54.5 ± 14.0	95.5 ± 17.8	< 0.001
Age, yr	34.6 ± 4.0	34.4 ± 3.9	0.8
Ethnic background, no. (%)			0.3
White	56 (58.9)	58 (74.4)	
Black	3 (3.2)	1 (1.3)	
Other	36 (37.9)	19 (24.4)	
Body mass index	24.8 ± 4.7	23.3 ± 3.8	0.02
Gravidity	0.8 ± 1.1	0.9 ± 1.3	0.6
Parity	0.2 ± 0.5	0.3 ± 0.6	0.5
Duration of infertility, mo	20.7 ± 15.1	17.5 ± 12.3	0.1
Follicle-stimulating hormone, baseline, IU/L	6.4 ± 1.9	6.8 ± 2.3	0.9
Antral follicle count, baseline, no. of follicles	15.9 ± 8.4	17.7 ± 10.3	0.2
Previous IVF cycles, no.	0.7 ± 1.0	0.6 ± 1.0	0.4
Season in which IVF cycle was performed, no. (%) of patients			0.8
Spring	50 (52.6)	41 (52.6)	
Summer	31 (32.6)	28 (35.9)	
Fall	14 (14.7)	9 (11.5)	

Note: IVF = in vitro fertilization, SD = standard deviation.
*Unless otherwise stated.
†χ^2 (categorical) and Student t or Mann–Whitney U (continuous) test; statistically significant, p < 0.05.

Interpretation

We found that the serum level of 25(OH)D, as a marker of vitamin D level, may be a predictor of IVF pregnancy among women undergoing treatment for infertility. Women in our cohort with sufficient vitamin D levels had significantly higher rates of clinical pregnancy following IVF compared with women with insufficient or deficient levels. This finding is clinically significant and may hold potential therapeutic implications because 54.9% of women in our study had insufficient or deficient levels.

The prevalence of vitamin D deficiency (< 25 nmol/L), insufficiency (25–74 nmol/L) and sufficiency (≥ 75 nmol/L) in our population was 1.2%, 53.8% and 45.1%, respectively. These findings are similar to those previously reported in a North American cohort of reproductive-age women.[7] The study by Ozkan and colleagues,[2] which included women of reproductive age with infertility, found a slightly higher preva-

Table 2: In vitro fertilization cycle parameters for 173 women, by vitamin D status

Characteristic	Vitamin D status, no. (%) or mean ± SD		*p* value*
	Insufficient or deficient *n* = 95	Sufficient *n* = 78	
IVF protocol, no. (%)			0.08
Long	29 (30.5)	34 (43.6)	
Antagonist	64 (67.4)	40 (51.3)	
Flare	2 (2.1)	4 (5.1)	
Day of hCG injection	12.1 ± 1.3	11.9 ± 1.4	0.4
Gonadotropin dose, IU	2602.8 ± 1287.3	2547.1 ± 1330.3	0.8
Follicle diameter > 14 mm, no.	11.9 ± 6.5	11.7 ± 5.8	0.8
Estradiol level on the day of hCG administration, pmol/L	8556.7 ± 6187.3	9776.15 ± 5902.6	0.2
Endometrial thickness on the day of hCG administration, cm	1.0 ± 1.6	1.0 ± 0.2	0.5
Oocytes retrieved, no.	12.6 ± 7.4	12.7 ± 6.6	0.9
Intracytoplasmic sperm injection, no. (%)	74 (77.9)	63 (80.8)	0.4
Embryos transferred, no.	1.8 ± 1.0	1.8 ± 0.9	0.9
Day of embryo transfer, no. (%)			
Day 3	31 (32.6)	19 (24.4)	0.2
Day 5	56 (58.9)	56 (71.8)	0.054
Implantation rate, %	25.6	34.5	0.6
Clinical pregnancy rate, per cycle start, %	34.7	52.5	< 0.001
Clinical pregnancy rate, per embryo transfer, %	37.9	54.7	< 0.001

Note: hCG = human chorionic gonadotropin, IVF = in vitro fertilization, SD = standard deviation.
*χ^2 (categorical) and Students *t* or Mann–Whitney U (continuous) test; statistically significant, $p < 0.05$.
†Follicle-stimulating hormone or human menopausal gonadotropin, depending on protocol.

Table 3: Predictors of clinical pregnancy among 173 women undergoing in vitro fertilization

Variable	Crude		Adjusted	
	Odds ratio (95% confidence interval)	*p* value	Odds ratio (95% confidence interval)	*p* value†
Age, yr	0.96 (0.89–1.04)	0.3	0.98 (0.90–1.07)	0.7
Body mass index	0.92 (0.86–1.00)	0.05	0.94 (0.87–1.01)	0.1
Day 5 embryos transferred (v. day 3)	1.50 (0.81–2.90)	0.2	1.28 (0.61–2.77)	0.5
25-Hydroxy-vitamin D, mmol/L	1.02 (1.00–1.03)	0.2	1.01 (1.00–1.03)	0.046

*Analyses adjusted for age, body mass index, number of day 5 embryos transferred and 25-hydroxy-vitamin D level.
†Statistically significant, $p < 0.05$.

lence of vitamin D insufficiency (50–74 nmol/L; 36%) and deficiency (< 50 nmol/L; 27%). There was a very low prevalence of vitamin D deficiency (< 25 nmol/L) in our study (1.2%). This may have been because of a difference in our definition of deficiency, because most women in our study were taking prenatal vitamins (400 IU vitamin D) or because our study did not extend over the winter months. However, the percentage of women with insufficient or deficient levels compared to those with sufficient levels did not significantly differ in the spring, summer or fall months or with respect to ethnic background (Table 1).

Body mass index was significantly higher among women in the insufficient 25(OH)D group (mean 24.8 [± SD 4.7]) than among those in the sufficient group (23.3 [± SD 3.8]; p = 0.02). This finding is consistent with existing knowledge about vitamin D metabolism and has been reported in the literature.[17] Vitamin D is a fat soluble vitamin, and adipose tissue is hypothesized to act as a reservoir for its storage, thus reducing its bioavailability.[17] Lagunova and colleagues[17] found a significant decrease in serum 25(OH)D levels in women as BMI increased. The prevalence of vitamin D deficiency (≤ 50 nmol/L) in their study was highest among women with a BMI of 40 or greater.

The mechanism by which vitamin D affects fertility is unclear. Postulated mechanisms include its effect on ovarian steroidogenesis and implantation.[2,3,8,13,14] We found no significant differences between women in the sufficient and insufficient 25(OH)D groups with respect to IVF cycle parameters including the day of human chorionic gonadotropin injection, gonadotropin dose, estradiol level on the day of human chorionic gonadotropin administration, endometrial thickness, the number of oocytes retrieved, or the number of embryos transferred. Therefore, it is unlikely that the observed difference in clinical pregnancy rates was caused by differences in ovarian steroidogenesis. The implantation rate was nonsignificantly higher among women with sufficient 25(OH)D levels (p = 0.6); however, the rate of clinical pregnancy per embryo transfer was significantly higher among women with sufficient levels (p < 0.001). One explanation for this finding may be that our study may have been underpowered to detect a difference in the implantation rate. We did find, however, that women with sufficient vitamin D levels were significantly more likely to have a day 5 embryo transfer. At our institution, the decision to transfer an embryo on day 3 versus day 5 is individualized and is based on a number of factors, including the number of good quality embryos. Therefore, the observed difference in the rate of clinical pregnancy between groups may be related to higher embryo quality among women with sufficient 25(OH)D levels. These results are in contrast to those of Anifandis and colleagues,[8] who found a higher clinical pregnancy rate among women with deficient or insufficient 25(OH)D levels in the follicular fluid, suggesting that excess vitamin D in follicular fluid may negatively affect IVF success. In their study, the mean embryo quality score, but not the cumulative embryo score, was significantly lower among women with sufficient vitamin D levels compared with those in the insufficient and deficient groups (p = 0.009).[8] Our prospective study is larger than the previously cited studies of the association between vitamin D and IVF outcomes, and we focused on serum (not follicular fluid) levels of vitamin D. Although BMI and the number of day 5 embryo transfers was significantly different between groups, only serum 25(OH)D level was an independent predictor of clinical pregnancy in our multivariable logistic regression analysis.

Limitations

This is the largest study in the literature with respect to the relation between vitamin D and infertility in human IVF populations; however, the interpretation is still limited by the sample size. A larger sample size could have perhaps resulted in a smaller p value and the 95% CIs may not have included 1. In this study, we focused on serum vitamin D levels and did not measure the level of 25(OH)D in follicular fluid. However, previous studies have shown that the levels in these fluids are highly correlated,[2] and serum levels have greater clinical utility. If embryo quality scores were available, we would have been better able to determine whether endometrial or embryo quality affected implantation and clinical pregnancy rates. Future studies should focus on determining the mechanism by which vitamin D affects clinical pregnancy, and they should include measures of embryo quality, implantation and uterine receptivity. Studies should also be undertaken to investigate whether vitamin D supplementation can improve the rates of pregnancy following IVF.

Conclusion

Our findings suggest that women with sufficient levels of vitamin D are significantly more likely than those with insufficient levels to achieve clinical pregnancy following IVF. Vitamin D supplementation may be an easy and cost-effective way of improving pregnancy rates and merits further investigation. There was a high prevalence of vitamin D insufficiency or deficiency in our study population. Therefore, it may be beneficial to determine vitamin D status as part of routine infertility assessment and before artificial reproductive treatment, especially in women with higher BMI.

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