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Characterizing the influence of vitamin D levels on IVF outcomes

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BACKGROUND: Vitamin D plays a role in reproductive capacity. Recently, several investigators have demonstrated higher IVF pregnancy rates in vitamin D replete women. The objective of this study was to validate these findings and to further elucidate the role of vitamin D in reproduction among a diverse group of women.

METHODS: This was a retrospective cohort study in an academic tertiary care center of 188 infertile women undergoing IVF. Serum levels of vitamin D (25OH-D) were measured in previously frozen serum samples. The main outcome measure was clinical pregnancy, defined as sonographic presence of a heartbeat following IVF.

RESULTS: The relationship between vitamin D status and pregnancy rates differed by race ($P < 0.01$). Among non-Hispanic whites, pregnancy rates declined with progressively lower levels of vitamin D, while in Asians, the reverse was true. Adjusting for age and number and quality of embryos transferred among non-Hispanic whites, the odds of pregnancy were four times higher in vitamin D replete versus deficient patients. Live birth rates mirrored pregnancy rates. Vitamin D status was not associated with ovarian stimulation parameters or with markers of embryo quality.

CONCLUSIONS: Vitamin D deficiency is associated with lower pregnancy rates in non-Hispanic whites, but not in Asians, possibly due to their lower IVF success rates. Vitamin D deficiency was not correlated with ovarian stimulation parameters or with markers of embryo quality, suggesting its effect may be mediated through the endometrium.

Key words: vitamin D / IVF / endometrium / 25-hydroxyvitamin D / race

Introduction

An epidemic of vitamin D deficiency has been emerging over the last decade among all racial groups in the USA, with the prevalence of vitamin D insufficiency nearly doubling from 1994 to 2004 (Looker *et al.*, 2008). Vitamin D deficiency has been implicated in a host of chronic diseases, including diabetes, obesity, autoimmune disease, cardiovascular disease and cancer. More recently, poor vitamin D status has been implicated as a contributing factor to poor pregnancy outcomes (Bodnar *et al.*, 2007) and infertility (Ozkan *et al.*, 2010).

The importance of vitamin D in reproduction is evident from murine models. Vitamin D receptor knockout mice demonstrate uterine hypoplasia, gonadal insufficiency, reduced aromatase gene expression, impaired folliculogenesis and infertility (Yoshizawa *et al.*, 1997; Kinuta *et al.*, 2000). Rats deficient in vitamin D demonstrate compromised mating behavior, reduced fertility, decreased litter sizes and impaired neonatal growth (Halloran and DeLuca, 1980; Kwiecinski *et al.*, 1989). Importantly, reproductive function can be normalized with vitamin D supplementation, but not with calcium alone, suggesting that vitamin D's role in reproduction lies outside of its classic endocrine role in regulating calcium homeostasis (Halloran and DeLuca, 1980; Kwiecinski *et al.*, 1989; Johnson and DeLuca, 2001).

The presence of the vitamin D receptor (VDR) in many tissues along the female reproductive axis, including the pituitary, ovary, uterus and placenta (Johnson and DeLuca, 2001), suggests that vitamin D is an important regulator of the female reproductive system. The active form of vitamin D (1,25 dihydroxyvitamin D3 or

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calcitriol), when bound to its receptor, acts as a transcription factor to regulate the expression of the CYP19 gene, which encodes aromatase, an essential enzyme in the production of estrogen (Kinuta et al., 2000). Serum calcitriol and estradiol levels track together, both in the normal menstrual cycle (Gray et al., 1982) and in stimulated IVF cycles (Potashnik et al., 1992); however, the main circulating form of vitamin D (25-hydroxyvitamin D or 25(OH)D) does not fluctuate throughout the menstrual cycle (Johnson and DeLuca, 2001).

Calcitriol is produced by the decidua in response to IL-1B secreted by the blastocyst (Vigano et al., 2006). Calcitriol regulates decidual expression of HOXA10, calbindin (Daftary and Taylor, 2006) and osteopontin (Vigano et al., 2006) genes, all integrally involved in embryo implantation. The decidua and placenta continue to secrete large quantities of calcitriol throughout pregnancy, which is important for regulating the immune response at the maternal–fetal interface. The presence of the blastocyst up-regulates the production of the active form of vitamin D in the endometrium (Vigano et al., 2006). In turn, calcitriol may help to support successful implantation by attenuating decidual T-cell function (Evans et al., 2004). Decidual NK cells treated with calcitriol show decreased synthesis of cytokines, CSF2, IL-1 and IL-6 and TNFα (Evans et al., 2006).

An IVF population provides valuable insight into the role of vitamin D since it is possible to examine each aspect of a single conception cycle from follicular development to implantation. A recent study found that women with higher 25(OH)D levels in their serum and follicular fluid were significantly more likely to achieve pregnancy from IVF compared with women with lower levels of vitamin D (Ozkan et al., 2010). The primary objective of our study was to verify this relationship between vitamin D status and IVF outcomes, and additionally to isolate the effect of vitamin D either to ovarian stimulation, embryo quality or endometrium. As a secondary objective, we aimed to evaluate the relationship between vitamin D and IVF outcomes in the context of patient race.

Materials and Methods

This was a retrospective cohort study of 208 infertile women who underwent their first IVF cycle at University of Southern California (USC) Fertility Clinic from January 2006 to August of 2009. The study protocol was approved by the USC Institutional Review Board. Patients were excluded if they had previous IVF cycles at USC Fertility or if they underwent zygote intra-Fallopian tube transfer. Patient characteristics and cycle parameters were identified from patient medical records. Patient race was categorized according to self-identified race/ethnicity on their initial patient questionnaire.

All patients underwent IVF cycles using standardized regimens for pituitary down-regulation and controlled ovarian hyperstimulation. The particular protocol was chosen according to patient diagnosis and age. In general, good prognosis patients underwent a Leuprolide acetate down-regulation protocol (Lupron; TAP Pharmaceuticals, North Chicago, IL, USA; Porter et al., 1984), whereas those patients judged to have a poor prognosis underwent either an antagonist protocol with flexible ganirelix acetate start (Antagon; Organon, Inc., West Orange, NJ, USA) (Oliviennes et al., 1994), or a microdose flare protocol (Scott and Navot, 1994).

Controlled ovarian hyperstimulation was initiated with either recombinant FSH alone or in combination with human menopausal gonadotrophins (Menopur, Ferring, Inc., Suffern, NY, USA). Starting dose was selected on the basis of age, Day 3 FSH levels, and number of antral follicles, with adjustments made according to patient response. Serial monitoring of ovarian response was assessed by transvaginal ultrasound and serum estradiol (E2) assays. When two to three follicles reached or exceeded 17–18 mm, hCG (10 000 IU IM) was administered. Serum samples collected the day after hCG administration were stored at −20°C until assayed.

Transvaginal ultrasound guided oocyte retrieval was performed 34–35 h following hCG injection. Conventional insemination and/or ICSI was performed as indicated. Ultrasound-guided fresh embryo transfer was performed on Days 3 through 5 after egg retrieval. The number of embryos transferred depended upon embryo development and number of embryos available.

Luteal phase supplementation with vaginal micronized progesterone in capsules and oral estrace was started 3 days following egg retrieval. Clinical pregnancy was defined by the sonographic presence of a heartbeat at 7–8 weeks of gestation.

Vitamin D status

Vitamin D status was measured by assessing circulating levels of 25(OH)D in frozen, never previously thawed serum samples using radioimmunoassay (RIA; DiaSorin, Stillwater, MN, USA; Hollis et al., 1993). Intra-and inter-assay coefficients of variation were 10.5 and 8.2%, respectively. Serum 25(OH)D was categorized according to clinically accepted ranges for vitamin D deficiency (<20 ng/ml), insufficiency (20–30 ng/ml) and replete (>30 ng/ml; Holick, 2007).

Statistical analysis

Continuous data were summarized as the mean ± SD, or as median, 25th and 75th quartiles if highly skewed, and categorical data as percentage (%). Univariate analyses were carried out using the Kruskal–Wallis test for continuous outcomes and χ^2 tests for categorical outcomes.

Multivariable logistic regression was used to evaluate predictors of clinical pregnancy and of live birth. Vitamin D status (deficient, sufficient, replete) was included in the model as an ordinal variable. Race/ethnicity was coded using dummy variables, and effect modification was evaluated by including race-by-vitamin D cross-product terms in the model and conducting a likelihood ratio test. Since race was found to be an effect modifier, race-specific results were obtained by evaluating appropriate linear combinations of regression coefficients in the interaction model. Adjusted results are presented as predicted probability of the outcome (clinical pregnancy or live birth), adjusted to mean levels of covariates included in the model.

All models were adjusted for maternal age. Additional covariates were included in the final model if they confounded the relationship between vitamin D and treatment outcome, as evidenced by a change in race-specific regression coefficients of at least 15%. Variables evaluated as potential confounders included age, BMI, obesity, parity, diagnosis, previous treatment failure, stimulation protocol, season of transfer, number of embryos transferred and markers of embryo quality. Mild confounding effects (10–15% change in at least one race-specific regression coefficient) were observed for number of embryos, embryo quality (the average number of blastomeres among transferred embryos) and diagnosis of diminished ovarian reserve. Taken jointly, these three covariates resulted in substantial confounding (>25% change in one or more race-specific regression coefficients) and thus they were retained in the final model. Model fit was evaluated using the Hosmer and Lemeshow test (Hosmer and Lemeshow, 1980).

Power was calculated based on results of the Ozkan study, in which patients with the highest levels of vitamin D had a clinical pregnancy rate of 47% and those with the lowest vitamin D levels had clinical pregnancy rates of 20%. Thus, in order to detect a 27% difference in clinical pregnancy rates, with 80% power and alpha of 0.05, we required at

least 102 patients. All *P* values are two sided and statistical significance was established as $P < 0.05$.

All analyses were conducted using Stata 11.0 (StataCorp, College Station TX).

Results

Of 208 eligible patients, 18 did not have available serum for testing and 2 had missing outcome data, leaving 188 study participants in the final analysis. Of the 188 patients, 21% (39/188) were vitamin D deficient (25(OH)D <20 ng/ml), 37% (70/188) were vitamin D insufficient (20–30 ng/ml) and only 42% (79/188) were vitamin D replete (25(OH)D >30 ng/ml).

Race was categorized as non-Hispanic white (53%), Asian (26%), Hispanic white (14%) or other (7%), according to guidelines outlined in the 2010 USA Census Bureau (http://www.census.gov/geo/www/2010 census) Asian race included those patients whose origins were from Southeast Asia as well as from the Indian subcontinent. Serum 25(OH)D levels varied by race ($P = 0.01$), being highest in non-Hispanic whites ($n = 100$; mean: 30.6 ng/ml), intermediate in Asians ($n = 49$; mean: 27.1 ng/ml) and lowest in Hispanic whites ($n = 26$; mean: 25.6 ng/ml). Baseline patient characteristics including infertility diagnosis, age and BMI were compared among the different racial groups. Hispanic whites were on average younger with a higher BMI compared with other races. No other significant differences were noted.

Table I depicts patient and IVF cycle characteristics by vitamin D status. Vitamin D deficient women were, on average, younger ($P = 0.03$) and heavier ($P = 0.03$), and were less likely to have a diagnosis of diminished ovarian reserve ($P = 0.01$). Although vitamin D deficient women were heavier ($P = 0.03$), all patients had BMI values within the normal range. Vitamin D status was not associated with other infertility diagnoses, with parity, or previous IVF failure. Of all patients, 53% underwent long lupron protocol, 37% underwent microdose flare and 11% underwent an antagonist protocol. Women with vitamin D deficiency were more likely to have been treated with the long lupron protocol ($P = 0.01$). Vitamin D status was not associated with ovarian stimulation parameters (dose of medications required, peak estradiol levels, number of oocytes retrieved and number of mature oocytes) as noted in Table I. Although the number of embryos transferred differed significantly by vitamin D status, there was no trend (more embryos were transferred to women in the intermediate vitamin D group). Vitamin D status was not associated with fertilization rates or markers of embryo quality (the mean number of blastomeres or mean percent fragmentation).

Table II depicts the patient and IVF cycle characteristics by pregnancy outcome. Clinical pregnancy rates were 43% in non-Hispanic whites, 38% in Hispanic whites and 35% in Asians. A similar pattern was seen for live birth rates: 35% in non-Hispanic whites, 27% in Hispanic whites and 26% in Asians. Clinical pregnancy was associated with lower dose of medication needed ($P = 0.05$), higher peak of E2 ($P = 0.03$), higher number of oocytes retrieved ($P = 0.05$) and higher mean number of cells on Day 3 both among the entire cohort of embryos ($P = 0.03$) and among the embryos that were selected for transfer ($P = 0.01$). The number of embryos transferred was greater in the clinical pregnancy group (3.6 versus 3.3, $P = 0.02$). Only dose of medication ($P = 0.02$) and the mean percent fragmentation among transferred embryos ($P = 0.05$) were significantly associated with live birth.

As shown in Table III, race significantly modified the relationship between cycle outcome and vitamin D status ($P < 0.01$, for clinical pregnancy and live birth). Among non-Hispanic whites, clinical pregnancy rates progressively decreased with declining vitamin D status, from 51% in those who were vitamin D replete, to 44% in those who were insufficient, to 19% in those who were vitamin D deficient ($P = 0.04$). However, the opposite trend was seen among Asians, with pregnancy rates increasing with worsening vitamin D status ($P = 0.01$). Results were similar after fitting multivariable logistic models to adjust for maternal age, number of embryos transferred, embryo quality and diagnosis of diminished ovarian reserve (Table III). A similar relationship was observed between vitamin D status and the live birth rate, with worsening vitamin D status, live birth rates decreased among non-Hispanic whites ($P = 0.03$), but increased in Asians ($P = 0.01$).

Discussion

In this ethnically diverse population, we were able to confirm that vitamin D status is related to IVF success in non-Hispanic white patients; pregnancy rates declined with progressively lower levels of vitamin D. The odds of pregnancy were four times higher in vitamin D replete compared with deficient patients, substantiating the findings of Ozkan *et al.* (2010) in a larger study population. However, among Asians the beneficial effect of sufficient levels of vitamin D was not observed and in fact, vitamin D status was inversely related to IVF success. The influence of race on the relationship between vitamin D and clinical pregnancy was statistically significant.

We believe that our diverse study population is representative of a typical IVF population; we observed the expected associations between typical predictors of successful IVF outcomes and pregnancy rates. Our observed prevalence of vitamin D insufficiency (37%) and deficiency (21%) is similar to the prevalence in Ozkan's study (36 and 27% respectively), but is slightly lower than in national studies in women of childbearing age (Holick, 2009). This may be due to IVF population demographics, which tends to be older, mostly Caucasian, higher in socioeconomic status and education level (Chandra, *et al.* 2005; Ginde *et al.*, 2010), and more likely to be taking prenatal vitamin supplementation. These demographics are associated with higher vitamin D status in population-based US surveys among reproductive aged women (Holick, 2009).

It is unclear why we saw an inverse relationship between 25(OH)D levels and clinical pregnancy rates among Asians. It is possible that though statistically significant, this is a chance finding. The number of Asians in the study was relatively small ($n = 49$). We could not identify any clinically important racial differences in patient characteristics or cycle parameters (including embryo quality) contributing to their lower pregnancy rates. It is possible that the influence of vitamin D status on pregnancy outcomes was overshadowed by other factors that contribute to the lower pregnancy rate observed among Asian patients (35% in Asians, 43% in non-Asians). Previous studies have demonstrated significantly lower live birth rates after IVF in Asian ethnicities compared with Caucasians (Hammoud *et al.*, 2009), despite a younger age among Asian subjects and similar embryo quality (Hammoud *et al.*, 2009; Butts and Seifer, 2010). Others have found a higher prevalence of diminished ovarian function in Asian-Chinese versus Caucasian oocyte donors, suggesting that oocyte factors may

Table I Patient and IVF cycle characteristics by vitamin D status.

Characteristic	Vitamin D >30 ng/ml, $n = 79$ (42%)	Vitamin D 20–30 ng/ml, $n = 70$ (37%)	Vitamin D <20 ng/ml, $n = 39$ (21%)	*P*-value[a]
Race/ethnicity				
Non-hispanic White	45 (57%)	39 (56%)	16 (41%)	0.38
Hispanic White	9 (11%)	7 (10%)	10 (26%)	
Asian	20 (25%)	18 (26%)	11 (28%)	
Other	5 (6%)	6 (9%)	2 (5%)	
Age (years)	36.5 ± 4.1	36.7 ± 3.7	34.7 ± 4.1	0.03
BMI (kg/m^2)	22.7 ± 3.9	23.4 ± 4.3	24.8 ± 4.5	0.03
Obese (BMI ≥30)	3 (4%)	7 (10%)	8 (21%)	0.02
Nulliparous	69 (87%)	60 (86%)	30 (77%)	0.32
Diagnosis				
DOR/age[b]	38 (48%)	35 (50%)	9 (23%)	0.01
Tubal	11 (14%)	11 (16%)	7 (18%)	0.85
Endometriosis	11 (14%)	6 (9%)	8 (21%)	0.21
Uterine	5 (6%)	5 (7%)	5 (13%)	0.45
Anovulatory/endocrine	13 (16%)	9 (13%)	10 (26%)	0.23
Male factor	36 (46%)	25 (36%)	14 (36%)	0.40
Technological[c]	5 (6%)	10 (14%)	1 (3%)	0.10
Unexplained	14 (18%)	4 (6%)	4 (10%)	0.07
Previous failed IVF	23 (29%)	15 (21%)	6 (15%)	0.22
Stimulation protocol				
Microdose flare	36 (45%)	24 (34%)	8 (20%)	0.04
Antagonist	10 (13%)	8 (11%)	3 (8%)	
Long lupron	33 (42%)	38 (54%)	28 (72%)	
Peak estradiol (pg/ml)	3020 ± 1922	3168 ± 1646	3186 ± 1679	0.56
Oocytes retrieved (*n*)[d]	11 (6.19)	14 (9.20)	17 (11.22)	0.10
Fertilization rate (%)	68 ± 25	71 ± 23	74 ± 17	0.79
Mean cells Day 3 (*n*)	6.1 ± 1.6	6.2 ± 1.1	6.4 ± 1.2	0.78
Mean fragmentation (%)	15.5 ± 13.8	16.9 ± 10.6	16.2 ± 11.8	0.44
Embryos transferred (*n*)	3.2 ± 1.2	3.8 ± 1.3	3.1 ± 1.0	<0.01
Mean cells Day 3 (*n*)	6.8 ± 1.5	7.1 ± 1.9	7.1 ± 1.1	0.73
Mean fragmentation (%)	12.2 ± 9.9	12.0 ± 6.8	11.7 ± 9.0	0.79

[a]Continuous data are presented as the mean ± standard deviation with *P* values obtained from Kruskal–Wallis test unless otherwise indicates. Categorical data are presented as *n* (%) with *P* values obtained from χ^2 tests (the Pearson or Fisher exact, as appropriate).
[b]Decreased ovarian reserve or advanced reproductive age.
[c]IVF chosen for the purposes of sex selection or PGD.
[d]Median (25th, 75th percentiles).

be involved in the lower IVF success rates in Asian populations (Gleicher *et al.*, 2007; Shaine *et al.*, 2009). Ideally, vitamin D levels in the recipients of donor IVF cycles could be studied to further eliminate confounding due to oocyte quality.

Finally, numerous studies have shown racial differences in the metabolism of vitamin D. In particular, southern Asians have been reported to have increased activity of the enzyme responsible for deactivating both 25(OH)D and calcitriol (Awumey *et al.*, 1998). There are also ethnic differences in VDR gene polymorphisms that may confound or modify the relationship between vitamin D levels and reproductive outcomes (Ingles *et al.*, 1997; Ingles, 2007). Our finding of significant heterogeneity by Asian ancestry suggests that the relationship between vitamin D and reproduction should be considered within the context of ethnicity.

Although vitamin D status was not associated with ovarian stimulation parameters or with markers of embryo quality, vitamin D levels were correlated with pregnancy outcomes in our non-Hispanic white population. It is possible that vitamin D may have an impact on embryo quality not captured using the current methods available to assess embryo viability. However, more plausible, is that vitamin D may exert an effect on IVF cycle outcomes via the endometrium. The VDR is expressed in the endometrium and plays a vital role in activating the innate immune response (Evans *et al.*, 2004; Vigano *et al.*, 2006). Additionally, vitamin D may play an important autocrine role through its regulation of the

Table II Patient and IVF cycle characteristics by pregnancy outcome.

Characteristic	Pregnant, *n* = 77 (39%)	Not pregnant, *n* = 111 (59%)	*P*-value	Live birth, *n* = 59 (31%)	No live birth, *n* = 129 (69%)	*P*-value
Age (years)	36.2 ± 3.64	36.2 ± 4.30	0.74	35.9 ± 3.66	36.3 ± 4.19	0.50
BMI (kg/m^2)	23.2 ± 4.35	23.5 ± 4.22	0.49	23.3 ± 4.57	23.4 ± 4.14	0.88
Obese (BMI ≥30)	9 (11%)	11 (9%)	0.65	7 (11%)	13 (9%)	0.68
Nulliparous	69 (83%)	102 (84%)	0.93	56 (88%)	115 (82%)	0.29
Diagnosis						
DOR/age[a]	31 (37%)	59 (48%)	0.12	22 (34%)	68 (48%)	0.06
Tubal	12 (14%)	19 (16%)	0.83	11 (17%)	20 (14%)	0.58
Endometriosis	10 (12%)	17 (14%)	0.70	8 (13%)	19 (13%)	0.85
Uterine	5 (6%)	11 (9%)	0.43	4 (6%)	12 (9%)	0.58
Anovulatory/endocrine	14 (17%)	21 (17%)	0.95	11 (17%)	24 (17%)	0.98
Male factor	35 (42%)	48 (39%)	0.69	27 (24%)	56 (40%)	0.74
Technological[b]	9 (11%)	9 (7%)	0.39	5 (8%)	12 (9%)	0.74
Unexplained	12 (14%)	13 (11%)	0.41	11 (17%)	14 (10%)	0.14
Previous failed IVF	21 (25%)	25 (20%)	0.42	16 (25%)	30 (21%)	0.55
Race/ethnicity[a]						
Non-Hispanic White	43 (56%)	57 (51%)	0.59	37 (58%)	74 (52%)	0.87
Hispanic White	10 (13%)	16 (14%)		9 (14%)	19 (13%)	
Asian	17 (22%)	32 (29%)		14 (22%)	38 (27%)	
Other	7 (9%)	6 (5%)		4 (6%)	10 (7%)	
Stimulation protocol						
Microdose flair	27 (35%)	41 (37%)	0.18	21 (36%)	47 (36%)	0.17
Antagonist	5 (7%)	16 (14%)		3 (5%)	18 (14%)	
Long lupron	45 (58%)	54 (49%)		35 (59%)	64 (50%)	
Peak estradiol (pg/mL)	3414 ± 1782	2897 ± 1730	0.03	3331 ± 1834	3008 ± 1730	0.27
Oocytes retrieved (*n*)	16.9 ± 9.5	14.4 ± 9.0	0.05	16.8 ± 9.9	14.8 ± 9.0	0.18
Fertilization rate (%)	74.1 ± 16.8	68.0 ± 25.6	0.21	74.2 ± 16.1	68.6 ± 24.9	0.37
Mean cells Day 3 (*n*)	6.5 ± 1.1	6.0 ± 1.5	0.03	6.5 ± 1.2	6.1 ± 1.4	0.08
Mean fragmentation (%)	15.3 ± 9.3	16.8 ± 14.0	0.96	15.6 ± 9.9	16.5 ± 13.3	0.93
Embryos transferred (*n*)	3.6 ± 1.2	3.3 ± 1.3	0.02	3.5 ± 1.2	3.4 ± 1.3	0.24
Mean cells Day 3 (*n*)	7.4 ± 1.5	6.7 ± 1.5	0.01	7.3 ± 1.7	6.9 ± 1.5	0.07
Mean fragmentation (%)	10.7 ± 6.6	13.0 ± 9.7	0.11	10.2 ± 6.4	12.9 ± 9.4	0.05

Continuous data are presented as the mean ± standard deviation with *P* values obtained from Kruskal–Wallis test unless otherwise indicates. Categorical data are presented as *n* (%) with *P* values obtained from χ^2 tests (the Pearson or Fisher exact, as appropriate).
[a]Decreased ovarian reserve or advanced reproductive age.
[b]IVF chosen for the purposes of sex selection or PGD.

transcription of genes such as HOXA10, critical for embryo implantation and placentation (Evans *et al.*, 2006).

Recent studies demonstrate that vitamin D is important throughout gestation as well, not just at the time of implantation. There are varying levels of vitamin D metabolites and HOXA10 expression throughout pregnancy in the endometrium, decidua and placenta (Evans *et al.*, 2006). In cultured syncytiotrophoblasts, calcitriol regulates hCG expression and secretion, and it stimulates estradiol and progesterone secretion from trophoblasts in a dose-dependent manner (Barrera *et al.*, 2007; Barrera et *al.*, 2008). Clinical studies demonstrate an association between lower vitamin D levels and increased risk of preeclampsia and gestational diabetes. Thus, vitamin D may have an important role in maintaining a healthy pregnancy (Bodnar *et al.*, 2007).

These data add clinical support to the growing body of evidence that vitamin D may play an important role in IVF success as well, possibly via localized effects in the endometrium. Research is needed to further elucidate the mechanism by which vitamin D acts, the ethnic heterogeneity in vitamin D metabolism and its subsequent effects on IVF success. The prevalence of vitamin D deficiency and insufficiency is alarmingly high in infertile patients. A role for vitamin D supplementation may exist as a means of improving one's natural fertility both among the fertile and the infertile. Regardless of potential fertility benefits, patients can be counseled regarding appropriate vitamin D supplementation for overall health benefits, pregnancy health and chronic disease risk reduction.

Table III Rates of clinical pregnancy, live birth and implantation by vitamin D status: unadjusted and multivariable adjusted[a].

Outcomes	Vitamin D replete (>30 ng/ml), *n* = 79	Vitamin D insufficient (20–30 ng/ml), *n* = 70	Vitamin D deficient (<20 ng/ml), *n* = 39	*P*-value
Clinical pregnancy rate				
All subjects	34 (43%)	29 (41%)	14 (36%)	0.48
Non-Hispanic Whites	23 (51%)	17 (44%)	3 (19%)	0.04
Hispanic Whites	5 (56%)	3 (43%)	2 (20%)	0.12
Asians	3 (15%)	7 (39%)	7 (64%)	0.01
Adjusted clinical pregnancy rate[a]				
Non-Hispanic Whites	55%	36%	21%	0.01
Hispanic Whites	68%	38%	15%	0.03
Asians	14%	34%	64%	0.01
Live birth rate				
All subjects	26 (33%)	22 (31%)	11 (28%)	0.62
Non-Hispanic Whites	20 (44%)	13 (33%)	2 (13%)	0.03
Hispanic Whites	3 (33%)	2 (29%)	2 (20%)	0.51
Asians	2 (10%)	5 (28%)	6 (55%)	0.01
Adjusted live birth rate[a]				
Non-Hispanic Whites	47%	27%	14%	0.01
Hispanic Whites	42%	26%	14%	0.19
Asians	9%	25%	53%	0.02
Implantation rate				
All subjects	19 ± 26%	18 ± 27%	16 ± 27%	0.38
Non-Hispanic Whites	24 ± 28%	18 ± 24%	7 ± 16%	0.02
Hispanic Whites	26 ± 29%	24 ± 37%	13 ± 32%	0.17
Asians	7 ± 17%	18 ± 31%	30 ± 32%	0.01
Adjusted implantation rate[a]				
Non-Hispanic Whites	25%	15%	9%	0.01
Hispanic Whites	31%	16%	9%	0.07
Asians	7%	14%	28%	0.01

Pregnancy and birth rates expressed as *n* (%); implantations rates expressed as the mean ± SD; adjusted rates expressed as %.
[a]Adjusted for maternal age, number of embryos transferred, embryo quality (average number blastomeres among transferred embryos) and diagnosis of diminished ovarian reserve.

Authors' roles

All authors were involved in the final design of the study. K.B. was the principal investigator of the study. All authors applied for IRB for approval. B.J.R., K.B., K.C., R.J.P. and S.A.I. coordinated the study and with F.S., analyzed the data. F.S. also provided technical support in running the blood samples. S.A.I. provided primary statistical support. K.B., SA.I. and B.J.R. were involved in the many first drafts of this paper. All authors contributed to the final draft of this paper. SA.I and K.B. contributed equally to this paper.

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Conflict of interest

None declared.

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