Replete vitamin D stores predict reproductive success following in vitro fertilization

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Objective: To determine whether 25OH-D levels in the follicular fluid (FF) of infertile women undergoing IVF demonstrate a relationship with IVF cycle parameters and outcome, hypothesizing that levels of 25OH-D in body fluids are reflective of vitamin repletion status.
Design: Prospective cohort study.
Setting: Academic tertiary care center.
Patient(s): Eighty-four infertile women undergoing IVF.
Intervention(s): Follicular fluid from follicles $\geq$14mm; serum (n = 10) and FF levels of 25OH-D.
Main Outcome Measure(s): Clinical pregnancy (CP), defined as evidence of intrauterine gestation sac on ultrasound, following IVF; IVF cycle parameters.
Result(s): Serum and FF levels of 25OH-D were highly correlated (r = 0.94). In a predominantly Caucasian population (66%), significantly lower FF 25OH-D levels were noted in Black versus non-Black patients. Significant inverse correlations were seen between FF 25OH-D levels and body mass index (r = –0.25). Significantly higher CP and implantation rates were observed across tertiles of FF25OH-D; patients achieving CP following IVF (n = 26) exhibited significantly higher FF levels of 25OH-D. Multivariable logistic regression analysis confirmed FF 25OH-D levels as an independent predictor to success of an IVF cycle; adjusting for age, body mass index, ethnicity, and number of embryos transferred, each ng/mL increase in FF 25OH-D increased the likelihood for achieving CP by 6%.
Conclusion(s): Our findings that women with higher vitamin D level in their serum and FF are significantly more likely to achieve CP following IVF–embryo transfer are novel. A potential for benefit of vitamin D supplementation on treatment success in infertile patients undergoing IVF is suggested and merits further investigation. (Fertil Steril® 2010;94:1314–9. ©2010 by American Society for Reproductive Medicine.)

Key Words: Vitamin D, 25OH-D, infertility, in vitro fertilization, clinical pregnancy, follicular fluid

Vitamin D, a steroid hormone, is well known to be involved in calcium-phosphate homeostasis and bone metabolism (1–3). Emerging data identify critical roles for vitamin D in a variety of other biological processes including regulation of cellular growth and differentiation and metabolic modulations specifically involving insulin action (4). Indeed, beneficial roles for vitamin D in a spectrum of pathologic processes, including autoimmunity, insulin resistance, cardiovascular disease, and malignancies, are emerging concomitantly with the appreciation of a global pandemic of vitamin D insufficiency (4–5). Biological actions of vitamin D are mediated through vitamin D receptor (VDR) (3, 6), which is a member of the steroid/thyroid nuclear hormone receptor superfamily and has been demonstrated in calcium-regulating tissues, intestines, the skeleton, parathyroid glands, and reproductive tissues including ovary, uterus, placenta, testis, and the hypophysis (7–10).

Among the many physiologic processes influenced by vitamin D, critical roles in reproductive physiology are suggested (1, 8–15). Experiments investigating the significance of vitamin D for fertility and reproductive capacity, while demonstrating that the vitamin may not be critical for successful female reproduction, demonstrate compromised mating behavior, reduced fertility rates, decreased litter sizes, and impaired neonatal growth in vitamin D–deficient female rats (1). Similar evidence of reproductive compromise in male rats deficient in vitamin D is identified (11). Data regarding the effects of vitamin D on reproductive physiology in nonpregnant subjects are limited to a few experimental investigations in animal models; specific human data in this context are sparse. Although able to reproduce, vitamin D–deficient rats demonstrate diminished mating success and fertility capacity (1); reduced litter sizes and impaired neonatal growth are also described as is an overall reduction

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FIGURE 1

Follicular fluid 25OH-D reliably reflects serum levels. r = 0.94; * $P < 0.001$.

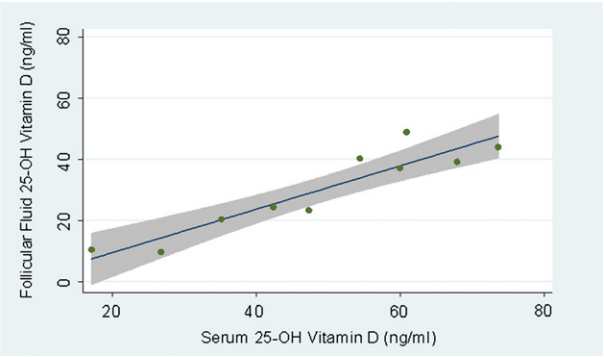

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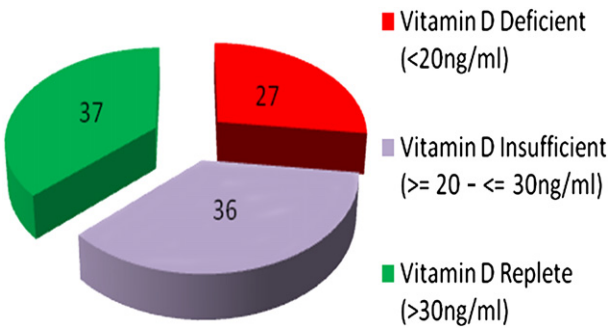
FIGURE 2

Population prevalence (%) of vitamin D status.



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in fertility by 75%, primarily attributed to decreased mating rates and increased pregnancy complications (1). Expression of VDR in reproductive tissues and a hamster ovarian cell line further demonstrated a potential role of vitamin D in female reproduction (6). Vitamin D has been shown to promote Ca transport in the placenta, stimulate lactogen expression, promote decidualization of the endometrium, and regulate *HOXA10* expression (a target gene related to the implantation process) contributing to data related with vitamin D reproduction (4, 16–18). Additional experimental studies with VDR-null mutant mouse models demonstrated gonadal insufficiency, reduced aromatase gene expression, low aromatase activity, hypergonadotropic hypogonadism (7), and features of estrogen deficiency, such as bone malformations, uterine hypoplasia, impaired folliculogenesis, and infertility (3). Vitamin D has thus been identified as a critical ingredient to reproductive success, at least in the murine model (12).

Whereas alterations in calcium-phosphate metabolism are suggested to partially explain the reproductive sequelae of vitamin D deficiency, adverse implications for ovarian steroidogenesis and uterine receptivity are also described (3, 6, 11, 14–15). Some investigators have attributed the reduced fertility rates seen in vitamin D–deficient animals as a direct effect of vitamin D rather than the hypocalcemia associated with vitamin D deficiency. However, others suggest that in male rats, fertility is most critically affected by calcium levels, independent of vitamin D. Indeed, calcium has been shown to affect sperm maturation, capacitation, and acrosome reaction (15), and in vitamin D–deficient rats, normalization of reproductive capacity has been reported by feeding a diet high in calcium and phosphate alone (14). However, others have demonstrated that consumption of a vitamin D–deficient diet before and during pregnancy in rats adversely affects fecundity rates independent of female age and body mass index (BMI).

The literature is supportive of roles for vitamin D in placental steroidogenesis, calcium transport through the placenta, expression of placental lactogen, and decidualization of the endometrium. In addition, vitamin D has been identified to regulate key target genes related with implantation and in establishment of the fetoplacental unit (9, 16–19). Decreased aromatase activity and reduced aromatase gene expression in the ovary, testes and epididymis of animals deficient in vitamin D are described (7). The available data thus identify vitamin D as a key player in processes involved in reproductive success and thereby suggest pathophysiological mechanisms for reproductive compromise in the setting of vitamin D deficiency.

Realizing the pandemic of vitamin D insufficiency, in the setting of an increasing appreciation of the myriad roles of vitamin D in health in general, and in reproductive physiology in particular, we herein hypothesized that abundant vitamin D stores will translate to improved reproductive success following in vitro fertilization (IVF). Given the proximity of the developing oocytes to the follicular fluid (FF), we further hypothesized that higher FF 25OH-D levels will be associated with improved ovarian response to controlled ovarian hyperstimulation (COH).

MATERIALS AND METHODS

A prospective cohort study was undertaken at the Montefiore Institute for Reproductive Medicine and Health. Eighty-four infertile women undergoing IVF were enrolled between March 2005 and December 2007. The study protocol was in accordance with the guidelines of Declaration of Helsinki, was approved by institutional review board at the Montefiore Medical Center, and included written consent from the participants.

All patients enrolled in the study underwent IVF cycles per standard clinical care. Standardized regimens for COH and pituitary down regulation were employed. Leuprolide acetate (Lupron; TAP Pharmaceuticals, North Chicago, IL), starting in the midluteal phase at a dose of 0.5 mg/day SC or ganirelix acetate (Antagon; Organon, Inc., West Orange, NJ) started following initiation of COH with E_2 levels reaching $\geq$400

TABLE 1

Participant and IVF cycle characteristics by outcome of IVF cycle.

	Clinical pregnancy (n = 26; 30.95%)	Not pregnant (n = 58; 69.04%)	*P* value
FF 25OH-D			
ng/mL	34.42 ± 15.58[a]	25.62 ± 10.53	0.013[b]
mmol/L	86.05 ± 38.96	64.04 ± 26.32	
Age (y)	33.88 ± 4.57	34.86 ± 5.08	0.565
BMI (Kg/m^2)	23.77 ± 4.00	26.37 ± 6.60	0.164
Race[c]			0.801
Black (%)	4/23 (17)	8/53 (15)	
White (%)	17/23 (74)	33/53 (62)	
Other (%)	2/23 (9)	12/53 (23)	
Baseline FSH (mIU/ml)	7.76 ± 3.08	8.16 ± 2.09	0.360
Gonadotropin dose (Amps[d])	28.10 ± 14.27	44.96 ± 27.19	0.001[b]
Days of COH	10.73 ± 1.43	11.93 ± 1.70	0.002[b]
E_2 day of hCG (pg/ml)	2297 ± 1171.86	2266.31 ± 1101.07	0.961
Oocytes retrieved (n)	12.88 ± 6.33	12.02 ± 6.72	0.525
Overall fertilization rate (%)	53.00 ± 21.00	45.00 ± 24.00	0.154
Embryos transferred (n)	2.56 ± 0.66	1.98 ± 1.16	0.011[b]

Note: Continuous data are presented as mean ± standard deviation.

[a] Values are expressed as mean ± SD.

[b] Statistically significant, $P < 0.05$.

[c] Information on race was not available for the entire cohort.

[d] Gonadotrophin dose per ampoule = 75IU.

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pg/mL or dominant follicle size (≥14mm), were instituted. Controlled ovarian hyperstimulation was initiated with recombinant follicle stimulating hormone (FSH), and the starting dose was selected on the basis of age, early follicular phase levels of FSH, and the number of antral follicles. Individualized step-down or step-up protocols were instituted, and serial monitoring of ovarian response was assessed by transvaginal ultrasound and serum E_2 assays. Nuclear maturation was triggered with hCG (10,000 IU IM) when three or more follicles >17 mm were achieved. Serum samples were collected on the day of hCG administration (approximately 34 hours before egg retrieval) and stored at –20°C until assayed.

Transvaginal ultrasound guided oocyte retrieval was performed 34 hours following the hCG injection. Follicular fluid was collected from follicles ≥14mm; following oocyte isolation, FF for each patient was pooled, centrifuged at 3000 × *g* for 15 minutes, and the supernatant was stored at –80°C until assayed. Fertilization was assessed 17–18 hours after insemination. Ultrasound-guided fresh embryo transfer (ET) was performed on day 3 after insemination. The luteal phase was supported by intramuscular P in oil (50 mg/day); positive serum hCG tested 12 days after embryo transfer was considered as evidence of implantation, and P supplementation was continued until documentation of fetal cardiac activity. Clinical pregnancy (CP) was defined as intrauterine gestational sac visible on transvaginal ultrasound.

Patient and cycle parameters were identified from clinical records including age, self-reported ethnicity, infertility etiology, infertility duration, body mass index (BMI), early follicular phase hormonal assessment of ovarian reserve (FSH and E_2), IVF cycle stimulation protocol (i.e., duration of COH in days, total FSH ampoules used for COH), number of ovarian follicles >14 mm on day of hCG, serum E_2 and P levels on day of hCG administration, total oocytes retrieved and number of mature oocytes, fertilization rate, number, and cleavage status of ET. Outcomes of interest were implantation rate (number of gestational sacs identified on ultrasound ÷ number of ET × 100) and CP following fresh ET.

Based on previously defined serum criteria (5), FF 25OH-D level >30ng/mL was defined to reflect "replete" vitamin D status; level between 20–30 ng/mL was taken to reflect vitamin D insufficiency, whereas 25OH-D level <20ng/mL defined evidence of vitamin D deficiency.

Statistical Analysis

Continuous data are reported as mean ± SD and categorical as percentage (%). Univariate analyses determined associations between FF 25OH-D levels with patient and cycle parameters (Student's *t* test, Mann-Whitney U test as appropriate). Tertiles of FF 25OH-D were computed (25OH-D levels from lowest to highest tertiles were 16.74 ± 3.38, 25.58 ± 3.17, and 43.01 ± 10.65 ng/dL, respectively);

FIGURE 3

Significantly higher follicular fluid 25OH-D levels are noted in IVF cycles achieving clinical pregnancy. * $P = 0.013$

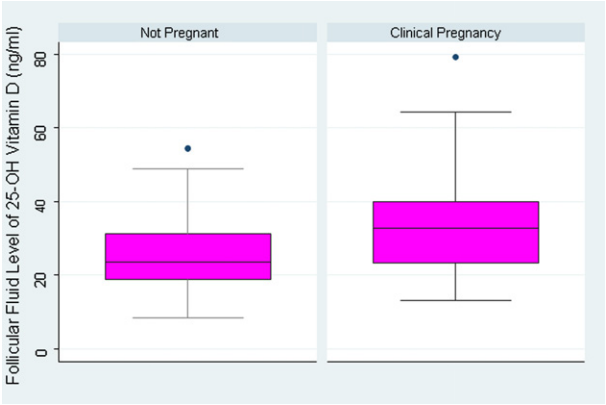

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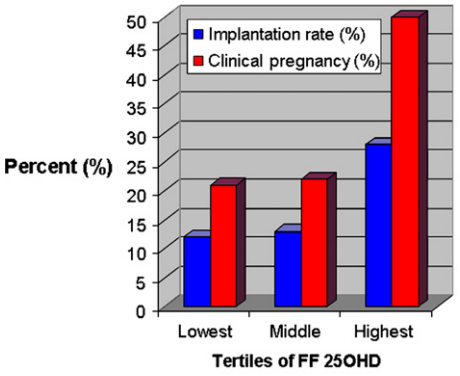
FIGURE 4

Increasing implantation and clinical pregnancy rates are observed across FF 25OH-D tertiles. $P = 0.041$.



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proportion of patients achieving CP and implantation rate across the tertiles of 25OHD distribution were assessed by Kruskall–Wallis rank test. Multivariable logistic regression analysis evaluated the relationship between FF 25OH-D and CP after adjusting for parameters known to influence success of an IVF cycle (age, BMI, ethnicity, and number of ET). Likelihood of CP is presented as odds ratio (OR) ± 95% confidence interval (95% CI). Because of the relatively small dataset and the few outcomes (i.e., CP), a propensity score analysis was used (20). The propensity score, derived from a separate multivariate logistic model incorporating the adjustment covariates, was used as a single adjustment variable (summarizing the covariates) within the logistic regression models determining an association between CP and FF 25OH-D levels. STATA IC 10 (StataCorp, College Station, TX) was used for the statistical analysis. Statistical significance was established as $P < 0.05$.

RESULTS

Serum and FF levels of 25OH-D were highly correlated (r = 0.94; $P < 0.001$; Fig. 1) demonstrating that FF levels of 25OH-D indeed were reliable reflectors of body stores of the vitamin. Per serum level criteria (5), only 37% of participants demonstrated replete 25OH-D stores (>30 ng/mL); 36% met criteria for insufficiency (20–30 ng/mL), whereas 27% were vitamin D deficient (<20 ng/mL; Fig. 2).

In a predominantly Caucasian population (66%), significantly lower FF 25OH-D levels were noted in Black (n = 12) compared with patients of non-Black ethnicity (n = 64; 18.88 ± 8.5 ng/mL vs. 30.51 ± 12.95 ng/mL; P=0.001). Significant inverse correlations were noted between FF 25OH-D levels and BMI (r = –0.25; P=0.035). Although lower FF 25OH-D levels were noted in eight patients with polycystic ovarian syndrome (PCOS) and in 20 women with diminished ovarian reserve (DOR) compared to those with other infertility etiologies (26.19 ± 11.22 in patients with PCOS vs. 28.57 ± 13.09 ng/mL in those without PCOS, and 24.16 ± 9.57 in DOR vs. 29.65 ± 13.5 ng/mL in patients with normal ovarian reserve), these differences were not of statistical significance ($P > 0.05$).

Table 1 describes the participant and IVF cycle characteristics by outcome of IVF cycle (i.e., CP vs. not pregnant). Those achieving CP (n = 26; 30.95%) demonstrated significantly higher FF 25OH-D levels compared to those with unsuccessful cycles (n = 58; 69.04%; P=0.013; Fig. 3), used significantly lower doses of gonadotropin ampoules for shorter durations (in days), and received a significantly higher number of transferred embryos compared with those whose IVF cycles were unsuccessful; the remaining IVF cycle characteristics did not differ significantly (Table 1), and the proportions of cycles using a GnRH agonist vs. antagonist were similarly comparable across the two groups (in those achieving CP, 55% of cycles used GnRH agonist, compared with 53% in those with failed outcome; P=0.857).

Although significant differences were observed in cycle parameters in patients achieving CP and those with failed outcomes as specified in Table 1, no direct relationship was observed between patient and cycle parameters and 25OH-D levels—that is, there was no correlation observed between FF 25OH-D levels and ovarian response parameters (i.e., duration of COH, number of follicles, number of eggs retrieved, maximal E2 levels attained during COH) nor with ovarian reserve parameters (age or FSH; data not shown). Although a trend toward increasing ovarian response (shorter duration of COH, increasing E2 levels) was observed across the tertiles for vitamin D, these differences were not statistically significant ($P > 0.05$; data not shown).

TABLE 2

Predictors of successful clinical pregnancy following IVF (associations presented as odds ratio ± 95% confidence intervals).

Clinical pregnancy	Unadjusted OR (95% CI)	P value	Adjusted OR[a] (95% CI)	P value
Age (y)	0.96 (0.87–1.06)	0.404	1.01 (0.84–1.20)	0.940
Race[b]	1.71 (0.58–5.57)	0.328	1.47 (0.30–7.30)	0.635
BMI (kg/m^2)	0.91 (0.81–1.02)	0.101	0.91 (0.78–1.06)	0.208
Embryos transferred (n)	1.75 (1.04–2.95)	0.034[c]	2.13 (1.12–4.05)	0.021[c]
FF 25OH-D (ng/mL)	1.06 (1.01–1.10)	0.007[c]	1.07 (1.01– 1.13)	0.013[c]

[a] Analyses adjusted for age, body mass index, race, number of embryos transferred and FF 25OH vitamin D level.
[b] White vs. other races.
[c] Statistically significant, $P < 0.05$.

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Significant increase in implantation and CP rates was observed across the tertiles of 25OH-D distribution (P=0.041 and 0.029, respectively; Fig. 4); all other patient and cycle parameters were comparable across tertiles of 25OH-D including patient age, BMI, FSH levels, dose of gonadotropins, duration of COH, maximal E_2, number of eggs retrieved, and number of ET (data not shown). Those in the highest tertile for 25OH-D were almost fourfold more likely to achieve CP compared with patients in the lowest tertile (OR for CP 3.83; 95% CI, 1.20–12.28; P=0.024).

Multivariable logistic regression incorporating propensity score analyses confirmed FF 25OH-D levels as an independent predictor of success of an IVF cycle; adjusting for age, BMI, ethnicity, and number of ET, each ng/mL increase in FF 25OH-D level enhanced the likelihood for achieving CP by 7% (P=0.013; Table 2). Alternatively, those with FF 25OH-D levels in the lowest to middle tertiles were 75% less likely to achieve CP compared to women with FF vitamin D levels in the highest tertile (OR for CP, 0.25; 95% CI, 0.07–0.84; P=0.026).

Of the 84 IVF cycles, 81 were first ART cycles whereas 3 were repeat cycles. Sensitivity analyses were conducted excluding the 3 repeat ART cycles and confirmed essentially unchanged magnitudes of association (ORs, CIs, and P values) in the previously observed relationships between 25OH-D and IVF cycle outcome (data not shown).

DISCUSSION

We herein demonstrate that FF levels of 25OH-D are reflective of body stores of vitamin D. While our study is limited by the relatively small sample size, our findings of significantly higher FF levels of vitamin D in those achieving clinical pregnancy following IVF are indeed novel, not previously described and may hold potential therapeutic implications. Improved COH parameters, in the context of higher vitamin D levels, are suggestive of facilitatory implications of FF 25OH-D on ovarian steroidogenesis, albeit insignificantly so; these observations are hence in keeping with published literature (3, 7, 8). However, in the absence of any significant relationship with ovarian response, our observations may identify endometrial receptivity as the potential target for beneficial influences of higher circulating vitamin D levels. Vitamin D has been previously identified as a regulator of endometrial expression of *HOXA10*, a target gene critical to implantation process (18), and our observations could thus be explained by this proposed relationship.

The magnitude of prevalent vitamin D insufficiency and the ethnic disparity in status of vitamin D repletion as seen in our data are consistent with prior reports. The prevalence of vitamin D insufficiency or deficiency in various communities is reportedly staggering (6), and our findings corroborate this impression. Socioeconomic disparity is a recognized contributor to nutritional deficiencies across populations and is also well identified as a hurdle for access to infertility care. Our findings thus suggest nuances other than socioeconomic inequality, such as lifestyle, that may be contributory to insufficient vitamin D stores in a population of otherwise healthy infertile women undergoing IVF (and thus deemed to be economically sound).

The observed prevalence of vitamin D insufficiency in an otherwise healthy population is concerning, especially in the context of accruing data on beneficial influences of replete vitamin D stores on multiple physiological processes and the emerging roles of vitamin D insufficiency in a spectrum of diseases. Given our findings, assessment of vitamin D status might be considered as a part of routine infertility workup since appropriate supplementation of those deemed depleted of vitamin D might translate to improved fertility outcome and improved overall health. These latter conjectures merit further assessment by appropriately designed longitudinal studies.

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