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Male vitamin D status and male factor infertility

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Abstract

Objective: To determine the association between vitamin D levels in the male partner and fertility outcomes in couples with mild male factor infertility.

Design: Secondary analysis of a randomized, controlled trial

Setting: Nine fertility centers in the United States

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Patient(s): Males (N = 154) with sperm concentration between 5 M/mL and 15 M/mL, motility ≤ 40%, or normal morphology ≤ 4% were eligible. Female partners were ovulatory, ≤ 40 years old, and had documented tubal patency.

Intervention(s): Men provided semen and blood at baseline for semen analysis and 25-hydroxyvitamin D (250HD) levels. They were randomly assigned to receive a vitamin formulation including Vitamin D 2000 IU daily or placebo for up to 6 months. Couples attempted to conceive naturally during the first 3 months and with clomiphene citrate with intrauterine insemination of the female partner in months 4 through 6.

Main Outcome Measure(s): Primary: sperm concentration, motility, morphology, and DNA fragmentation at baseline; Secondary: cumulative pregnancy, miscarriage, and live birth rates

Result(s): Semen parameters and sperm DNA fragmentation were not statistically significantly different in males with vitamin D deficiency compared to males with 25OHD levels ≥ 20 ng/mL. Clinical pregnancy and live birth rates were also similar. Male 25OHD level < 20 ng/mL was associated with a higher pregnancy loss rate [adjusted OR 9.0 (95% CI 1.3 to 61.3), p = 0.024]

Conclusion(s): Vitamin D deficiency in the male partner did not significantly impact semen parameters or treatment outcomes. Further study is warranted to better characterize the rate of miscarriage in couples with male vitamin D deficiency.

Capsule:

Vitamin D deficiency in the male partner did not significantly impact semen parameters or fertility treatment outcomes in a secondary analysis of a randomized controlled trial.

Keywords

Vitamin D; 25-Hydroxyvitamin D; Male factor infertility

Introduction

Vitamin D deficiency, defined as a 25-hydroxyvitamin D (25OHD) level less than 20 ng per milliliter (< 50 nmol/1), is highly prevalent worldwide, but the health implications are poorly understood (1–3). While the importance of vitamin D in bone health and metabolism is generally accepted, no consensus exists regarding vitamin D's importance in male reproduction. Vitamin D receptors (VDR) and enzymes for vitamin D metabolism have been identified in testes, the male reproductive tract, and sperm, lending biologic plausibility to a potential role of vitamin D deficiency or insufficiency in male factor infertility (4). Additionally, mice lacking the VDR have decreased sperm counts and motility, with only partial rescue observed with calcium supplementation (5–7).

Existing observational data have been mixed, with comparisons of vitamin D levels and semen parameters showing positive correlations in some, but not all studies (8–21). Differences in populations studied (vitamin D deficient vs. vitamin D replete, fertile vs. subfertile) further complicate interpretation of the available data. Additionally, few studies have included data on pregnancy outcomes, with conflicting results (18–19). One randomized trial of 330 men with low circulating 250HD found that supplementation

increased the live birth rate among oligospermic men but did not observe any improvement in semen parameters (22).

The purpose of this study was to investigate the association between male vitamin D deficiency and fertility outcomes in couples with mild male factor infertility participating in a large multicenter clinical trial.

Methods

This is a secondary analysis of data from the randomized clinical trial, Males, antioxidants, and Infertility (MOXI) trial. Only men who provided a serum sample for analysis of 25OHD and semen for semen analysis at baseline were included in this analysis.

Study cohort

The study design and methods for the Reproductive Medicine Network MOXI trial have been previously described (23). In brief, MOXI was a double-blind, multi-center randomized controlled trial of an antioxidant formulation for males use in couples with mild male factor infertility, conducted from December 2015 to December 2018. Males with isolated male infertility, with at least one abnormal semen parameter on a semen analysis within 6 months of study participation, were randomized to receive placebo or a commercially available antioxidant formulation containing 500 mg of vitamin C, 400 mg of vitamin E, 0.20 mg of selenium, 1,000 mg of L-carnitine, 20 mg of zinc, 1,000 μg of folic acid, 10 mg of lycopene, and 2,000 IU of vitamin D daily. Males with sperm concentration < 5 million/mL were excluded. Female partners were ≥18 and ≤ 40 with regular menstrual cycles, evidence of ovulation, and evidence of a normal uterus and at least one patent fallopian tube. IRB approval was obtained through the University of Pennsylvania.

Male serum 25OHD levels, a semen analysis, and sperm DNA fragmentation testing were obtained at baseline and 3 months after initiation of the study antioxidant formulation or placebo. Serum 25OHD were measured with the Eagle Biosciences ELISA assay kit. Semen samples were analyzed according to World Health Organization (WHO) standards (24). Pregnancy and live birth rates were determined after participating couples attempted conception for 3 months, followed by clomiphene citrate with intrauterine insemination for 3 months. Final analysis of the MOXI trial did not show improvement with the antioxidant formulation compared to placebo for live birth rate, pregnancy rate, miscarriage rate, time to pregnancy, semen parameters or sperm DNA fragmentation. The MOXI trial is registered with ClinicalTrials.gov, number NCT02421887.

Outcomes

The primary outcomes of interest for this secondary analysis were sperm concentration, motility, morphology and DNA fragmentation at the baseline visit. Secondary outcomes were pregnancy, live birth, and pregnancy loss rates after 6 months of trying to conceive per the MOXI protocol. Pregnancy was defined by a positive home pregnancy test within 6 months of treatment. Live birth was defined as delivery of a live infant after 20-weeks gestation. Pregnancy loss was defined as the difference between pregnancy and live birth.

Analysis of sperm motility by 25OHD categories <10 ng/mL, 10-19 ng/mL, 20-29 ng/mL, 30-39 ng/mL, 40-49 ng/mL and ≥ 50 ng/mL was conducted to interrogate the data to better understand potential differences between optimal vitamin D levels in male reproductive function versus vitamin D levels for bone health. No difference was noted across the 25OHD categories (Supplemental table 1). Vitamin D cutoffs were therefore determined according to the Endocrine Society Guidelines (3). Vitamin D deficiency was defined as a 25OHD level < 20 ng/mL, while insufficiency was characterized by a 25OHD level between 20 and 29 ng/mL. Vitamin D sufficiency was defined as a 250HD level 30 ng/mL and above. The primary analysis dichotomized vitamin D status at the level of deficiency (25OHD level < 20 ng/mL vs. ≥ 20 ng/mL).

Statistical analysis

Continuous data were analyzed using the Wilcoxon rank-sum test and categorical data were analyzed using the χ^2 or Fisher's exact test. Multivariable logistic regression and general linear regression were used to determine the association between vitamin D levels and semen parameters and treatment outcomes, respectively. Male ethnicity, BMI, season of blood draw, and total sun exposure, as measured by the Sun Exposure and Behaviour Inventory (SEBI) questionnaire, were included as covariates with biologically plausible influence on vitamin D levels. Male age and race were included in the regression analysis of semen parameters due to the presence of significant differences between the groups dichotomized at 25OHD level < 20 ng/mL and ≥ 20 ng/mL. Female ethnicity and race were highly correlated with male ethnicity and race and were consequently not included in the regression analysis of pregnancy outcomes. Analyses were conducted using SAS 9.4. A p-value < 0.05 was considered statistically significant.

Results

Initial vitamin D levels were available for 154 male participants. Baseline characteristics of male participants with available vitamin D levels were not different from participants lacking a vitamin D level (Supplemental table 2). Seventeen percent (N=26) of participants had a 25OHD level < 20 ng/mL, consistent with vitamin D deficiency. Fifty-nine percent (N=91) had a 25OHD level < 30 ng/mL, consistent with vitamin D insufficiency. Baseline demographics for male participants and their female partners are summarized in Table 1. Male race and age were associated with vitamin D deficiency in male subjects. Black males represented 8.4% of the population studied and 30.8% of males with a 25OHD < 20 ng/mL. Female race and ethnicity were associated with male vitamin D deficiency. Male and female race and male and female ethnicity were highly correlated (Cramer's V Coefficient = 0.58 and 0.47, indicative of a very strong correlation). Vitamin D levels measured after 3 months of treatment with the antioxidant formulation containing 2000 IU of vitamin D were not significantly different from baseline levels [0.8 IU change (95% CI −2.4 to 7.3, p = 0.47)]. Visit 3 vitamin D levels were significantly higher in males with a baseline 25OHD < 20 ng/mL, but this change was irrespective of randomization to the treatment or control group (Supplemental table 3).

Baseline visit 1 semen analysis parameters and DNA fragmentation did not statistically significantly differ by the presence or absence of vitamin D deficiency (Table 2). These results did not change after adjusting for season of blood draw, male age, ethnicity, race, BMI, and total sun exposure (Supplemental table 4). Analysis using a 25OHD cut-off of < or ≥ 30 ng/mL did not impact the results (data not shown).

Of the 154 couples analyzed, 39 (25%) achieved a pregnancy during the study. There were no significant differences in pregnancy or live birth rates by vitamin D status. This finding was not affected by whether the couple was randomized to the treatment or the placebo group (Table 3). These results did not change after adjusting for season of blood draw, male age, ethnicity, race, and total sun exposure. Pregnancy loss occurred in nine couples (23%). Male 25OHD level < 20 ng/mL was associated with a higher pregnancy loss rate (5/8 vs. 4/31, p = 0.009). This finding was consistent across the entire cohort, the placebo group, and the treatment group (Table 3). These results did not change after adjusting for male age and race, with an adjusted odds ratio of 9.042 (95% CI 1.333 to 61.345, p = 0.024) for pregnancy loss for male 25OHD level < 20 ng/mL. Analysis was also performed using a 25OHD cut-off of < or ≥ 30 ng/mL. All nine pregnancy losses occurred in couples with a male 25OHD level < 30 ng/mL (9/25 vs. 0/14, p = 0.015).

Discussion

This secondary analysis of the MOXI prospective randomized controlled trial did not detect a relationship between semen parameters or sperm DNA fragmentation and vitamin D deficiency, using a 25OHD cutoff of 20 ng/mL. Additionally, clinical pregnancy and live birth rates were similarly unrelated to male 25OHD levels. Male 25OHD level < 20 ng/mL was, however, associated with a higher pregnancy loss rate.

The small sample size and potential confounding factors must be considered in the interpretation of this finding.

There is little consensus in the literature on the impact of vitamin D on male fertility. Vitamin D receptors have been localized to the human testis, epididymis, seminal vesicle and prostate, as well as in the human sperm, at the mid-piece and the sperm nucleus (25–28). It is still unclear whether vitamin D deficiency or insufficiency in men impact male fertility. Studies based on semen analysis parameters tend to yield inconsistent data, which may in part reflect the relatively poor predictive value of semen analysis for male fertility (29). Existing studies are also plagued by significant heterogeneity in study design, populations studied, vitamin D cutoff values, and adjustment for potential confounders such as age, sun exposure and BMI (30).

Our study failed to find differences in semen analysis and sperm DNA fragmentation between men with mild male factor infertility with and without vitamin D deficiency. These data conflict with several prior observational studies (8–17). A cross-sectional study of 300 men from the general population found that vitamin D correlated positively with sperm motility and progressive motility, although median motility and progressive motility were in the normal range for men with vitamin D deficiency (8). In a cross-sectional study of 186

infertile men and 79 fertile men, Zhu, et al. compared fertile and infertile men by 25OHD and 1-25 dihydroxyvitamin D [1,25(OH)2D]. The authors found no difference in 25OHD levels but did find lower 1,25(OH)2D levels in men with abnormal semen parameters compared to fertile men. They also showed a correlation between 1,25(OH)2D levels and progressive motility and total sperm number, but no correlation between 25OHD levels and semen parameters (10). Our study did not measure 1,25(OH)2D levels. The study with the largest sample size to date, 1248 infertile men, also reported lower sperm motility and number of total motile sperm in men with vitamin D deficiency (defined as 25OHD level < 10 ng/mL) (11). This study included 99 men with serum 25OHD < 10 ng/mL, whereas our study included 18 men. It is possible that very low vitamin D has a greater impact on sperm motility and our study failed to detect this due to the smaller number of severely deficient men assessed.

Our study is not the first to find a lack of a relationship between vitamin D and semen parameters (18–21). One cross-sectional study of 307 healthy young men analyzed by "low," "medium" and "high" 25OHD levels found that high 25OHD was associated with lower total sperm and morphology, but this finding was not significant after adjustment for potential confounders (20). Another cross-sectional study of 198 university students from southern Spain and found no relationship between vitamin D and semen parameters (21). Both studies focused on young healthy men who had mostly not yet tried to conceive, and both had relatively few men with vitamin D deficiency. These differences make comparisons with studies focused on men with infertility, including our study, more difficult. Two smaller studies, discussed in more detail below, focused on men with infertility and also found no association between 25OHD and semen parameters, consistent with our study (18–19).

While multiple observational studies have examined semen parameters and vitamin D, pregnancy outcome data are sparse. Tartagni, et al., performed an observational study of 102 couples with unexplained infertility undergoing gonadotropin mono-follicular ovulation induction with timed intercourse after repletion of female vitamin D insufficiency and analysis dichotomized at male vitamin D insufficiency. Their results showed no difference in semen parameters, but a significantly higher pregnancy rate per couple and per treatment cycle in the males with normal vitamin D (19). Neville, et al., reported a cross-sectional study of 73 men undergoing IVF/ICSI, with 25OHD levels for both male and female study participants. They found no differences in male semen parameters or ongoing pregnancy rates by male 25OHD levels (18). Differences in study populations may contribute to the conflicting results. Our secondary analysis of the MOXI trial focused on couples with mild male factor infertility undergoing non-IVF treatment, but unlike the study by Tartagni, et al, we did not find any difference in pregnancy or live birth rates. The smaller sample size and focus on unexplained infertility in the Tartagni, et al. study, as well as difference in treatment protocol may have influenced study findings.

A recent single-center triple-blinded randomized clinical trial randomized 330 men with male factor infertility and vitamin D insufficiency (defined as 25OHD < 20 ng/mL) to high dose vitamin D supplementation plus calcium or placebo. The authors did not find an overall difference in semen parameters or live birth rates between the groups. The chance of spontaneous pregnancy resulting in live birth in the treatment group compared to the

placebo group approached, but did not reach, statistical significance (7.3% vs. 2.4%; 95% CI, −0.6% to 10.5%). Subgroup analysis of oligospermic men did, however, demonstrate an increased live birth rate in the treatment group (35.6% vs. 18.3%; 95% CI, 1.6% to 32.9%) (22). The study's overall negative findings are consistent with the MOXI trial results, which showed no benefit of an antioxidant formulation that included vitamin D, although circulating 25OHD levels in the MOXI trial were unchanged with treatment and the amount of vitamin D3 provided in the antioxidant formulation was limited (2000 IU).

In the MOXI trial, couples in which the male was vitamin D deficient were more likely to experience a pregnancy loss. Prior studies have not found an association between male vitamin D deficiency and subsequent pregnancy loss (18, 19, 22). Tartagni, et al. found an increased number of miscarriages in the low vitamin D group, with a 60% miscarriage rate, but this finding did not reach statistical significance ($p = 0.23$). Vitamin D may play a role in genomic stability in somatic cells (31–32). Localization of VDRs to the sperm nucleus may suggest a role of vitamin D in maintenance of sperm DNA integrity (27). Our finding of higher miscarriage rates in couples with male vitamin D deficiency and insufficiency could be explained by a role of vitamin D in maintenance of sperm DNA integrity, although we did not find an association between DNA fragmentation and vitamin D status. This analysis did not include female vitamin D levels. Data on couple vitamin D concordance are lacking (33). In our data, we found a moderate correlation between male and female BMI (Spearman Correlation Coefficient 0.31, P-value < 0.001). Considering BMI is a strong predictor of vitamin D levels, it is likely that there is at least some correlation between male and female partners for vitamin D levels. The impact of female vitamin D levels on pregnancy loss rates are not clear, with some studies showing a correlation between low vitamin D and miscarriage, and others not (34–38). Even if the correlation between males and females for vitamin D deficiency is high, it is unlikely that female vitamin D levels completely explain our findings, as the data to date do not show a strong association between female vitamin D insufficiency or deficiency and miscarriage. Future studies would be significantly strengthened by inclusion of female partner vitamin D levels.

Our study found no difference in semen parameters by vitamin D level, but did find a higher pregnancy loss rate. Similarly, Tartagni, et al., found a higher pregnancy rate in couples with normal male vitamin D levels, but no concomitant improvement in semen parameters (19). Finally, Jensen, et al. showed that vitamin D supplementation did not improve semen parameters, but, in certain groups, may improve live birth rate (22). Taken together, vitamin D may improve pregnancy and birth outcomes through mechanisms that do not lend themselves well to clinical interrogation. Additionally, these findings challenge the utility of the semen analysis in predicting fertility treatment outcomes.

The quality of our data, collected during the MOXI prospective RCT across multiple participating clinics, distinguishes this analysis from prior observational studies. Our study benefitted from reginal diversity, strict inclusion criteria, and evaluation of female partners to rule-out female causes of infertility. Use of data from the MOXI trial limited biases inherent in most observational studies. Our study's relatively modest sample size may have impacted our ability to detect subtle differences in semen parameters. Despite this, it is unlikely that small variations in semen parameters would be clinically significant. Based upon the

multi-center design, our study findings are widely applicable for couples with isolated mild male-factor infertility. The overall low total number of pregnancies and miscarriages makes it difficult to interpret our data on the impact of male vitamin D levels and pregnancy loss rates. The total percentage of pregnancy loss is comparable to expected population rates, but our finding of significantly higher rates in couples with male vitamin D deficiency and insufficiency should be confirmed with larger studies.

Conclusions

Vitamin D deficiency does not appear to negatively impact semen parameters or DNA fragmentation. Couples in which the male partner has vitamin D deficiency do not appear to have a lower likelihood of in vivo conception or subsequent live birth. Our finding of higher pregnancy loss among couples with male vitamin D deficiency should be confirmed with larger studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Characteristics at screening for male subjects by male vitamin D status

	Male Vitamin D < 20 ng/mL (n=26)	Male Vitamin D ≥ 20 ng/mL (n=128)	P value
Age, median (IQR), years	37.0 (32.0-40.0)	33.0 (30.0-36.0)	0.029
Body mass index, median (IQR), kg/m^2	29.1 (25.2-31.6), n=24	27.6 (24.2-31.0), n=128	0.400
Obese (BMI ≥ 30 kg/m^2)	9/24 (37.5%)	38/128 (29.7%)	0.447
Ethnicity, No. (%)			0.418
Hispanic or Latino	2/26 (7.7%)	7/128 (5.5%)	
Non-Hispanic	22/26 (84.6%)	116/128 (90.6%)	
Unknown	2/26 (7.7%)	5/128 (3.9%)	
Race, No. (%)			<0.001
White	12/26 (46.2%)	107/128 (83.6%)	
Black	8/26 (30.8%)	5/128 (3.9%)	
Asian	2/26 (7.7%)	7/128 (5.5%)	
American Indian or Alaska Native	0/26 (0.0%)	1/128 (0.8%)	
Unknown	3/26 (11.5%)	7/128 (5.5%)	
Mixed Race	1/26 (3.8%)	1/128 (0.8%)	
Duration of infertility, median (IQR), months	24.0 (16.0-36.0)	24.0 (16.0-36.0)	0.973
History of smoking, No. (%)			0.406
Never	13/26 (50.0%)	75/128 (58.6%)	
Current	5/26 (19.2%)	13/128 (10.2%)	
Former	8/26 (30.8%)	40/128 (31.3%)	
Blood Draw Season, No. (%)			0.876
Spring	2/6 (33.3%)	12/45 (26.7%)	
Summer	0/6 (0.0%)	7/45 (15.6%)	
Fall	1/6 (16.7%)	10/45 (22.2%)	
Winter	3/6 (50.0%)	16/45 (35.6%)	
Total Sun Exposure (Score)	3.5 (3.0-5.0)	3.0 (3.0-4.0)	0.553
Female Age, median (IQR), years	32.0 (29.0-36.0)	32.0 (29.0-34.5)	0.563
Female Body mass index, median (IQR), kg/m^2	26.2 (21.9-28.3), n=25	24.0 (22.3-27.3), n=126	0.622
Female Obese (BMI ≥ 30 kg/m^2)	5/25 (20.0%)	17/126 (13.5%)	0.399

	Male Vitamin D < 20 ng/mL (n=26)	Male Vitamin D ≥ 20 ng/mL (n=128)	P value
Female Ethnicity, No. (%)			0.023
Hispanic or Latino	3/26 (11.5%)	6/128 (4.7%)	
Non-Hispanic	20/26 (76.9%)	119/128 (93.0%)	
Unknown	3/26 (11.5%)	3/128 (2.3%)	
Female Race, No. (%)			<0.001
White	14/26 (53.8%)	106/128 (82.8%)	
Black	10/26 (38.5%)	3/128 (2.3%)	
Asian	2/26 (7.7%)	11/128 (8.6%)	
American Indian or Alaska Native	0/26 (0.0%)	0/128 (0.0%)	
Unknown	0/26 (0.0%)	2/128 (1.6%)	
Mixed Race	0/26 (0.0%)	5/128 (3.9%)	

Table 2.

Semen parameters at baseline by male vitamin D status

Parameters	Vitamin D < 20 ng/mL (n=26)	Vitamin D ≥ 20 ng/mL (n=128)	P value
Sperm concentration (million/ml)	19.0 (11.0-52.0)	19.0 (10.2-38.2)	0.504
Sperm concentration ≤ 15 million/ml	11/26 (42.3%)	52/128 (40.6%)	0.874
Normal morphology (%)	6.5 (4.0-10.0), n=22	5.0 (2.0-8.0), n=94	0.133
Normal morphology ≤ 4%	7/22 (31.8%)	41/94 (43.6%)	0.312
Total motility (%)	44.7 ± 20.2	43.8 ± 16.1	0.908
Total motility ≤ 40%	14/26 (53.8%)	58/128 (45.3%)	0.427
DNA fragmentation (SCSA, DNA fragmentation index) (%)	20.4 (13.6-35.1), n=23	19.7 (14.2-27.9), n=112	0.518
DNA fragmentation > 25%	9/23 (39.1%)	35/112 (31.3%)	0.463

Table 3.

Pregnancy outcomes

	Vitamin D < 20 ng/mL	Vitamin D ≥ 20 ng/mL	P value
Entire Cohort			
Achieved pregnancy	8/26 (30.8%)	31/128 (24.2%)	0.484
Live birth	3/26 (11.5%)	27/128 (21.1%)	0.415
Pregnancy loss	5/8 (62.5%)	4/31 (12.9%)	0.009
Placebo Group			
Achieved pregnancy	3/12 (25.0%)	21/68 (30.9%)	1.000
Live birth	1/12 (8.3%)	18/68 (26.5%)	0.276
Pregnancy loss	2/3 (66.7%)	3/21 (14.3%)	0.099
Treatment Group			
Achieved pregnancy	5/14 (35.7%)	10/60 (16.7%)	0.110
Live birth	2/14 (14.3%)	9/60 (15.0%)	1.000
Pregnancy loss	3/5 (60.0%)	1/10 (10.0%)	0.077