HHS Public Access

Author manuscript

Minerva Endocrinol. Author manuscript; available in PMC 2020 June 01.

Published in final edited form as:

Minerva Endocrinol. 2019 June ; 44(2): 176–184. doi:10.23736/S0391-1977.18.02824-9.

Associations between vitamin D levels and polycystic ovary syndrome (PCOS) phenotypes

Erin.M. Davis, Ph.D[1,*], **Jennifer D. Peck, Ph.D**[2], **Karl R. Hansen, M.D., Ph.D**[3], **Barbara.R. Neas, Ph.D**[2], and **LaTasha B. Craig, M.D.**[3]

[1]University of Louisville, Department of Anatomical Sciences and Neurobiology, Louisville, KY, USA

[2]University of Oklahoma Health Sciences Center, Department of Biostatistics and Epidemiology, Oklahoma City, OK, USA

[3]University of Oklahoma Health Sciences Center, Department of Obstetrics and Gynecology, Section of Reproductive Endocrinology and Infertility, Oklahoma City, OK, USA

Abstract

Background: Studies comparing serum 25-hydroxyvitamin D concentrations in women with and without PCOS have produced inconsistent results. Additionally, no previous studies have evaluated associations between vitamin D and specific PCOS phenotypes.

Methods: This case-control study was conducted among women undergoing intrauterine insemination. Cases (n=137) were diagnosed with PCOS and then further classified into 3 diagnostic phenotypes based on combinations of the Rotterdam criteria [ovulatory dysfunction +polycystic ovaries (n=55); ovulatory dysfunction +androgen excess (n=15); and ovulatory dysfunction, +polycystic ovaries, +androgen excess (n=67)]. Controls (n=103) were ovulatory women without PCOS who were undergoing IUI. Serum total 25-hydroxyvitamin D concentrations were categorized as deficient (≤20 ng/ml), insufficient (21–29 ng/ml), and sufficient (≥30 ng/ml). Prevalence odds ratios (PORs) were calculated using logistic regression.

Results: A higher proportion (59.9%) of PCOS cases lacked sufficient vitamin D levels compared to controls (47.6%; p-value=0.06). The odds of vitamin D deficiency in all PCOS cases were twice that of controls (POR=2.03, 95% CI 0.97–4.26); however, the association was attenuated after adjusting for body mass index (BMI) and race/ethnicity ($_{adj}$POR=1.43, 95% CI 0.62, 3.26). When examining PCOS phenotypes exhibiting androgen excess, crude associations were observed for deficient vitamin D levels ($_{unadj}$POR=2.93, 95% CI: 1.27, 6.77); however, the

[*]Correspondence address: Erin M. Davis, Ph.D., University of Louisville, School of Medicine, 511 South Floyd, Room 111, Louisville, KY 40202, USA, erin.davis@louisville.edu.

Author's Contribution: EM Davis: Project development, Data collection/management, Data analysis, Manuscript writing
JD Peck: Project development, Data management, Results interpretation, Manuscript writing
KR Hansen: Results interpretation, Manuscript editing
BR Neas: Data analysis, Results interpretation, Manuscript editing
LB Craig: Project development, Data collection/management, Results interpretation, Manuscript writing

NOTES

Conflicts of Interest: The authors declare that they have no conflicts of interest.

association decreased after adjustment for BMI and race/ethnicity ($_{adj}$POR=2.03, 95% CI: 0.79, 5.19).

Conclusions: Vitamin D deficiency occurred more frequently in PCOS cases with androgen excess, but associations were attenuated after adjusting for BMI and race/ethnicity. Combining etiologically distinct PCOS subgroups may obscure associations with lower vitamin D levels and other potential risk factors.

Keywords

Vitamin D; 25-hydroxyvitamin D; polycystic ovary syndrome; PCOS

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility, affecting approximately 6 to 12% of reproductive aged women [1,2]. First described by Stein and Leventhal in 1935, PCOS results from a malfunctioning hypothalamus-pituitary-gonadal (HPG) axis and produces symptoms such as oligo- or amenorrhea, hirsutism, and increased acne [3,4]. Women with PCOS may also exhibit symptoms of metabolic syndrome, including central obesity and insulin resistance [3,5]. Typically, PCOS symptoms begin around menarche [6]; however women may not be diagnosed until years later when seeking medical treatment for infertility. Short and long-term health concerns with PCOS include obesity [7], hypertension [8], diabetes [9,10], and endometrial cancer [11]. Thus, studies are needed to improve our limited understanding of PCOS and issues that could contribute to the associated co-morbidities.

Vitamin D is considered essential for reproductive health and for achieving and supporting a healthy and viable pregnancy. Fertility issues, poor pregnancy outcomes, and pregnancy complications are associated with decreased levels of vitamin D [12,13]. According to the National Health and Nutrition Examination Survey (NHANES) 2003–2006 data, vitamin D deficiency (≤20 ng/ml) and insufficiency (21–30 ng/ml) are relatively high in women of reproductive age (20–44 years) with a prevalence of 11% and 26%, respectively [14,15]. Therefore, this lack of vitamin D sufficiency may negatively impact the health of reproductive age women.

The prevalence of vitamin D deficiency has been shown to be even higher in women with PCOS. Li et al found that 72% of PCOS women were either deficient or insufficient [16]. Further support for this relationship is demonstrated by associations between vitamin D deficiency and symptoms of PCOS, including increased BMI [5,17–19], higher hirsutism score, and hyperandrogenemia [5,18,19]. However, the existing literature comparing vitamin D concentrations in women with and without PCOS is inconsistent [20], with studies reporting higher [21,22], lower [23,24], and no observed differences [17,25–27] in serum 25 hydroxyvitamin D (25OHD) levels. The discrepancy in these results could be due to variation in study design including differences in case definition, control selection, type of serum vitamin D analyses, and analytical methods.

To address this unresolved question, we conducted a case-control study to evaluate the relationship between vitamin D deficiency and PCOS among infertility patients undergoing intrauterine insemination (IUI). The objective of our study was to compare serum 25OHD concentrations in women with PCOS to levels in non-PCOS ovulatory women undergoing IUI for male factor infertility. We aimed to evaluate associations with PCOS diagnoses overall and by diagnostic phenotypes.

MATERIALS AND METHODS

Patients

We conducted a prevalence case-control study among women undergoing fertility treatment at a university-based fertility clinic. We identified women with PCOS (cases) and non-PCOS ovulatory women with male factor infertility (controls) who underwent an IUI from January 2008 to June 2012. Subjects were ineligible if records were unavailable or incomplete, frozen serum prior to IUI treatment was unavailable, or the patients had other female infertility diagnoses (e.g., endometriosis, tubal issues, unexplained infertility, fibroids, recurrent miscarriage) or underlying known medical conditions identified as affecting vitamin D levels and absorption (e.g., anti-phospholipid antibody syndrome, hyperprolactinemia, or hypothyroidism).

The case women were diagnosed with PCOS using the Rotterdam criteria (PCOS Consensus Workshop 2004) (n=137). Based on the 2012 NIH-sponsored Evidence-based Methodology Workshop on Polycystic Ovary Syndrome [28], the clinical records for each potential PCOS case were reviewed for the symptoms identified at diagnosis to differentiate between specific PCOS phenotypes: 1) ovulatory dysfunction and polycystic appearing ovaries on ultrasound (US) (n=55); 2) ovulatory dysfunction and androgen excess (n=15); 3) ovulatory dysfunction, polycystic ovaries, and androgen excess (n=67). No women with the Rotterdam phenotype of androgen excess and polycystic ovaries were identified because all patients with these characteristics also exhibited oligo or anovulation. The control group were women attending the same fertility clinic due to male factor infertility issues (n=103). This included women whose male partners had WHO IV defined semen deficiencies [29–31], as well as women seeking donor sperm insemination.

25-hydroxyvitamin D Assessment

Vitamin D concentrations were analyzed using serum collected at the initial fertility clinic appointment and stored at −80°C until analysis. Serum was analyzed by the Diagnostic Laboratory of Oklahoma (Quest Diagnostics) for 25-hydroxyvitamin D (25OHD) concentrations, using tandem mass spectrometry (LC-MS/MS). Total 25OHD concentrations (the sum of $25OHD_2$ and $25OHD_3$), as well as separate concentrations of $25OHD_2$ and $25OHD_3$, were measured. The analysis was conducted in three batches, with the first analysis completed as part of a pilot study in October 2011 (n=42). The remaining samples were separated into two batches (n=62 and 136) for analysis in June 2013. Statistical analyses examined the potential impact of batch variability and long-term freezer storage on serum 25OHD measurements. Vitamin D concentrations were categorized using Institute of

Medicine (IOM) recommended cut-points for total 25OHD: ≤20 ng/mL (deficient), 21–29 ng/mL (insufficient), and ≥30 ng/mL (sufficient) [32,33].

Covariate Assessment

Covariates were extracted from patient medical records. These variables were recorded at the first clinical evaluation for each patient and included demographic factors such as age (≥35, <35), race/ethnicity (non-Hispanic white, other), and body mass index (BMI) (<25, 25–29, 30–34, ≥35), as well as fertility factors such as years of infertility (<2, ≥2), parity (yes/no), and gravidity (yes/no). Tobacco use (yes/no), alcohol use (yes/no), and exercise habits (yes/no) were also available, but these were limited to binary indicators of current behaviors. Factors such as season of blood draw (Winter, Spring, Summer, Fall), IUI treatment year (2008–2012), and batch of serum analysis (1–3) were also considered as covariates.

Statistical Analysis

Data analyses were conducted using SAS v9.2 (Cary, NC, USA). Median (range) vitamin D levels for women with PCOS and women undergoing IUI due to male factor infertility were compared using a Wilcoxon Rank Sum Test. Covariate distributions were examined by case status and compared by calculating prevalence odds ratios. Differences in the proportion of cases and controls with sufficient, insufficient or deficient vitamin D levels were evaluated using chi-square tests.

Associations between vitamin D concentrations and PCOS were evaluated using logistic regression, with prevalence odds ratios (POR) and 95% confidence intervals (CI) reported. Using directed acyclic graphs (DAGs), we selected age, race/ethnicity, alcohol, smoking, and BMI for evaluation as potential confounders. Quantitative assessment for confounding included these covariates in the logistic regression model and used backward elimination to evaluate the change in the POR for vitamin D. Only adjustment for BMI accompanied by race/ethnicity altered the POR by more than 15% and met our criteria for inclusion in the adjusted models. To acknowledge the uncertainty surrounding obesity as a potential consequence of inadequate vitamin D levels [34–36], which would introduce bias by inappropriately adjusting for causal intermediates, the results are reported for both unadjusted associations and for associations controlling for BMI and race/ethnicity. Effect modification between the exposure and covariates was explored by entering interaction terms in the logistic regression models. No interaction terms had p-values ≤0.05; thus no covariates were identified as effect modifiers. Similar analyses were also conducted for the PCOS diagnostic phenotypes with unadjusted and adjusted results reported for each.

Variability by batch of serum analysis was assessed using mixed effects logistic regression, modeling vitamin D exposure as a fixed effect and assay batch as a random effect. Accounting for laboratory batch had minimal impact on the study results; therefore batch was not included in the final models.

RESULTS

Between January 2008 and June 2012, we identified 168 cases with PCOS and 146 controls with male factor infertility issues undergoing their first IUI cycle at the study site. After excluding women without available serum (22 cases and 43 controls) or without records available for diagnostic confirmation (9 cases), the study population consisted of 240 women, with 137 cases of confirmed PCOS and 103 controls seeking IUI for male factor infertility. Covariate distributions for cases and controls are reported in Table I. The study population was predominately non-Hispanic white (85%), with no racial/ethnic differences observed among the cases and controls. Compared to controls, cases were younger, had higher BMI, and were also more likely to experience infertility for two or more years. Current exercise, alcohol use, and smoking status were similar among the cases and controls. In terms of factors related to the serum collection and storage, season of serum collection and year of IUI treatment did not differ by case status.

Evaluation of Vitamin D Exposure and PCOS Diagnosis

Women with PCOS had lower median levels of total vitamin D (26 ng/mL, range: 6–70) when compared to controls (30 ng/mL, range: 11–63) However, this difference was not statistically significant (p= 0.07). Concentrations of $25OHD_3$ were similar to total vitamin D levels with median concentrations of 25 ng/mL (range: 2–70) for cases and 30 ng/mL (range: 11–63) for controls (p-value=0.13). Approximately 96% of the study population had $25OHD_2$ levels below the levels of detection (LOD) (4 ng/mL) (n=230). Therefore, $25OHD_2$ levels were not evaluated further. Likewise, since results evaluating $25OHD_3$ levels were nearly identical to results analyzed using total vitamin D concentrations, further study results are reported using only total 25OHD levels.

A higher proportion of vitamin D deficiency was observed among the cases although it did not reach statistical significance (21.2% vs. 13.6%, p-value=0.13) (Table II). Additionally, 59.9% of the cases lacked sufficient vitamin D levels (i.e. combining insufficient and deficient categories) compared to 47.6% of the controls (p-value=0.06).

Using vitamin D sufficiency as the referent, the unadjusted odds of vitamin D deficiency were 2.03 (95% CI: 0.97, 4.26) times higher in women with PCOS compared to controls (Table II). Significant associations with vitamin D insufficiency were not observed but the point estimates were in the same direction ($_{unadj}$POR=1.49, 95% CI: 0.84, 2.63). Adjusting for BMI and race/ethnicity attenuated the results (Table II). A pattern of increasing odds ratios with decreasing vitamin D levels was noted; however the confidence intervals contained the null value.

Evaluation of Vitamin D Exposure and PCOS Phenotypes

Associations between vitamin D exposure and PCOS phenotypes are reported in Table III. Vitamin D deficiency and insufficiency were positively associated with phenotype 3 (ovulatory dysfunction, polycystic appearing ovaries, and androgen excess) in the unadjusted analyses, but associations were attenuated after adjusting for BMI and race/ethnicity. Vitamin D insufficiency, but not deficiency, was associated with an increased odds of

phenotype 2 (ovulatory dysfunction and androgen excess) in the unadjusted analyses, but again the odds ratio was attenuated after controlling for BMI and race/ethnicity. The precision of associations with phenotype 2, however, was limited by the small number of cases with this phenotype (n=15). When phenotypes 2 and 3 were combined to examine all 82 cases with androgen excess, crude associations with both insufficient ($_{unadj}$POR=2.35, 95% CI: 1.21, 4.54) and deficient ($_{unadj}$POR=2.93, 95% CI: 1.27, 6.77) vitamin D levels were observed. However, the magnitude of association decreased and the confidence intervals included 1.0 after adjusting for BMI and race/ethnicity (Insufficient: $_{adj}$POR=1.72, 95% CI: 0.84, 3.50; Deficient: $_{adj}$POR=2.03, 95% CI: 0.79, 5.19). In contrast, no associations with vitamin D deficiency or insufficiency were observed for phenotype 1 (ovulatory dysfunction and polycystic appearing ovaries).

DISCUSSION

The prevalence of vitamin D deficiency was greater among PCOS subjects when compared to controls; however, this relationship, which approached marginal significance in unadjusted analyses, was not statistically significant in adjusted models. We chose to present the study results both with and without adjustment for BMI due to concerns that the temporal relationship between obesity, vitamin D levels and PCOS remains unclear. For instance, individuals with higher BMI have been reported to have lower vitamin D levels due to the sequestration of vitamin D in the larger adipocytes [37,38]. In contrast, associations between lower vitamin D concentrations and incident obesity have also been observed [34–36]. Additionally, since the etiology of PCOS remains unknown, the directionality of the relationship between PCOS and obesity remains unclear [5,17–19]. Thus, if BMI resides on the causal path between vitamin D and development of PCOS, it would not be appropriate to control for BMI as a confounder and doing so would lead to overadjustment bias toward the null value. Ultimately the results of our study suggest that BMI adjusted associations for vitamin D deficiency and insufficiency were similar to the unadjusted associations, but attenuated.

Four previously conducted studies also reported lower serum 25OHD levels in women with PCOS [17,23–25,27], however only one of those studies calculated a measure of association [23]. The case-control study by Mazloomi, et al. reported that the odds of vitamin D deficiency were 1.6 times higher among women with PCOS when compared to control women (95% CI: 1.2, 2.0) who were described as healthy, ovulatory women, and free of any systemic disease that may affect their reproductive physiology [23]. Associations with vitamin D insufficiency were not reported nor were any analyses for confounding variables. Despite our study having similar sample sizes of approximately 200 women, our unadjusted measures of association failed to reach statistical significance and when adjusted for BMI and race/ethnicity the results were further attenuated. These differences in results may be due, in part, to differences in our control populations. In the Mazloomi study, controls were recruited from an endocrine clinic and the infertility status was unknown [23]. Although it is difficult to discern, it appears that these controls were not women seeking infertility treatment like those selected for our study population.

We used a similar study design to Li et al., which included a control group of women also seeking fertility treatments, but lacking a female infertility diagnosis [17]. Bloom et al. [39] addresses the common misconception that healthy controls are optimal for PCOS case-control comparisons, when in fact control selection should represent the exposure distribution among the source population at risk in order to avoid introducing bias. The source population at risk for PCOS is, thus, determined by the case definition [39]. Our controls who were seeking IUI due to male infertility were selected to represent the vitamin D exposure distribution in the source population from which the PCOS cases were drawn. Controls selected from the general population of fertile women may not reflect the exposure experience of women who would have sought treatment if they experienced an inability to conceive a pregnancy, thereby introducing a potential selection bias. Therefore, the use of a community-based control group in other studies that ascertained PCOS cases from an infertility clinic [21] may have introduced a selection bias if factors that influence access to fertility treatment, such as socioeconomic factors are also associated with vitamin D status. The generalizability of our results from this clinic-based population, however, is limited to women seeking treatment for infertility.

The heterogeneous nature of PCOS and use of various diagnostic criteria [28,40,41] contributes to the difficulty in elucidating its etiology. To address the complexity of PCOS, a 2012 NIH-sponsored Evidence-based Methodology Workshop on Polycystic Ovary Syndrome reviewed the criteria used in diagnosing PCOS, recommending the use of the more broad Rotterdam diagnostic criteria [28,40]. Our study follows the panel's recommendations for specifying the PCOS diagnostic phenotype in an effort to improve outcome classification and to shed light on the associations between etiologically distinct PCOS subgroups. In our study population, the majority of cases were characterized by all three Rotterdam criteria (ovulatory dysfunction, polycystic ovaries by US, and androgen excess) (49%), followed closely by cases with ovulatory dysfunction and polycystic ovaries (40%). No women were identified as having the PCOS phenotype of polycystic ovarian appearance on US and androgen excess only. Since this study population was recruited from a fertility clinic, it is reasonable that ovulation issues would be a defining feature of PCOS for these women.

The results from our analyses of PCOS phenotypes indicate that women diagnosed with PCOS due to androgen excess (either increased serum testosterone levels or evidence of hirsutism) had higher odds of vitamin D insufficiency and deficiency when compared to controls. In our study 60% of PCOS women had phenotypes 2 or 3, which included androgen excess. Further support regarding the relationship between the PCOS symptom of androgen excess and decreased vitamin D levels is demonstrated by studies that observed negative correlations between PCOS symptoms, such as higher hirsutism scores and increased testosterone levels, and vitamin D levels [5,18,19]. Yet, no other studies have addressed the association between vitamin D levels and PCOS phenotypes. Additional research is needed to further investigate this association between androgen excess and vitamin D concentration.

While the prevalence case-control design was a practical choice for studying women with PCOS, use of this design leaves us unable to establish the temporality of the association between PCOS and serum 25OHD. Evaluating risk factors for chronic disorders such as

PCOS is challenging, given that disease onset is difficult to establish and thus incident cases are rarely identified or diagnosed. Thus, this study is limited by the measurement of serum 25OHD at the time of PCOS evaluation, which may not accurately reflect vitamin D concentrations present at the time of PCOS development. Studies of vitamin D variability, however, have reported a relatively high degree of correlation (>0.70) between 25OHD measures when repeated in women over one, two and three years [42,43].

An additional concern regarding the vitamin D measurement results from the potential overestimation of total 25OHD levels that may result when using the tandem mass spectrometry analytical method. Tandem mass spectrometry is the method of choice for large population studies, such as the NHANES [44], and allows for individual quantification of the $25OHD_3$ form. During the high-pressure liquid chromatography (HPLC) process, analyses completed without a specialized column result in the co-elution of $25OHD_3$ and the epimeric version of this molecule. This co-elution results in inflated measures of total 25OHD, with an estimated 3% misclassification of vitamin D sufficiency [45]. However, our assessment of this potential source of misclassification using quantitative bias analyses [46] indicated it had no meaningful impact on study conclusions (data not shown).

Serum 25OHD is known to be extremely stable, with reports of continued stability after undergoing up to 4 freeze-thaw cycles [47,48]. Additionally, the Vitamin D External Quality Assessment Scheme (DEQAS), a vitamin D quality assessment organization, ships serum samples to quality assessment laboratories by ground mail with no refrigeration, failing to observe issues with 25OHD concentrations [49]. Long-term freezer storage stability has also been observed when stored for up to 50 years [50]. We observed no association between vitamin D levels and year of IUI treatment with serum stored for up to 5 years. This suggests that sample degradation or desiccation during the storage period was not likely to contribute to exposure misclassification.

CONCLUSIONS

Although we failed to observe a statistically significant association between vitamin D exposure and PCOS among the entire case group, positive, but less precise, associations were observed in PCOS women exhibiting androgen excess. Thus, a mixing of etiologically distinct PCOS conditions may obscure associations with vitamin D deficiency and other potential risk factors. Future research should continue to evaluate relationships with more homogenous subgroups of PCOS women sharing similar phenotypes. The use of refined case definitions may provide more definitive information regarding PCOS development and potential treatment for women suffering from this disorder.

Acknowledgements:

We would like to give special thanks to Christy Zornes, Mitch Trammell, Kammy Wardlaw, Yanet Figueroa, and Dr. Drew Rainer, for their assistance in the data collection process and to Drs. Laura Beebe and Christina Shay for their assistance in the editing process.

Funding: This project was supported by the Oklahoma Shared Clinical and Translational Resource Institute NIGMS U54 GM104938

REFERENCES

1. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. The Journal of Clinical Endocrinology and Metabolism 2004;89:2745–9. [PubMed: 15181052]
2. March WA, Moore VM, Willson KJ, Phillips DIW, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Human Reproduction 2009;25:544–51. [PubMed: 19910321]
3. Erhmann DA. Polycystic Ovary Syndrome. The New England Journal of Medicine 2005;352:1223–36. [PubMed: 15788499]
4. Azziz R, Adashi E. Stein and Leventhal: 80 years on. American Journal of Obstetrics and Gynecology 2016:247–56.
5. Wehr E, Pilz S, Schweighofer N, et al. Association of hypovitaminosis D with metabolic disturbances in polycystic ovary syndrome. European Journal of Endocrinology 2009;161:575–82. [PubMed: 19628650]
6. Franks S Adult polycystic ovary syndrome begins in childhood. Best Practice and Research Clinical Endocrinology and Metabolism 2002;16:263–72. [PubMed: 12064892]
7. Azziz R, Ehrmann D, Legro RS. Troglitazone improves ovulation and hirsutism in the polycystic ovary syndrome: a multicenter, double blind, placebo-controlled trial. J Clin Endocrinol Metab 2001;86:1626–32. [PubMed: 11297595]
8. Glueck C, Papanna R, Wang P, Goldenberg N, L S-S. Incidence and treatment of metabolic syndrome in newly referred women with confirmed polycystic ovarian syndrome. Metabolism 2003;52:908–15. [PubMed: 12870169]
9. Ehrmann D, Barnes R, Rosenfield R, Cavaghan M, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care 1999;22:141–6. [PubMed: 10333916]
10. Legro RS, Kunselman A, Dodson W, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. J Clin Endocrinol Metab 1999;84:165–9. [PubMed: 9920077]
11. Balen A Polycystic ovary syndrome and cancer: an update. Hum Reprod Update 2001;7:522–5. [PubMed: 11727859]
12. Barrett H, McElduff A. Vitamin D and pregnancy: an old problem revisited. Best Practice and Research Clinical Endocrinology and Metabolism 2010;24:527–39. [PubMed: 20832734]
13. Rojansky N, Brzezinski A, Schenker J. Seasonality in human reproduction: an update. Human Reproduction 1992;7:735–45. [PubMed: 1323571]
14. Ginde AA, Liu MC, A. CJC. Demographic differences and trends of vitamin D insufficiency in the US population, 1988–2004. Arch Intern Med 2009;169:626–32. [PubMed: 19307527]
15. Zhao G, Ford ES, Tsai J, Li C, Croft JB. Factors associated with vitamin D deficiency and inadequacy among women of childbearing age in the United States. International Scholarly Research Network Obstetrics and Gynecology 2012;2012:1–9.
16. Li C, Ford E, Mokdad A, Cook S. Recent trends in waist circumference and wasit-height ratio among US children and adolescents. Pediatrics 2006;118:345–53.
17. Li H, Brereton R, Anderson R, Wallace A, Ho C. Vitamin D deficiency is common and associated with metabolic risk factors in patients with polycystic ovary syndrome. Metabolism 2011;60:1475–81. [PubMed: 21550088]
18. Hahn S, Haselhorst U, Tan S, et al. Low serum 25-hydroxyvitamin D concentrations are associated with insulin resistance and obesity in women with polyscystic ovary syndrome. Experimental and Clinical Endrocrinology and Diabetes 2006;114:577–83.
19. Yildizhan R, Kurdoglu M, Adali E, et al. Serum 25-hydroxyvitamin D concentrations in obese and non-obese women with polycystic syndrome. Arch Gynecol Obstet 2008;280:559–63.
20. He C, Lin Z, Robb SW, Ezeamama AE. Serum Vitamin D levels and polycystic ovary syndrome: a systematic review and meta-analysis. Nutrients 2015;7:4555–77. [PubMed: 26061015]

21. Mahmoudi T, Gourabi H, Ashrafi M, Yazdi R, Ezabadi Z. Calciotropic hormones, insulin resistance, and the polycystic ovary syndrome. Fertility and Sterility 2010;93:577–83.
22. Ngo D, Chan WP, Rajendran S, et al. Determinants of insulin responsiveness in young women: Impact of polycystic ovarian syndrome, nitric oxide, and vitamin D. Nitric Oxide 2011;1149:1120–25.
23. Mazloomi S, Sharifi F, Hajihosseini R, Kalantari S, Mazloomzadeh S. Association between hypoadiponectinemia and low serum concentrations of calcium and vitamin D in women with polycystic ovary syndrome. International Scholarly Research Network 2012:1–6.
24. Nestler JE, Reilly ER, Cheang KI, Bachmann LM, Downs RW Jr.. A Pilot Study: Effects of Decreasing serum insulin with diazoxide on vitamin D levels in obese women with polycystic ovary syndrome. Trans Am Clin Climatol Assoc 2012;123:209–20. [PubMed: 23303986]
25. Ozkan S, Jindal S, Greenseid K, et al. Replete vitamin D stores predict reproductive success following in vitro fertilization. Fertility and Sterility 2010;94:1314–9. [PubMed: 19589516]
26. Panidis D, Balaris C, Farmakiotis D, et al. Serum Parathryoid Hormone Concentrations Are Increased in Women with Polycystic Ovary Syndrome. Clin Chem 2005;51:1691–97. [PubMed: 16037412]
27. Sahin S, Eroglu M, Selcuk S, et al. Intrinsic factors rather than vitamin D deficiency are related to insulin resistance in lean women with polycystic ovary syndrome. European Review for Medical and Pharmocological Sciences 2014;18:2851–6.
28. Evidence-based Methodology Workshop on Polycystic Ovary Syndrome Washington D.C.: Office of Disease Prevention and the Eunice Kennedy Shriver National Institute of Child Health; 2012.
29. WHO Laboratory Manual for the Examination of Human Semen and Sperm-cervical mucus interaction Cambridge: Cambridge University Press; 1999.
30. WHO laboratory manual for the Examination and processing of human semen Cambridge: Cambridge University Press; 2010.
31. Cooper T, Noonan E, von Eckardstein S, et al. World Health Organization reference values for human semen characteristics. Human Reprod Update 2010;16:231–45.
32. Institue of Medicine Committee to Review Dietary Reference Intakes for Vitamin D and Calcium Washington DC: The National Academies Press; 2011
33. Holick MF, Binkley N, Bischoff-Ferrari H. Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911–30. [PubMed: 21646368]
34. Gilbert-Diamond D, Baylin A, Mora-Plazas M, et al. Vitamin D deficiency and anthropometric indicators of adiposity in school-age children: a prospective study. Am J Clin Nutr 2010;92.
35. Gonzalez-Molero I, Rojo-Martinez G, Morcillo S, et al. Hypovitaminosis D and incidence of obesity: a prospective study. European Journal of Clinical Nutrition 2013;67:680–2. [PubMed: 23422920]
36. Mai X-M, Chen Y, Camargo CA Jr., Langhammer A. Cross-sectional and prospective cohort study of serum 25-hydroxyvitamin D level and obesity in adults. Am J Epidemiol 2012;175:1029–36. [PubMed: 22312120]
37. Arunabh S, Pollack S, Yeh J, Aloia JF. Body fat content and 25-hydroxyvitamin D levels in healthy women. The Journal of Clinical Endocrinology and Metabolism 2003;88:157–66. [PubMed: 12519845]
38. Wortsman J, Matsuoka LY, Chen TC. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2000;72:690–3. [PubMed: 10966885]
39. Bloom M, Schisterman E, Hediger M. Selecting controls is not selecting “normals”: Design and analysis issues for studying the etiology of polycystic ovary syndrome. Fertility and Sterility 2006;86:1–12. [PubMed: 16750830]
40. Legro RS, Arslanian S, Ehrmann D, et al. Diagnosis and treatment of polycystic ovary syndrome: an endocrine society clinical and practical guideline. J Clin Endocrinol Metab 2013;98:4565–92. [PubMed: 24151290]
41. Franks S Diagnosis of polycystic ovarian syndrome: in defence of the Rotterdam criteria. J Clin Endocrinol Metab 2006;91:786–9. [PubMed: 16418209]

42. Major J, Graubard B, Dodd K. Variability and Reproducibility of Circulating Vitamin D in a Nationwide U.S. Population. The Journal of Clinical Endocrinology and Metabolism 2013;98:97–104. [PubMed: 23144464]
43. Kotsopoulos J, Tworoger S, Campos HC, FL, et al. Reproducibility of plasma and urine biomarkers among premenopausal and postmenopausal women from the Nurses' Health Studies. Cancer Epidemiology Biomarkers Prevention 2010;19:938–46.
44. Yetley EA, Pfeiffer CM, Schleicher RL, et al. NHANES monitoring of serum 25-hydroxyvitamin D: a roundtable summary. J Nutr 2010;140:2030S–45S. [PubMed: 20881084]
45. Strathmann F, Sadilkova K, Laha T, LeSourd S, Bornhorst J, Hoofnagle AN. 3-epi-25 hydroxyvitamin D concentrations are not correlated with age in a cohort of infants and adults. . Clin Chim Acta 2012;413:203–6. [PubMed: 21983164]
46. Greenland S, Lash T. Bias analysis. In: Rothman KG S, Lash T, eds. Modern Epidemiology 3rd ed. Philadelphia: Lippincott, Williams & Wilkins; 2008.
47. Ersfeld D, Rao D, Body J. Analytical and clinical validation of the 25 OH vitamin D assay for the LIAISON automated analyzer. Clin Biochem 2004;37:867–74. [PubMed: 15369717]
48. Antoniucci D, Black D, Sellmeyer D. Serum 25-hydroxyvitamin D is unaffected by multiple freeze-thaw cycles. Clin Chem 2005;51:258–61. [PubMed: 15613728]
49. Hollis B Measuring 25-hydroxyvitamin D in a clinical environment: challenges and needs. Am J Clin Nutr 2008;88:S5075–108.
50. Bodnar L, Catov J, Wisner K, Klebanoff M. Racial and seasonal differences in 25-hydroxyvitamin D detected in maternal sera frozen for over 40 years. British J of Nutr 2009;101:278–84.

Table I.

Covariate distributions and associations among PCOS cases and controls.

Variable	PCOS Cases (n=137)	Male Factor Controls (n=103)	Unadjusted POR[a] 95% CI
Age at IUI			
<35	121 (88.3)	64 (62.1)	Referent
≥35	16 (11.7)	39 (37.9)	0.22 (0.11, 0.42)
Race/Ethnicity			
Non-Hispanic white	115 (83.9)	89 (86.4)	Referent
Other	22 (16.1)	14 (13.6)	1.22 (0.59, 2.51)
BMI			
<25	35 (25.6)	44 (42.7)	Referent
25–29	30 (21.9)	29 (28.2)	1.30 (0.66, 2.56)
30–34	30 (21.9)	17 (16.5)	2.22 (1.06, 4.66)
≥35	42 (30.7)	13 (12.6)	3.39 (1.89, 8.72)
Number of years of infertility			
<2	32 (23.4)	38 (36.9)	Referent
≥2	105 (76.6)	65 (63.1)	1.92 (1.09, 3.37)
Gravid			
Yes	38 (27.7)	42 (40.8)	0.55 (0.32, 0.94)
No	99 (72.3)	60 (58.3)	Referent
Missing	0 (0.0)	1 (1.0)	----------
Parous			
Yes	23 (16.8)	21 (20.4)	0.78 (0.40, 1.50)
No	114 (83.2)	81 (78.6)	Referent
Missing	0 (0.0)	1 (1.0)	----------
Reported exercising at first clinic visit			
Yes	97 (70.8)	77 (73.8)	0.76 (0.40, 1.40)
No	35 (25.5)	21 (20.4)	Referent
Missing	5 (3.6)	5 (5.8)	----------
Reported smoking at first clinic visit			
Yes	21 (15.3)	11 (10.7)	1.51 (0.69, 3.31)
No	111 (81.0)	88 (85.4)	Referent
Missing	5 (3.6)	4 (3.9)	----------
Reported alcohol use at first clinic visit			
Yes	85 (62.0)	67 (65.0)	0.81 (0.46, 1.42)
No	47 (34.3)	30 (29.1)	Referent
Missing	5 (3.6)	6 (5.8)	----------
IUI treatment year			
2008	12 (8.8)	2 (1.9)	4.36 (0.86, 22.25)

Variable	PCOS Cases (n=137)	Male Factor Controls (n=103)	Unadjusted POR[a] 95% CI
2009	31 (22.6)	31 (30.1)	0.73 (0.32, 1.64)
2010	32 (23.4)	33 (32.0)	0.71 (0.32, 1.58)
2011	40 (29.2)	21 (20.4)	1.39 (0.60, 3.19)
2012	22 (16.1)	16 (15.5)	Referent
Season of serum collection			
Winter (Dec-Feb)	36 (26.3)	28 (27.2)	1.00 (0.49, 2.06)
Spring (Mar-May)	35 (25.6)	22 (21.4)	1.24 (0.59, 2.62)
Summer (Jun-Aug)	34 (24.8)	28 (27.2)	0.95 (0.46, 1.96)
Fall (Sep-Nov)	32 (23.4)	25 (24.3)	Referent

[a]POR = prevalence odds ratio

Table II.

Crude and adjusted prevalence odds ratios[a] (PORs) and 95% confidence intervals (CI) evaluating associations between vitamin D categories and PCOS.

Variable	PCOS Cases (n=137)	Male Factor Controls (n=103)	Crude POR (95% CI)	Adjusted[b] POR (95% CI)
Vitamin D categories (ng/mL)				
Sufficient (≥30)	55 (40.2)	54 (52.4)	Referent	Referent
Insufficient (21–29)	53 (38.7)	35 (34.0)	1.49 (0.84, 2.63)	1.15 (0.63, 2.11)
Deficient (≤20)	29 (21.2)	14 (13.6)	2.03 (0.97, 4.26)	1.43 (0.62, 3.26)

[a] Prevalence odds ratios were calculated using logistic regression.

[b] Adjusted for BMI and race/ethnicity

Table III.

Crude and adjusted prevalence odds ratios[a] (PORs) and 95% confidence intervals (CI) evaluating associations between vitamin D categories and PCOS phenotypic subgroups[b].

	PCOS phenotype #1 (n=55) ovulatory dysfunction polycystic ovaries		PCOS phenotype #2 (n=15) ovulatory dysfunction androgen excess		PCOS phenotype #3 (n=67) ovulatory dysfunction polycystic ovariesandrogen excess	
Vitamin D Exposure Categories (ng/mL)	**Crude POR (95% CI)**	**Adjusted POR[c] (95% CI)**	**Crude POR (95% CI)**	**Adjusted POR[c] (95% CI)**	**Crude POR (95% CI)**	**Adjusted POR[c] (95% CI)**
Vitamin D categories						
Sufficient (≥30)	Referent	Referent	Referent	Referent	Referent	Referent
Insufficient (21–29)	0.77 (0.36, 1.64)	0.64 (0.29, 1.41)	3.86 (1.12, 13.26)	2.98 (0.80, 11.06)	2.06 (1.01, 4.18)	1.47 (0.68, 3.17)
Deficient (≤20)	1.29 (0.51, 3.25)	0.97 (0.35, 2.64)	0.96 (0.10, 9.32)	0.94 (0.09, 9.61)	3.31 (1.40, 7.82)	2.19 (0.83, 5.80)

[a] Prevalence odds ratios were calculated using logistic regression.

[b]There were no women in our study who exhibited only polycystic ovaries and hyperandrogenism. All PCOS women were oligo or anovulatory.

[c]Adjusted for BMI category and race/ethnicity