Vitamin D Status Relates to Reproductive Outcome in Women with Polycystic Ovary Syndrome: *Secondary Analysis of a Multicenter Randomized Controlled Trial*

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Context: Experimental evidence supports a relevance of vitamin D (VitD) for reproduction; data in humans are however sparse and inconsistent.

Objective: To assess relationship of VitD status with ovulation induction (OI) outcomes in women with polycystic ovary syndrome (PCOS).

Design: Retrospective cohort

Setting: Secondary analysis of randomized controlled trial (RCT) data.

Participants: Pregnancy in PCOS-I (PPCOS I) RCT (n=540); participants met the NIH diagnostic criteria for PCOS.

Interventions: Serum 25OHD (ng/ml; for conversion to SI units [nmol/L], multiply by 2.5) levels were measured in stored sera.

Main outcome measures: Primary (Live birth- LB); secondary (ovulation-OV and pregnancy loss-PL) following ovulation induction (OI)

Results: Likelihood for LB was reduced by 44% for women if 25OHD level was <30 ng/ml (<75 nmol/L, OR 0.58 [0.35–0.92]). Progressive improvement in the odds for LB was noted at thresholds of ≥38ng/ml, (≥95 nmol/L, OR 1.42 [1.08- 1.8]), ≥40ng/ml (≥100 nmol/L, OR1.51 [1.05–2.17] and ≥45ng/ml (>112.5 nmol/L, OR 4.46 [1.27–15.72]). On adjusted analyses, VitD status was an independent predictor of LB and OV following OI.

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Abbreviations:

Conclusions: In women with PCOS, serum 25OHD was an independent predictor of measures of reproductive success following OI. Our data identify reproductive thresholds for serum 25OHD that are higher than recommended for the non-pregnant population.

Nearly 10% of reproductive age women (6.1 million) in the U.S. have difficulty achieving pregnancy with ovulatory dysfunction being a major cause of female infertility (1–2). Characterized by clinical and biochemical hyperandrogenism, menstrual irregularities and ovulatory dysfunction, polycystic ovary syndrome (PCOS) is the most common cause of ovulatory infertility (3).

While vitamin D (VitD) has long been recognized for its importance in skeletal biology (4), an appreciation of its relevance for reproductive physiology is also evident, particularly in animal models (5–6). Evidence supporting a critical role for VitD signaling in human reproduction however is sparse, almost entirely observational, and data are inconsistent (7–15). A growing body of literature suggests mechanistic implications of VitD deficiency for insulin resistance, inflammation, dyslipidemia and obesity, ie, clinical and metabolic phenomena commonly encountered in PCOS (16–17), implying pathophysiological relevance of VitD insufficiency for PCOS. To that end, multiple observational as well as small sample trials, have explored the role of VitD in PCOS (18–22).

Given that ovulation induction (OI) is a first line approach for management of PCOS related ovulatory infertility (23) and in light of the above summarized data relating VitD to both PCOS and fertility, we hypothesized that in women with PCOS, VitD insufficiency (serum 25OHD $<$ 30 ng/ml), is associated with lower rates of live birth following OI.

Materials and Methods

Serum 25OHD levels were assessed in stored samples from participants in the PPCOS I (Pregnancy in Polycystic Ovary Syndrome) randomized controlled trial (RCT) (24). Briefly, 626 reproductive age women aged 18–39 years, with elevated testosterone levels and oligomenorrhea (thus meeting the 1990 NIH criteria for PCOS diagnosis (3)) and seeking pregnancy, with at least one patent fallopian tube(s), normal uterine cavity, and a partner with sperm concentration of at least 20 million/mL in at least one ejaculate, were randomized to one of three different treatment arms: 1) clomiphene citrate (CC) 50 mg every day for 5 days; 2) metformin XR (M) 1000 mg twice/d or 3) a combination of CC and M. Women continued on study medications for 30 weeks or 6 treatment cycles; treatment related live birth was the primary outcome of interest. Ovulation (a secondary outcome) was determined by weekly or every other week assessment of serum progesterone (P) levels and was confirmed by a serum P concentration above 5 ng/mL (24). This trial demonstrated superiority of CC (either alone or in combination with M) over M alone as an OI agent with live-birth rates of 22.5% in the CC group (47 of 209) 26.8% in CC+M group (56 of 209) and only 7.2% (15 of 208) in the M alone group ($P < .001$ for M vs. both CC and CC+M) (24).

Stored sera (either unthawed or previously thawed no more than 3 times) maintained at the Reproductive Medicine Network (RMN) bio repository at –80°C, from a time point before initiation of trial drugs, were utilized for assessment of 25OHD levels for the present study; 25OHD analyte stability over protracted period of storage at temperatures of –20°C or less, and following repeat thaw-freeze cycles is well established (25–26). Approvals were obtained from the RMN Repository Committee and the Human Investigation Committee at Yale University for utilization of deidentified samples as well as clinical and biochemical data collected as part of PPCOS I RCT, that included: 1) Outcomes: Live birth (LB, on intent to treat analysis), OV (ovulatory response achieved at least once over six cycles or up to 30 weeks) and attainment of pregnancy (positive pregnancy test); 2) Participant characteristics: age, parity, BMI, ovulatory infertility as the only evident contributor to infertility (Yes/No); hirsutism (Yes/No) was identified based on the modified Ferriman Gallwey (FG) pictorial assessment tool, a visual scoring method to quantify presence and severity of hair growth in nine androgen sensitive hair growth areas; FG score $\geq$ 8 was taken as evidence of hirsutism (27), 3) Two time variables (cycles to OV and days in study), and 4) Baseline hormonal and metabolic parameters: total testosterone (TT, ng/dL), sex hormone binding globulin (SHBG, mg/dL), fasting glucose (mg/dL), fasting insulin (mIU/L), HOMA-IR, serum creatinine (meq/L), and hepatic transaminases (AST and ALT, mg/dL). The following variables were created for analyses: Pregnancy loss (PL-difference between positive pregnancy test and LB), glucose:insulin ratio (GIR) and free androgen index (FAI, total testosterone in nmol/L/SHBG in nmol/L x 100).

Assays for 25OHD were performed in duplicate at the Endocrinology and Metabolism laboratory at Yale University using competitive equilibrium RIA (Diasorin RIA, sensitivity 5ng/ml (multiplication of value in ng/ml by 2.5 allows conversion to SI units, expressed in nmol/L), intra-assay CV 11% and interassay CV 17%, Diasorin Stillwater, MN) and mean value was used for analyses. As per The Endocrine Society of North America guidelines (28), 25OHD $\geq$ 30 ng/ml ($\geq$75 nmol/L) was defined as VitD sufficiency; levels between 20–29.9 ng/ml (50–74.9 nmol/L) were considered inadequate, $<$20 ng/ml ($<$50 nmol/L) defined VitD deficiency and $<$ 10 ng/ml ($<$25 nmol/L) characterized severe VitD deficiency.

Attainment of ovulation (OV, Yes/No), LB (Yes/No, primary outcome) and PL (Yes/No) were evaluated as outcomes of interest. Relationships between 25OHD (continuous) with dichotomous outcomes (OV, LB and PL) were graphically assessed using Lowess curves (Supplemental Figures 1 and 2) for visual identification of inflection points (if any) at which association between 25OHD levels with one or more of the specified outcomes became exaggerated; VitD level was then dichotomized at these visually discerned threshold values for individual outcomes (20 ng/ml [50 nmol/L] for OV, 45 ng/ml [112.5 nmol/L) for LB and 39 ng/ml [97.5 nmol/L] for PL).

Correlation between serum 25OHD levels with baseline endocrine (fasting insulin, TT, SHBG, FAI) and metabolic (fasting glucose, GIR, serum creatinine, hepatic transaminases and HOMA-IR) parameters were additionally undertaken using Pearson or Spearman correlation analyses as appropriate (based on data distribution). Univariate analyses examined baseline characteristics; parametric tests (student's T test and ANOVA) compared normally distributed continuous data across two or more groups, respectively; nonparametric (Mann Whitney-U and Kruskal Wallis Rank Test) tests compared continuous data of skewed distribution across two or more groups respectively, and proportions were compared by χ^2 test. Multivariable logistic regression analyses assessed relationship between serum 25OHD (as continuous and as categorized variable) with the specified outcomes. Key covariates included in model building were age, BMI, smoking status, race and hirsutism; additionally variables demonstrating $P \leq .20$ for relationship with outcome of interest on univariate analysis were incorporated in model building. Stepwise backward elimination of nonsignificant variables was then undertaken, and the final model represented one demonstrating the best "fit" for each outcome. Interaction between OI treatment and VitD status, and between BMI and VitD for specified outcomes were examined; interaction terms were created and included in respective logistic regression models for each specified outcome. Goodness of model fit was assessed (29). Sensitivity, specificity, positive and negative predictive values (PPV and NPV) for specified 25OHD thresholds were also calculated (30).

Continuous data are presented as mean (± standard deviation [SD]) or median (interquartile range [IQR]) and categorical data as percentage (%). Magnitude of associations are presented as odds ratio (OR) and 95% confidence interval (CI). P value $<$ 0.05 was deemed statistically significant; p values are reported up to 3 decimal points, except as otherwise specified. STATA (College St. TX) version 12 was used for statistical analyses.

Sensitivity analyses

Given the lipophilic propensity of VitD metabolites, we hypothesized that an individual's body mass needs to be considered when interpreting VitD status based on circulating 25OHD level. A new variable "BMI adjusted D (BMI^{a}D)" was created (ratio of serum 25OHD to BMI). Correlation analyses assessed directionality and magnitude of relationship of BMI^{a}D with baseline endocrine and metabolic parameters. Multivariable logistic analyses assessed relationship of BMI^{a}D with specified outcomes after adjusting for previously specified covariates.

Power analysis

The overall LB rate for the PPCOS I cohort was almost 19% (118/626). Based on an assumption that participants in the lowest quartile of serum 25OHD would have half the LB rate as those in the highest quartile, we hypothesized a LB rate of 13% for subjects in the lowest and 26% for those in the highest 25OHD quartile. These assumptions provided $>$ 80% power to demonstrate such a difference, if it indeed existed, in a two-sided test with an alpha of 0.05.

Results

Stored sera collected prior to initiation of study drugs were available for 540 of the 626 PPCOS I participants (86%). Population characteristics are presented in Table 1. VitD status was comparable for subjects in the three study treatment arms (serum 25OHD levels were 22.85 ± 10.12 ng/ml [57.13 ± 25.3 nmol/L], 24.11 ± 9.8 ng/ml [60.27 ± 24.5 nmol/L] and 23.71 ± 9.76 ng/ml [59.28 ± 24.4 nmol/L] for CC, M and CC+M groups respectively, P = .473).

Racial differences in VitD status were apparent; 25OHD levels were the highest in White (26.0 ± 9.2ng/ml [64.82 ± 23 nmol/L]), and the lowest in Black women (16.3 ± 10.2ng/ml [40.83 ± 25.4 nmol/L]) with intermediate levels for women of other races ($P < .01$ for racial differences in serum 25OHD). Relationship between race and VitD was independent of BMI. Adjusting for BMI, Black women were 14 times (OR 14.5, 95% CI 7.2, 29.5) more likely to be severely deficient, and 63% (OR 0.37, 95% CI 0.19, 0.70) less likely to have normal (≥30ng/ml or ≥ 75 nmol/L) vitamin D levels.

Association of 25OHD with ovulation (OV)

Evidence of OV was observed in 74% of the cohort over 6 month trial duration (402/540). The probability of achieving OV varied directly with VitD status (68%, 77%, 78% in those with VitD deficiency, insufficiency and normal status, P = .050). VitD deficient women were significantly less likely to achieve OV compared to those with 25OHD levels ≥ 20ng/ml (P = .006, Table 2). Advancing age, higher GIR (ie, better insulin sensitivity), higher SHBG, and use of CC (either alone or with M) were associated with higher chances of OV; conversely, higher BMI, ovulatory dysfunction as the sole attributed cause for infertility, higher FAI and a longer time to OV were associated with reduced likelihood of OV (Table 2). Unlike FAI, hirsutism was unrelated to the chance of achieving OV (P = .467). There was no evidence of interaction between VitD status and OI treatment categories, nor between VitD and BMI for OV outcome.

On adjusted analyses, VitD deficiency, higher BMI, FAI, and length of time in study were identified as negative predictors of OV response; advancing age and use of CC (either alone or with M) were predictive of a higher likelihood for OV (Table 2). Final statistical model demonstrated an 89% sensitivity for the specified outcome.

Figure 1 demonstrates attainment of OV and time to OV during the course of PPCOS I trial by VitD status.

Association of 25OHD with Live Birth (LB)

Overall LB rate was almost 19% (112/540). Serum 25OHD was significantly higher in women achieving LB (25.34 ± 10.39) compared to those failing to attain LB (23.16 ± 9.71), P = .046. Each 1 ng/ml (2.5 nmol/L) increase in 25OHD increased the likelihood of LB by 2%

Table 1. Population characteristics for PPCOS I subjects on whom stored sera were available (n = 540)

Variable		
Age (years)		28.07 (3.98)
BMI (kg/m^2)		35.29 (8.66)
≥35		270 (50%)
30–34.99		118 (22%)
<30		152 (28%)
Parity	0	362 (67%)
	1	178 (37%)
Race n (%)		
White		372 (69)
Black		88 (16)
Asian		16 (2.97)
American Indian		61 (11.34)
Native Hawaiian		1 (0.19)
Ethnicity n (%)		
Hispanic		148 (27.41)
Non-Hispanic		392 (72.59)
Hirsutism[a] n (%)		
Yes		436 (81)
No		104 (19)
Ovulatory dysfunction [b] n (%)		
Yes		415 (77)
No		125 (23)
Smoking history [c] n (%)		
Yes		205 (38)
No		335 (62)
Baseline total T (ng/dl)		61.72 (27.74)
Baseline SHBG[a] (nmol/liter)		29.53 (18.24)
Baseline FAI[d]		9.51 (6.45)
Baseline HOMA[e]		3.52 (1.90–6.15)
Baseline GIR[f]		5.38 (3.24–8.48)
Baseline creatinine		0.76 (0.13)
Serum 25OHD (ng/ml)[g]		23.56 (9.88)
Categories of vitamin D n (%)		
<10 ng/ml [g]		42 (8)
10–19.99 ng/ml [g]		143 (27)
20–29.99 ng/ml[g]		207 (38)
≥30 ng/ml [g]		148 (27)
Serum 25OHD >45 ng/ml[g]		
Yes		10 (2)
No		530 (98)
Study drug allocation		
Clomid alone n (%)		175 (32)
Clomid + metformin n (%)		183 (34)
Metformin alone n(%)		182 (34)
Live birth n(%)		102 (19)
Ovulation[h] n(%)		402 (74)
Pregnancy loss[i] n(%)		42 (29)
Days in study		180.70 (66.21)
Cycles to Ovulation n(%)		2.45 (1.87)
1		235 (43)
2		142 (26)
3		39 (7)
4		37 (7)
5		13 (2)
6		46 (8)
7[j]		26 (5)
8[j]		2 (0.4)
>2 cycles to ovulation n (%)		
Yes		163 (30)
No		377 (70)

[a] Ferriman Gallwey score ≥8;[b] Ovulatory dysfunction as attributable cause for infertility; [c] Sex hormone binding globulin; [d] Free androgen index: total testosterone (nmol/liter) /SHBG (nmol/liter) × 10; [e] Homeostasis Model Assessment-IR: Glucose (mg/dl) x Insulin (microIU/ml/405; [f] Baseline Glucose:Insulin; [g] multiply by 2.5 for conversion to SI units (nmol/liter); [h]Patient ovulated during the six treatment cycle; [i] Pregnancy loss; [j] Maximum of 6 treatment cycles over a 30 week period

(OR 1.02, 95% CI 1.00, 1.04, P = .046). A dose response directionality was suggested when examining relationship between LB and VitD status as defined by specified thresholds of 25OHD; compared to a LB rate of 26% in those sufficient in VitD (38/148), the likelihood of LB declined progressively in the settings of VitD insufficiency (33/207, OR 0.74, 95% CI 0.57, 0.96), deficiency (25/143, OR 0.61, 95% CI 0.35, 1.08) and severe deficiency (6/42, OR 0.48, 95% CI 0.19, 1.23). The proportion of women with VitD inadequacy (<30 ng/ml or < 75 nmol/L) was significantly higher among those failing to achieve compared to those attaining LB (75% vs 63%, P = .013).

Supplemental Figure 1 offers visual representation of relationship between LB and serum 25OHD and demonstrates an inflection point at and above 38 ng/ml (95 nmol/L), beyond which the association between serum 25OHD with LB is progressively magnified. The likelihood for LB was increased fourfold (OR 4.5, 95% CI 1.27, 15.72, P = .02), for women with 25OHD levels greater than 45 ng/ml (>112.5 nmol/L, Table 3). Conversely, the likelihood for achieving LB was reduced by 44% for women with 25OHD level < 30 ng/ml (<75 nmol/L, OR 0.58, 95% CI 0.35, 0.92). The magnitude of association between 25OHD level and LB was exaggerated when analyses were restricted to participants assigned to CC treatment (alone or in combination with M) wherein each ng/ml increase in 25OHD increased the likelihood of LB by 3% (OR 1.03, 95% CI 1.01, 1.06). Progressive improvement in the odds for LB was noted at 25OHD thresholds of ≥ 38 ng/ml or > 95 nmol/L (n = 27, OR 1.42, 95% CI 1.08, 1.8, P = .013), and ≥ 40 ng/ml or ≥ 100 nmol/L (n = 20, OR 1.51, 95% CI 1.05, 2.17, P = .027)

On univariate analyses, advancing age, higher BMI, Black race, hirsutism, attainment of ovulatory cycles after more than two attempts at OI, and higher baseline serum creatinine levels were associated with reduced odds of LB (Table 3). Higher SHBG levels were associated with significantly higher likelihood for achieving LB (OR 1.02, 95% CI 1.01, 1.03). Although smoking history reduced the odds of LB by 22%, this association was not of statistical significance. There was no evidence of interaction between VitD status and OI treatment categories, nor between VitD and BMI for LB.

On adjusted analyses, 25OHD > 45 ng/ml (>112.5 nmol/L), and use of CC (either alone or in combination with M) were associated with increased likelihood of LB (Table 3); conversely, Black race, increasing BMI, pres-

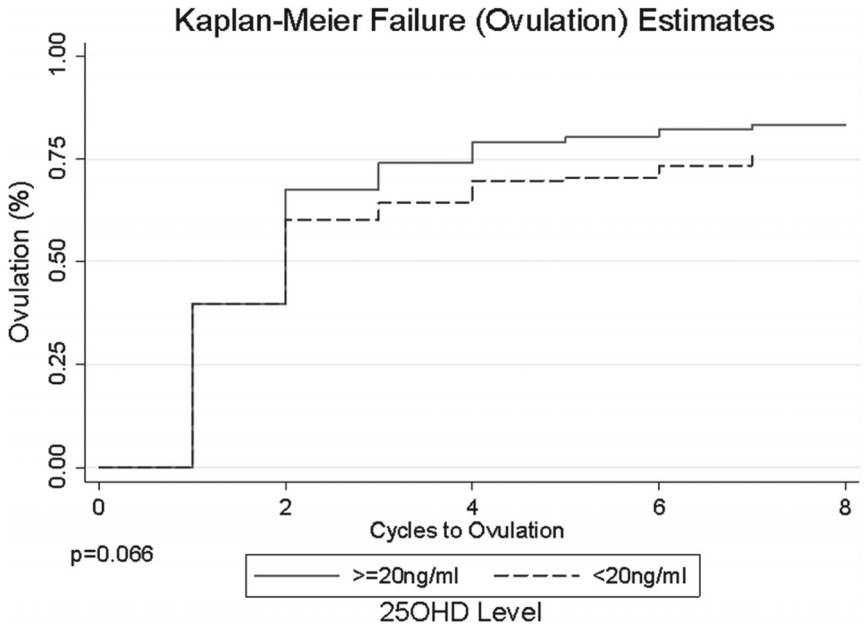

Figure 1. Kaplan Meier curve for ovulation in the PPCOS I cohort by vitamin D status (OV in women with 25OHD < 20ng/ml vs in women with higher levels). *For conversion to SI units (nmol/L), multiply value in ng/ml by 2.5.*

ence of hirsutism, and attainment of ovulation following more than 2 attempts at OI were predictive of reduced likelihood of LB (Table 3). Statistical significance of the associations between advancing age, serum creatinine and smoking history with LB disappeared on adjusted analyses; these variables however were retained in the final model due to biological plausibility (age and smoking history) and borderline statistical significance (serum creatinine). Association between SHBG and LB was no longer present on multivariable adjustment ($P > .50$). The final statistical model presented in Table 3 demonstrated 78% sensitivity for LB.

Figure 2 demonstrates attainment of LB achieved during the course of PPCOS I trial in participants across categories of VitD status (normal, insufficient, deficient, and severely deficient).

Association of 25OHD with pregnancy loss

Positive pregnancy test was followed by PL in 29% (42/144). Serum 25OHD level ≥ 38 ng/ml (≥95 nmol/L) was associated with an 82% reduced likelihood of PL compared to lesser levels (OR 0.18, 95% CI 0.02, 0.90, $P = .020$). There was no evidence of interaction between VitD status and OI treatment categories, nor between VitD and BMI for PL.

Association of serum 25OHD with hormonal and metabolic features of PCOS

Serum 25OHD levels demonstrated inverse associations with BMI (r −0.21, $P < .01$, Supplemental Figure 3), fasting insulin (r=-0.15, $P < .01$), and HOMA (r=-0.11, $P < .01$), and positive associations with SHBG (r = 0.15, $P < .01$) and fasting GIR (r = 0.18, $P < .01$). VitD status did not exhibit any relationship with TT ($P = .93$), FAI (r=-0.05, $P = .27$), fasting glucose ($P = .59$), serum creatinine or hepatic transaminases (data not shown).

Predictive value of vitamin D status for outcome of OI

Sensitivity, specificity, PPV and NPV of serum 25OHD < 20ng/ml (<50 nmol/L) for *failed ovulation* for the entire cohort were 48%, 65%, 32% and 78% respectively (Supplemental Table 1). When analyses were restricted to CC treated population (CC alone or CC +M), respective values were marginally improved: sensitivity 53%, specificity 62%, PPV 23% and NPV 86%.

Serum 25OHD level ≤ 45ng/ml (≤112.5 nmol/L) demonstrated 99% sensitivity and 82% PPV for *no LB*; respective specificity and NPV were 5% and 50% respectively (Supplemental Table 1).

Serum 25OHD level of < 39 ng/ml (<97.5 nmol/L) demonstrated 100% sensitivity and 100% NPV for PL; values for specificity and PPV were 10% and 31% respectively (Supplemental Table 1). Not a single case of PL was observed in women with serum 25OHD level ≥ 39 ng/ml (≥97.5 nmol/L).

Sensitivity analyses substituting BMI adjusted 25OHD as independent variable of interest

Compared to 25OHD, BMI^{a}D demonstrated more robust associations with fasting hormonal and metabolic variables including insulin (r=-0.26, $P < .01$), glucose (r=-0.11, $P = .01$), GIR (r = 0.39, $P < .01$), HOMA (r=-0.21, $P < .01$) and SHBG (r = 0.41, $P < .01$). Unlike 25OHD, BMI^{a}D demonstrated an inverse correlation with hepatic ALT (r=-0.18, $P < .001$).

Supplemental Figure 2 presents visual representation of the relationship between LB with BMI^{a}D. Association between BMI^{a}D with LB is a more uniform slope than seen for 25OHD (Supplemental Figure 1) and this relationship was more robust when analyses were restricted to CC users (CC alone plus CC+M) wherein each unit increase in BMI^{a}D was associated with a threefold increase in the likelihood of LB (OR 3.15, 95% CI 1.80–5.54).

Association of VitD with specified outcomes was reassessed by substituting BMI^{a}D for 25OHD in the previ-

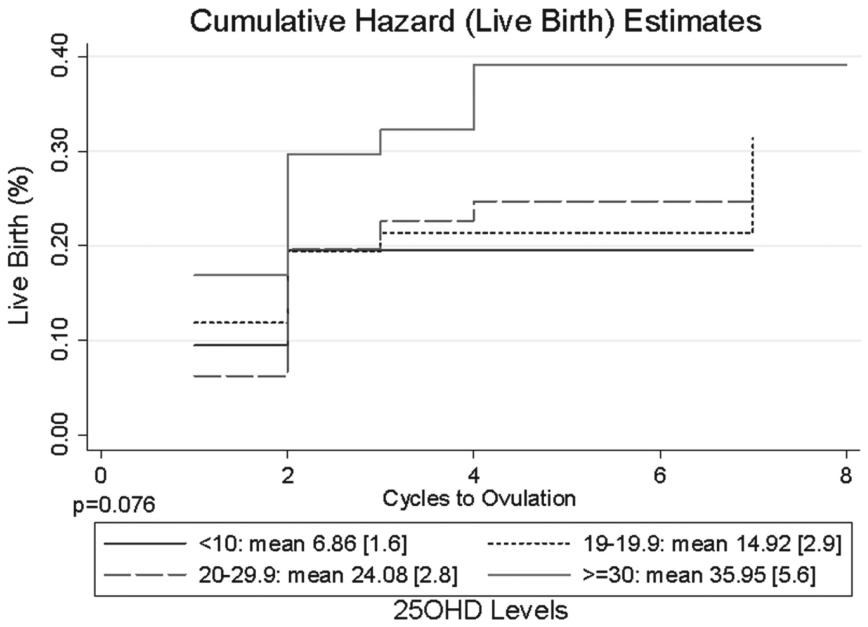

Figure 2. Kaplan Meier curves for live birth in the PPCOS I cohort by vitamin D status based on specified serum levels of 25OHD (ng/ml). *For conversion to SI units (nmol/L), multiply values in ng/ml by 2.5.*

ously discussed multivariable logistic regression analyses (Supplemental Tables 2 and 3). Of the 5 of 540 participants with BMI[a]D of > 2, ie, their serum 25OHD value was ≥twice that of their BMI, (mean serum 25OHD 47 ± 4.45 and BMI 22.3 ± 2.39) all achieved OV (100%), 4/5 (80%) achieved pregnancy with all pregnancies (100%) proceeding to LB.

Adjusted models exhibited sensitivities of 78% and 89% for outcomes of OV and LB respectively (essentially identical to seen when 25OHD values were substituted for BMI[a]D, and BMI included as an additional covariate (Supplemental Tables 2 and 3 respectively).

Discussion

In a cohort of infertile women undergoing in vitro fertilization (IVF), we had previously identified facilitatory implications of *replete* VitD stores on IVF success (7). Our current study reaffirms a relevance of adequate 25OHD for procreative success in women with PCOS undergoing OI. Beyond reaffirming a consistency in directionality of the previously observed associations, we have additionally noted that this association becomes apparent at serum 25OHD levels that are well beyond the threshold of 30ng/ml (75 nmol/L) that is currently deemed as a target "normal" level. Highest likelihood for LB was evident in women with serum 25OHD level > 45 ng/ml (>112.5 nmol/L); in contrast, 25OHD levels < 20 ng/ml (<50 nmol/L), were predictive of a dampened OV response to OI strategies. These observations allow us to propose that circulating 25OHD level of 45 ng/ml (112.5 nmol/L) or higher be considered as "optimal" for women attempting to conceive (Supplemental Figure 1).

Based on observations in this work, we propose concepts of distinct "reproductive thresholds" of VitD below which OV to OI is blunted (**Lower Reproductive Threshold-*LRT*** <20 ng/ml [<50 nmol/L]) and beyond which (**Upper Reproductive Threshold –*URT*** >45 ng/ml [>112.5 nmol/L]) likelihood of LB may be optimized and propensity for PL is reduced. While the study design does not allow us to comment on pathophysiological mechanisms, our data suggest that PL risk is mitigated at 25OHD levels of ≥ 39ng/ml (≥97.5 nmol/L). These latter observations are in line with recent suggestions that VitD deficiency, by alterations in the status of cellular and autoimmunity, may be contributory to PL (31). Notably, the observed *URT* is higher than 25OHD levels of 20 ng/ml (50 nmol/L) and 30 ng/ml (75 nmol/L) that are identified by IOM (32) and The Endocrine Society (28) respectively, to reflect normal VitD status. Recapitulation of our earlier findings of higher VitD levels associating with a higher likelihood of fertility treatment success in the large sample size of the PPCOS I cohort, and consistency with similar observations reported by others (8–12) reinforces a relevance of an individual woman's VitD status for fertility.

Racial disparities in fertility treatment success rates are well described with lower pregnancy rates observed in infertile women of color following infertility treatments (33). The observed spectrum of VitD status across spectrum of races represented in PPCOS I is in line with prior observations, and VitD deficiency has been previously suggested as a mechanism for lower treatment related fertility rates in the Blacks (34). The PPCOS I cohort was predominantly comprised of Caucasians; however, the racial and ethnic composition of enrollees still allowed exploration of relationship between specified outcomes with VitD status and race. Our analyses reaffirm that OI related LB rates are lower in Black compared to Caucasian women with PCOS and that this relationship is independent of VitD status. Our study design however does not allow postulation on mechanisms that could explain the observed racial differential in OI related live births.

Inverse correlations between serum 25OHD levels with

Table 2. Predictors of ovulation on multivariable logistic regression analysis of PPCOS I RCT data. Magnitude of associations is presented as odds ratio (95% CI)

Variables	UOR[a] (95% CI)	P value	AOR[b] (95% CI)	P value
Age (years)	1.07 (1.02–1.123)	0.006	1.12 (1.04–1.20)	0.002
25OHD <20 ng/ml[c]	0.58 (0.39–0.86)	0.006	0.43 (0.25–0.76)	0.003
BMI (kg/m^2)	0.94 (0.91–0.96)	<0.001	0.95 (0.92–0.98)	0.003
Ovulatory dysfunction[d]	0.48 (0.28–0.81)	0.006	0.46 (0.23–0.91)	0.025
Baseline FAI	0.999 (0.998–1.00)	0.060	1.002 (1.0001–1.003)	0.031
Cycles to ovulation (n)	0.44 (0.38–0.50)	<0.001	0.45 (0.38–0.52)	<0.001
Clomid[e]	1.36 (0.89–2.08)	0.157	2.68 (1.41–5.09)	0.003
Clomid + Metformin[e]	3.07 (1.89–4.98)	<0.001	3.69 (1.86–7.30)	<0.001

Model sensitivity: 89% with all specified covariates included in the model

[a] *Unadjusted Odds Ratio (95% confidence interval)*

[b] *Adjusted Odds Ratio (95% confidence interval)*

[c] *Multiply value by 2.5 for conversion to SI units (nmol/liter)*

[d] *vs. other infertility diagnoses*

[e] *vs. metformin*

Table 3. Predictors of live birth on multivariable logistic regression analysis of PPCOS I RCT data. Magnitude of associations is presented as odds ratio (95% CI)

Variables	UOR[a] (95% CI)	P value	AOR[b] (95% CI)	P value
Age (Years)	0.95 (0.90–1.01)	0.081	0.94 (0.89–1.00)	0.076
25OHD >45 ng/ml[c]	4.46 (1.27–15.72)	0.020	5.35 (1.02–28.15)	0.048
BMI (kg/m^2)	0.95 (0.92–0.97)	<0.001	0.97 (0.94–0.99	0.044
Black race[d]	0.43 (0.21–0.89)	0.023	0.44 (0.20–0.96)	0.039
Hirsutism (Yes/No)	0.95 (0.93–0.98)	0.002	0.45 (0.26–0.78)	0.005
Clomid[e]	1.44 (0.93–2.26)	0.104	3.99 (1.92–8.30)	<0.001
Clomid + Metformin[e]	2.23 (1.44–3.46	<0.001	5.79 (2.82–11.88)	<0.001
>2cycles to ovulation	0.15 (0.07–0.33)	<0.001	0.20 (0.10–0.43)	<0.001
Baseline creatinine	0.24 (0.04–1.27)	0.092	0.23 (0.03–158)	0.135
History of Smoking	0.78 (0.49–1.23)	0.285	0.85 (0.51–1.40)	0.521

Model sensitivity 78% with all specified covariates included in the model.

[a] *Unadjusted Odds Ratio (95% confidence interval)*

[b] *Adjusted Odds Ratio (95% confidence interval)*

[c] *Multiply value by 2.5 for conversion to SI unit (nmol/liter)*

[d] *vs. other races*

[e] *vs. metformin alone*

BMI and with fasting insulin are recognized (5, 17) and are affirmed in our analyses. Associations between serum 25OHD with hyperandrogenemia and hyperandrogenism are described and reductions in TT achieved with VitD supplementation, albeit inconsistently (19–20, 35–36). We did not observe any association between serum 25OHD with either TT, FAI, or with hirsutism.

VitD signaling holds the potential of negating a spectrum of pathophysiological corollaries to obesity (4, 37), and hence our rationale to adjust VitD status by BMI. We observed an increase in the magnitude of observed associations between VitD status with LB and OV (Supplemental Tables 2 and 3). Given a recognized inverse relationship between BMI and serum 25OHD, and the higher inflammatory and metabolic burden of obesity, conceptually, one can make a case that burden of obesity be considered when defining norms for VitD status. Indeed, compared to 25OHD, BMI[a]D demonstrated more robust associations with hormonal and metabolic variables. Given the improved magnitude of association of BMI[a]D status for the various outcomes discussed, we propose that future efforts aimed at assessing a relationship between VitD status and disease states incorporate such a paradigm to better understand relevance of VitD in health and disease.

A retrospective approach, lacking information on a number of variables that may modulate efficiency of VitD signaling (such as VitD binding protein (VDBP) levels that would allow a more precise calculation of bioavailable 25OHD, (38) population's VitD receptor genotype status

and VDBP gene polymorphisms (39–40), missing information on seasonality, and on intake of VitD supplements are obvious limitations of our work. Given small numbers of participants with 25OHD levels above the specified thresholds for defined outcomes, ie, only 39/540 (7%) had level > 38 ng/ml (>95 nmol/L) associated with PL, and only 10 (2%) had level > 45 ng/ml (associated with LB), the possibility of alpha errors being reflected in the observed associations is plausible. However, the directionality of and consistency in the observed associations that held on analyses that utilized BMI[a]D instead of 25OHD wherein 118 subjects demonstrated a value greater than 1 (ie, serum 25OHD level was greater than the value of individual's BMI) is reassuring (Supplemental Figure 2). Robustness of PPCOS I, a randomized double blind controlled trial, and assessment of VitD status in a large sample of women meeting NIH criteria for PCOS are all strengths of this work.

In a large sample representative of the PCOS population, we have systematically assessed and demonstrated relevance of VitD for OV response and LB with commonly utilized OI strategies. Our interpretation of VitD status based on BMI consideration is in line with the concept of "individualized medicine".

In summary, our data suggest that for infertile women with PCOS, VitD status, as reflected by serum levels of 25OHD is relevant for procreative success. We hypothesize that decline in circulating 25OHD below the *LRT* may be contributory to ovulatory dysfunction whereas at levels at and above an *URT,* achieved through supplementation, may result in improved endometrial receptivity, as has been previously suggested (41–42), thus yielding improved treatment LB rates and reduce risk of PL in women with PCOS, a population that is already an enhanced risk for pregnancy wastage (43). These functional observations provide support for the biological plausibility of our conclusions. The discrepancy between what may be an appropriate reproductive threshold and the currently defined norms for VitD status in adults merits further consideration. Future studies are needed to systematically assess these notions, because the clinical, public health, and financial implications of such a simple, safe and inexpensive strategy can be substantial.

Acknowledgments

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