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25-hydroxyvitamin D (25(OH)D) and biomarkers of ovarian reserve

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Abstract

Objective—To examine the associations between 25(OH)D and biomarkers of ovarian reserve in a large community-based sample of women.

Methods—In 2010–2016, women aged 30–44 without any known fertility problems were recruited from the Chapel Hill, North Carolina area for a prospective time-to-pregnancy cohort study. At enrollment 561 women provided a blood sample that was used to measure 25(OH)D, AMH, FSH, and inhibin-B. Unadjusted associations were estimated with Spearman correlation coefficients. Multivariable linear regression was used to estimate associations of 25(OH)D with ovarian reserve biomarkers, after adjusting for age, race, body mass index, smoking history, and recent use of hormonal birth control.

Results—The mean 25(OH)D was 36 ng/ml (SD=11 ng/ml). 25(OH)D was not correlated with AMH, FSH, or inhibin-B (all $r < 0.03$). Multivariable results with continuous hormonal outcomes were also null. For dichotomous outcomes there was a tendency for insufficient 25(OH)D (<30 ng/ml) to be associated with low AMH (<0.7 ng/ml) (Odds ratio (95% Confidence Interval): 1.8 (0.9, 4)).

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Conclusions—For the most part, 25(OH)D was not associated with ovarian reserve biomarkers in a group of women trying to become pregnant. We found some evidence that low 25(OH)D (<30 ng/ml) was associated with low AMH, but this should be confirmed in studies with a higher prevalence of low 25(OH)D.

Keywords

inhibins; follicle stimulating hormone; fertility; ovary; vitamin D; vitamin D deficiency

Introduction

Diminished ovarian reserve (a low quantity of oocytes) is associated with a poor response to ovarian stimulation among women seeking fertility treatment¹. Diminished ovarian reserve with subsequent early menopause not only limits a woman's reproductive life span but has also been linked to increased risk of osteoporosis, cardiovascular disease, and all-cause mortality^{2–6}.

Ovarian senescence results in a decline in both the quantity^{7, 8} and quality⁹ of ovarian follicles and oocytes. The aging ovary produces smaller amounts of the granulosa cell hormones, Anti-Mullerian Hormone (AMH)¹⁰ and inhibin B¹¹ which leads to a rise in early follicular phase follicle stimulating hormone (FSH).¹² Previous studies have shown associations between AMH, FSH, and inhibin B and ovarian aging.^{13–18} AMH and inhibin B decline and FSH rises as a woman moves through the menopausal transition.^{19–21} The levels of AMH and FSH mark later stages of reproductive aging such as early perimenopause, late perimenopause, and postmenopause.^{13, 22–26} AMH is predictive of time to menopause.²⁷

Vitamin D, which is well-known for its role in maintaining bone health²⁸, has also been associated with reproductive health (reviewed in²⁹) and menstrual function.^{30–32} In a previous study of women aged 35–44, lower plasma total 25-hydroxyvitamin D (25(OH)D) was associated with increased FSH.³³

The gene for AMH contains a vitamin D response element, which suggests that vitamin D may regulate AMH expression^{34, 35}. Treatment of isolated follicles from rhesus macaques with active vitamin D increases AMH concentrations.³⁶ Human studies of AMH and vitamin D (as measured by 25(OH)D) have shown mixed results. One study reported a positive correlation³⁷, while others report no correlation^{38–41}. One intervention study reported that supplementation with vitamin D prevented seasonal fluctuations in AMH⁴², while another study found no seasonal variation in AMH.³⁸ No previous studies have examined the associations between 25(OH)D and inhibin-B.

Our objective was to examine the association between 25(OH)D and three biomarkers of ovarian reserve (FSH, AMH, and inhibin-B) in a large community-based cohort of women attempting to conceive.

Methods

Study design

Time to Conceive was a prospective, time-to-pregnancy cohort study of biomarkers of ovarian reserve (2008–2016).⁴³ Women who were intending to become pregnant were recruited through mass emails, introductory letters, and web and radio advertising. Eligible women had to be between the ages of 30 and 44 and trying to conceive naturally for 3 months or less. Attempt time was self-reported as the amount of time they had been “having regular intercourse without doing anything to prevent pregnancy”. Women were excluded if they reported a history of infertility, polycystic ovarian syndrome, endometriosis, a partner with infertility, or current breastfeeding. Women completed a self-administered questionnaire that included demographic data, reproductive history, contraceptive history, tobacco use, and other behaviors.

Women were instructed to schedule a study visit at the beginning of their first menses after study recruitment (cycle-day 2, 3, or 4). If they missed this window, women were asked to come in at the next menstrual cycle. At the study visit, women gave informed consent and provided a venous blood sample. In 2010, the study protocol was amended to add the collection of whole blood spots at the study visit, which were dried and stored frozen.

Ethical approval

All women provided informed consent, and all study activities were approved by the University of North Carolina IRB.

25(OH)D

25(OH)D was extracted from 6mm punches from stored blood spots using previously described methods.⁴⁴ 25(OH)D₃ and 25(OH)D₂ were quantified through liquid chromatography-tandem mass spectrometry. 25(OH)D measured in dried blood spots shows good agreement with plasma measures.⁴⁵ Blinded samples indistinguishable from test samples were also sent to the lab. Based on these samples, the intra-assay coefficient of variation was 6.3% and the inter-assay coefficient of variation was 7.7%. Of the 618 women enrolled in or after 2010, 562 women provided a blood-spot measure of vitamin D (Figure 1).

We examined 25(OH)D both as the continuous, quantified measure, and as a dichotomized category of “insufficient”, which was defined based on the Endocrine Society guidelines as <30 ng/ml.⁴⁶ Finer categories of 25(OH)D were examined to determine the shape of the associations with ovarian reserve biomarkers. This model was compared with a linear fit to 25(OH)D using Akaike’s Information Criterion (AIC) and the model with the lowest AIC was chosen, which was the linear fit. It would be expected that biologic effects of 25(OH)D on ovarian reserve would accrue over time, and thus the average level of 25(OH)D over the whole previous year, averaged across seasonal fluctuations, might be associated with the measured ovarian reserve biomarkers. To investigate this, we also estimated the annual mean 25(OH)D level, according to the method of Sachs et al.⁴⁷ Briefly, a model is estimated that predicts 25(OH)D based on the season of the blood draw. This model is used to predict each

woman's yearly average 25(OH)D level. To accomplish this, the difference between the woman's date-specific predicted and actual 25(OH)D is calculated as her "residual". The intercept of the predictive model, which is the overall annual mean across women, was added to the woman's residual to obtain the woman's estimated annual mean 25(OH)D.

AMH, FSH, Inhibin-B

AMH was measured in serum samples that were stored at -30°C until analysis. Samples were shipped frozen in a single batch to the University of Southern California Reproductive Endocrinology Laboratory. There they were assayed using sensitive and specific assays for FSH (Immulite analyzer, Siemens, Deerfield, IL), inhibin-B (ELISA, Ansh Labs, Webster, TX), and AMH (Ultrasensitive AMH ELISA, Ansh). Interassay coefficients of variation ranged from 4–5% for FSH, 5–8% for inhibin-B, and 9–11% for AMH. Values below the limit of detection, 0.078 ng/ml for AMH (N=10) and 9 pg/ml for inhibin-B (N=42) were replaced with the limit of detection divided by the square root of two.⁴⁸

Of the 567 women with a 25(OH)D measure, 566 also had an AMH level. Samples from fifty-nine women who enrolled near the end of the study were sent to the lab for quantification of AMH, but the other hormones were not measured. Thus, of the 567 women, 507 had FSH, and 507 had inhibin-B.

Covariates

Variables examined as potential confounders were selected based on previous studies,^{49, 50} and included age at the time of the blood draw, race, body mass index, smoking and number of months since the participant had used an estrogen-containing hormonal contraception (categorized as one month or less, two months, three months, or more than three months or never). The multivariate analyses were also run without controlling for race, and the results were not substantially different. Five women were excluded from the multivariable analyses because they were missing covariate information.

Statistical analysis

We calculated means and standard deviations or, where appropriate, geometric means and geometric ranges. The geometric range is bounded by two quantities: the geometric mean divided by the geometric standard deviation, and the geometric mean multiplied by the geometric standard deviation⁵¹. The geometric range will contain approximately two-thirds of the data. This is analogous to the standard confidence interval calculation.

We calculated Spearman correlation coefficients among untransformed 25(OH)D, AMH, FSH, and inhibin-B with their associated Fisher 95% confidence intervals. We used multivariable linear regression to estimate the association between 25(OH)D (not log-transformed) and each hormone measure independently while adjusting for covariates. AMH and FSH were natural log transformed to achieve normality of the regression residuals. Inhibin-B did not need log-transformation. Additionally, outliers for some of the hormonal measurements influenced model fit. We addressed this with a sensitivity analysis in which values below the limit of detection (0.078ng/ml) for AMH were excluded from the linear regression model (N=10), values less than 1.5 ng/ml were excluded from the FSH model

(N=9), and values >200pg/ml were excluded from the inhibin-B linear regression (N=6) (Supplemental Table 1). In the main results, these values were included in the models. We carried out an additional sensitivity analysis in which, instead of being excluded, values of FSH under 1.5ng/ml were set to 1.5, values of AMH less than 0.078ng/ml were set to 0.078, and inhibin-B values above 200pg/ml were truncated to 200. These results are also shown in Supplemental Table 1.

We created dichotomous variables for AMH and FSH. For AMH, “low” was less than 0.7 ng/ml⁴³ and “high” was greater than 8.5 ng/ml (the upper 10th percentile of the cohort). For FSH, “high” was greater than 10 ng/ml.⁵² Outliers that were excluded from the linear regression were included in the analyses of these dichotomous variables. We used multivariable logistic regression models to estimate the associations between 25(OH)D and high FSH (compared with normal FSH), between 25(OH)D and high AMH (compared with normal AMH), and between 25(OH)D and low AMH (compared with normal AMH), while adjusting for covariates.

All analyses were completed with SAS software, version 9.4.

Results

The median 25(OH)D was 35 ng/ml (IQR: 29, 41). Insufficient levels (<30ng/ml) were seen in 30% of the women, further deficiency was infrequent with 4% at <20 ng/ml and one woman <10ng/ml. Levels tended to be lower in women ages 31–40, in African-American women, and in women with high BMI. (Table 1). Recent users of hormonal birth control had higher 25(OH)D.

25(OH)D was not correlated with AMH, FSH, or inhibin-B (all correlations <0.05, Table 2). After adjustment for age and other covariates, women who were insufficient in 25(OH)D had 17% lower AMH levels than women who were sufficient, although this was not statistically significant. Neither 25(OH)D nor the estimated yearly average 25(OH)D was associated with the other continuous measures of any of the hormones (Table 3). Supplemental Table 1 shows the same models as in Table 3, but with the outlying values excluded or included at the range (see Table, Supplemental Digital Content 1). Season was not hypothesized to be an independent predictor of ovarian reserve and thus was not considered a potential confounder in this analysis, however, none of the associations were altered with adjustment for season (see Table, Supplemental Digital Content 2).

When examined categorically, there was a tendency for decreasing 25(OH)D to be associated with decreased odds of high FSH (OR(CI): 0.81 (0.61, 1.1)), but this was not statistically significant. In contrast, women with vitamin D deficiency tended to have increased odds of high FSH, but the confidence interval was wide (Table 4). Insufficient 25(OH)D was associated with increased odds of low AMH (OR:1.8 (0.91, 3.6)), but the confidence interval was wide (Table 4). We also examined an alternative cutpoint for “high” AMH, 7.75 ng/ml (rather than 8.5ng/ml).⁵³ When high AMH was defined as > 7.75 ng/ml (rather than >8.5)⁵³, the estimate was unchanged, OR (CI): 1.8 (0.9, 3.6).

The associations between decreasing 25(OH)D and high FSH and insufficient 25(OH)D and low AMH were unchanged with adjustment for season; for FSH, OR(CI): 0.79 (0.59, 1.1), for AMH (1.8 (0.91, 3.6). Similarly, adjustment for physical activity (which was only available for a subset of the population, N=473) also did not alter results, high FSH, 0.79 (0.58, 1.1) and low AMH, 1.7 (0.82, 3.4).

A previous study reported that the association between 25(OH)D and AMH was limited to women who were at least 40 years of age³⁷. In our study the estimate for insufficient 25(OH)D in association with low AMH was stronger in this group, OR (CI): 5.2 (0.49, 55), but there were only 38 women in this age category.

Discussion

In this cohort of women of late reproductive age, there was little evidence of association between vitamin D and biomarkers of ovarian reserve. When the measures were categorized, there was some suggestion that insufficient 25(OH)D was associated with low AMH, but the confidence interval was broad and associations were not consistent across all analyses. There were no associations between 25(OH)D and inhibin-B.

AMH declines and FSH rises as a woman moves through the menopausal transition,^{19–21} and these hormones mark the later stages of reproductive aging such as early perimenopause, late perimenopause, and postmenopause.^{13, 22–26} The literature is mixed regarding vitamin D and ovarian reserve biomarkers. In one study, increasing vitamin D was associated with increasing AMH³⁷ and in another study with decreasing FSH.³³ However other observational studies report no association.^{38–41} The studies reporting no association are difficult to interpret as some do not report point estimates (only r-square or p-values) and it is not clear that age and BMI were accounted for in each analysis presented.^{38–41} All four of these null studies sampled women from fertility clinic populations, while the studies reporting associations^{33, 37} each sampled from a broader target population.

An intervention study of 33 women, reported that AMH varied across seasons, and that supplementation with cholecalciferol (vitamin D₃) was associated with apparent suppression of seasonal AMH changes.⁴² However, seasonal fluctuations were not found in a subsequent observational study.³⁸ In another intervention study, supplementation with active vitamin D (1,25 dihydroxyvitamin D) decreased AMH levels among women with PCOS (who typically have elevated AMH levels), but there was no effect among normally ovulatory women.⁵⁴

It is possible that there is racial or ethnic effect modification of the association between vitamin D and AMH or FSH. In two of the studies that found an association between vitamin D and either AMH or FSH the majorities of the study populations (~57%) were African-American^{33, 37}, while most of the studies that did not detect an association included primarily white women, including the results reported in the current analysis. One explanation for this could be that African-Americans have lower 25(OH)D, and it is possible that the association with AMH is strongest at low levels of 25(OH)D. Though one small study that reported no association included women with 25(OH)D levels as low as those seen in the two previously referenced studies, that study only reported the results as

unadjusted correlations.⁴⁰ Our analysis is the largest in the literature to date, and it was drawn from a community-based sample, but very few of the women were vitamin D deficient.

Conclusions

In this cohort of late reproductive-aged women, we did not find strong evidence of associations between 25(OH)D and biomarkers of ovarian reserve. We found some evidence that low 25(OH)D (<30 ng/ml) was associated with low AMH, but there were few women who had both of these characteristics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Time to Conceive participants included in this analysis.

Table 1

Ovarian reserve biomarkers and 25(OH)D, stratified by participant characteristics.

Variable	25(OH)D (ng/ml)			AMH (ng/ml)			Inhibin-B (pg/ml)			FSH (mIU/ml)		
	N	Geometric Mean	Geometric Range <i>a</i>	N	Geometric Mean	Geometric Range <i>a</i>	N	Mean	Standard Deviation	N	Geometric Mean	Geometric Range <i>a</i>
Age												
29 – 30	112	35.2	(27, 46)	112	4.0	(1.9, 8.4)	104	69.6	38.5	104	6.0	(3.8, 9.6)
31 – 32	157	34.2	(24, 48)	157	3.1	(1.2, 8.1)	144	88.2	47.3	144	6.5	(4.3, 9.8)
33 – 35	164	33.5	(26, 44)	164	2.6	(0.93, 7.3)	140	73.5	45.7	140	6.5	(3.8, 11)
36 – 40	106	33.9	(24, 47)	106	1.6	(0.53, 4.8)	93	70.0	42.3	93	6.5	(3.8, 11)
>40	22	36.1	(26, 51)	22	0.6	(0.12, 3.1)	21	59.0	51	21	8.8	(5.5, 14)
BMI												
<20	75	35.5	(27, 46)	75	2.3	(0.72, 7.4)	66	90.3	57.4	66	7.0	(4.4, 11)
20 – 25	292	35.8	(28, 47)	292	2.7	(0.93, 7.8)	260	73.0	42.9	260	6.3	(3.7, 11)
>25 – 30	110	32.4	(23, 45)	110	2.9	(1.1, 7.8)	99	74.9	42.4	99	6.4	(4.6, 9.0)
>30	84	30.4	(22, 43)	84	2.0	(0.65, 6.2)	77	61.8	36.4	77	6.7	(3.9, 11)
Race												
African-American	49	26.0	(17, 39)	49	2.7	(1, 7.3)	45	75.8	46.8	45	6.2	(3.1, 12)
White	438	36.2	(28, 47)	438	2.5	(0.86, 7.3)	388	73.2	44.5	388	6.5	(4.1, 10)
Other	74	29.6	(21, 41)	74	2.8	(0.93, 8.4)	69	77.1	44.1	69	6.6	(4.1, 10)
Smoking status												
Never	430	34.1	(24, 48)	430	2.6	(0.90, 7.5)	383	76.5	45.6	383	6.5	(4.1, 10)
Former	123	34.5	(27, 45)	123	2.5	(0.83, 7.5)	111	66.7	40.5	111	6.2	(3.9, 10)
Current	8	32.9	(25, 43)	8	1.6	(0.59, 4.3)	8	55.1	38.4	8	8.1	(4.8, 14)
Months since hormonal estrogen use												
One or less	32	40.1	(31, 52)	32	2.7	(1.1, 6.5)	27	62.2	69.2	27	5.2	(2.6, 10)
Two	20	37.4	(29, 49)	20	1.4	(0.4, 4.9)	19	65.2	42.8	19	7.4	(5.3, 10)
Three	41	37.3	(29, 48)	41	2.5	(0.76, 8.3)	37	72.5	45.7	37	6.9	(4.3, 11)
More than three	468	33.5	(24, 47)	468	2.6	(0.90, 7.5)	419	75.2	42.6	419	6.5	(4.1, 10)

^aThe geometric range is defined as: (geometric mean/geometric standard deviation, geometric mean x geometric standard deviation). Two-thirds of all observations lie within this range.

Table 2

Spearman correlation coefficients between 25(OH)D and ovarian reserve biomarkers

	Mean (SD)	25(OH)D	AMH	Inhibin-B
25(OH)D	36 (11)	--	--	--
AMH	4.0 (3.6)	-0.002 (-0.08, 0.08)	--	--
Inhibin-B	74 (45)	0.02 (-0.07, 0.10)	0.24 (0.16, 0.32) *	--
FSH	7.1 (3.1)	0.01 (-0.08, 0.10)	-0.36 (-0.43, -0.28) *	0.005 (-0.08, 0.09)

*
p<0.0001

Table 3Adjusted associations between 25(OH)D and ovarian reserve biomarkers.^a

	AMH, ^b Percent Difference (CI)	FSH, ^b Percent Difference (CI)	Inhibin-B ^c , Beta pg/ml (CI)
	N=561	N=502	N=502
25(OH)D, per 10ng/ml decrease	-3.3 (-12, 4.8)	0.53 (-3.8, 4.7)	-1.0 (-3.0, 5.1)
Annual mean 25(OH)D, per 10ng/ml decrease	-3.0 (-12, 5.1)	0.54 (-3.8, 4.7)	-1.2 (-2.8, 5.2)
Insufficient 25(OH)D ^d	-17 (-31, 0.2)	8.7 (-0.86, 19)	3.2 (-5.5, 12)

^a Adjusted for age, race, smoking history, BMI and recent use of hormonal birth control^b Natural log-transformed.^c Inhibin-B is not log-transformed.^d 25(OH)D less than 30 ng/ml, the Endocrine Society guideline for sufficiency, compared with at least 30 ng/ml

Table 4Adjusted associations between 25(OH)D and dichotomous measures of ovarian reserve biomarkers.^a

	AMH		FSH
	Low (<0.7 ng/ml) (N=561) OR (CI)	High (>8.5 ng/ml) (N=561) OR (CI)	High (>10 ng/ml) (N=502) OR (CI)
25(OH)D, per 10ng/ml decrease	1.0 (0.77, 1.4)	0.97 (0.73, 1.3)	0.81 (0.61, 1.1)
Annual mean 25(OH)D, per 10ng/ml decrease	1.0 (0.76, 1.4)	0.97 (0.73, 1.3)	0.82 (0.62, 1.1)
Insufficient 25(OH)D ^b	1.8 (0.91, 3.6)	0.71 (0.36, 1.4)	1.0 (0.52, 1.9)
Insufficient 25(OH)D ^c	1.5 (0.73, 2.9)	0.81 (0.42, 1.6)	0.90 (0.45, 1.8)

^a All regression models adjusted for age, race, smoking history, BMI and recent use of hormonal birth control^b Measured 25(OH)D less than 30 ng/ml, the Endocrine Society guideline for sufficiency, compared with at least 30 ng/ml^c Annual mean 25(OH)D less than 30 ng/ml, the Endocrine Society guideline for sufficiency, compared with at least 30 ng/ml