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Preconception serum 25 hydroxyvitamin D levels are associated with increased live birth and reduced pregnancy loss: a prospective cohort study

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Trial Registration: The trial was registered on [ClinicalTrials.gov](https://clinicaltrials.gov) (#NCT00467363).

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Summary

Background—Vitamin D deficiency during pregnancy is associated with adverse pregnancy outcomes, though the association between preconception levels and live birth is unknown. We aim to assess the relationship between preconception vitamin D and pregnancy outcomes among women with proven fecundity.

Methods—A secondary analysis of a prospective cohort from the EAGeR Trial (recruited from June 15, 2007 to July 15, 2011) which followed women for up to six cycles while attempting pregnancy and throughout pregnancy if they conceived. Serum 25-hydroxyvitamin D (25(OH)D) was measured at baseline (preconception) and 8 weeks gestation. Relative risk (RR) and 95% confidence intervals (CIs) for live birth, pregnancy, and loss were estimated using weighted log-binomial regression. Discrete time Cox proportional hazard models were used to calculate fecundability odds ratios.

Findings—46.6% of women (n=555/1191) had vitamin D concentrations ≥ 75 nmol/L and were more likely to achieve live birth (RR 1.15, 95% CI 1.02, 1.29) and clinical pregnancy (RR 1.10, 95% CI 1.01, 1.20), than women with 25(OH)D < 75 nmol/L. Among women who achieved pregnancy, preconception 25(OH)D, but not 8 weeks gestation, was associated with lower risk of pregnancy loss (preconception RR 0.88, 95% CI 0.77, 0.99; 8 weeks RR 0.98, 95% CI 0.95, 1.01, per 25 nmol/L). No association was observed with fecundability.

Interpretation—Sufficient preconception 25(OH)D was associated with higher live birth and pregnancy rates. Higher preconception, but not early pregnancy, concentrations were associated with reduced pregnancy loss.

Clinical Trials Registration—[ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00467363), number NCT00467363.

INTRODUCTION

Vitamin D is hypothesized to play a role in normal reproductive function as vitamin D receptors are found in reproductive organs including the ovary, uterus, placenta, hypothalamus, and pituitary gland.¹ Animal models show that vitamin D deficiency is associated with impaired ovarian function and decreased fertility.² However, studies in humans have been limited to women undergoing fertility treatments such as *in vitro* fertilization (IVF), and suggest that vitamin D concentrations in both serum and follicular

fluid are predictive of IVF success via impacts on endometrial receptivity and implantation.^{3,4} Further, given the role of vitamin D as an immune modulator, vitamin D is thought to help prevent pregnancy loss by facilitating maternal immune tolerance during pregnancy.^{5,6} Through these mechanisms vitamin D may influence fecundity, live birth, and pregnancy loss, though only two small studies have evaluated the role of preconception vitamin D on these outcomes among women not undergoing fertility treatment.^{7,8} Though several studies of pregnancy outcomes in non-IVF populations have suggested a beneficial role of vitamin D, these studies typically recruit during early pregnancy and are therefore limited in their ability to detect early pregnancy losses, and also fail to consider the importance of preconception health.

Therefore, the objective of this study was to evaluate associations between preconception serum 25-hydroxyvitamin D (25(OH)D) concentrations and fecundability, pregnancy loss, and live birth in women with proven fecundity, as well as serum 25(OH)D measured in early pregnancy on pregnancy loss.

MATERIALS & METHODS

Study Population

This was a prospective cohort from the Effects of Aspirin in Gestation and Reproduction (EAGeR) trial, a multi-center, block-randomized, double-blind, placebo-controlled clinical trial to evaluate the effect of preconception-initiated daily low dose aspirin (LDA, 81 mg) on reproductive outcomes in women with a history of pregnancy loss.^{9,10} The trial design and main findings have been described.¹⁰ In brief, LDA was not associated with live birth or fecundability overall, though was associated with higher probability of live birth among women with a single recent loss, and among women with low-grade inflammation.^{9,11} Participants were 1228 women attempting pregnancy, ages 18–40 years, with one to two prior pregnancy losses. This analysis included 1191 women with available preconception 25(OH)D concentrations (Figure 1). Exclusion criteria included a known history of infertility treatment, pelvic inflammatory disease, tubal occlusion, endometriosis, anovulation and polycystic ovarian syndrome, or uterine abnormality. Participants were recruited at four clinical sites in the United States from 2007–2011 and followed for six menstrual cycles while attempting pregnancy or throughout pregnancy if they conceived. Fertility monitors assisted visit scheduling and were used to time intercourse (Clearblue Easy Fertility Monitor; Inverness Medical Innovations, Waltham, MA, USA). Baseline data on demographic characteristics and reproductive history were obtained via questionnaire. The institutional review board at each study site (Salt Lake City, Utah; Denver, Colorado; Buffalo, New York; Scranton, Pennsylvania) and data coordinating center approved the trial protocol and all participants provided written informed consent prior to enrolling. The trial was registered with [ClinicalTrials.gov](https://clinicaltrials.gov), number NCT00467363.

Vitamin D Assessment

Serum samples were collected at baseline prior to randomization to LDA, and at 8 weeks gestation among women who conceived, and stored at –80°C until analysis. Combined concentrations of 25-hydroxyvitamins D2 and D3 (25(OH)D) were measured using the

25(OH)D ELISA solid phase sandwich enzyme immunoassay which is equipotent for both D2 and D3 (BioVendor R&D, Ashville, NC, USA) with demonstrated acceptability.¹² Further, immunoassays have not been shown to cross-react with 3-epi-25(OH)D3.¹³ The interassay laboratory coefficient of variations were 15.8 and 13.1% at mean concentrations of 38.7 and 103.8 nmol/L, respectively, for lyophilized manufacturer's controls, and 17% for an in-house pooled serum control. All values were above the lower limit of detection of 4.0 nmol/L.

Outcome Assessment

The outcomes of interest included hCG pregnancy, clinical pregnancy, time to pregnancy (TTP), pregnancy loss, and live birth. As previously described,¹⁴ pregnancy status was determined via positive hCG using daily first-morning urine collection and spot urine pregnancy tests at monthly visits. Clinical pregnancies were confirmed by ultrasound at 6–7 weeks' gestation. TTP was defined as the number of menstrual cycles until hCG pregnancy.

Pregnancy losses were defined as a 1) positive urine hCG pregnancy test at home or the clinical site followed by absence of signs of clinical pregnancy at the study ultrasound; 2) positive hCG from batched augmented urine testing followed by the absence of a positive pregnancy test at home or in the clinic;¹⁴ or 3) loss after ultrasound confirmation. Live birth was defined as a living infant born after 23 weeks' gestation.

Statistical Analysis

Women were classified as vitamin D insufficient (<75 nmol/L 25(OH)D) or sufficient (≥ 75 nmol/L 25(OH)D).¹⁵ Demographic and reproductive history characteristics were compared by baseline preconception vitamin D status using Student's t-tests and chi-square tests, as appropriate. Mean 25(OH)D concentrations were compared by pregnancy outcome, and frequency of outcomes by vitamin D status.

Relative risk (RR) and 95% confidence intervals (CIs) for live birth, hCG and clinical pregnancies, and pregnancy losses were estimated using log-binomial regression models adjusting for age, body mass index (BMI; kg/m²), race, physical activity, season of blood draw, treatment assignment, vitamin use, number of previous losses, and number of prior live births. Variables were included in the regression models based on prior literature and a priori assumptions regarding their associations with both vitamin D concentrations and pregnancy outcomes. For pregnancy loss, we restricted the analysis to women with hCG detected pregnancies, as pregnancy loss is conditional upon becoming pregnant; inverse probability weights were used to control for potential selection bias introduced by restricting to women who became pregnant as vitamin D may influence the probability of pregnancy. Weights included factors associated with the probability of pregnancy, including age, BMI, race, number of prior losses, physical activity, parity, season, treatment assignment, and 25(OH)D concentrations. Though vitamin D was measured prior to randomization and therefore should not be associated with the treatment, we included treatment assignment in the regression models to account for a potential influence of LDA. The weighted analysis also accounted for the potential role of LDA on pregnancy loss.

To assess the relationship between 25(OH)D and TTP, Cox proportional hazard regression models for discrete survival time, accounting for cycles trying prior to study entry (left truncation) and right censoring, were used to calculate fecundability odds ratios (FOR) and 95% CI. Models were adjusted for the same factors listed above.

We further assessed the relationship between 25(OH)D at 8 weeks gestation and pregnancy loss to evaluate potential effects on pregnancy losses occurring after 8 weeks. Models were adjusted for the same factors listed above, with weighted models adapted to account for pregnancy losses occurring prior to 8 weeks gestation.

Potential interactions with BMI and treatment assignment were considered though no significant interactions were observed; as such, overall model estimates are provided. Models evaluated continuous 25(OH)D concentrations (per 25 nmol/L) and were compared with models comparing vitamin D status (sufficient versus insufficient) to aid in clinical interpretation and comparison with other studies. The vitamin D cutoffs used in this analysis were based on levels designated by the Endocrine Society (<30 ng/mL equivalent to 75 nmol/L)¹⁵ and represent a cutpoint near the median (72.6 nmol/L). Of note, the Endocrine Society cutoffs were developed with regards to bone health, not for reproductive endpoints specifically. As such continuous models and other cutpoints, including quintiles, were evaluated, yielding similar results and supporting the findings of the continuous models. Multiple imputation with the fully-conditional specification method¹⁶ was used to address missing exposure and covariate data in all regression models, with models accounting for the imputation. Details are provided in the Appendix. Analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

Role of the Funding Source

The study sponsors played no role in the study design, collection, analysis, or interpretation of the data, in the writing of the report or in the decision to submit the paper for publication. The corresponding author had full access to the data in the study and had final responsibility for the decision to submit the paper for publication.

RESULTS

Of 1191 participants with measured baseline preconception 25(OH)D concentrations, 555 women (46.6%) had sufficient concentrations (≥ 75 nmol/L). The mean $\pm$ standard deviation 25(OH)D concentration in the study population was 76.9 ± 30.5 nmol/L with median (interquartile range, IQR) of 72.6 (32.2). Women who did not become pregnant were followed for a median of 5.6 months (IQR 1.5), and pregnant women for 9.6 months (IQR 2.7).

Women with sufficient preconception vitamin D concentrations on average had lower BMI, were more likely to be white, have more than a high school education, and be employed (Table 1). Vitamin D was not associated with age, vitamin use, smoking, or number of previous live births.

The percentage of hCG pregnancies, clinical pregnancies, and live births was higher among women with sufficient vitamin D, compared to insufficient (Table 2), with corresponding higher mean 25(OH)D concentrations among women with these outcomes (Table 3). After adjustment for potential confounders, women with sufficient vitamin D had a higher risk of clinical pregnancy (RR 1.10, 95% CI: 1.01, 1.20) and live birth (RR 1.15, 95% CI: 1.02, 1.29) (Table 4).

Though the percentage of pregnancy losses was similar between women with sufficient and insufficient vitamin D (Table 2), 25(OH)D concentrations were lower in women with pregnancy losses (Table 3). After adjustment for confounders, higher 25(OH)D concentrations were associated with reduced risk of pregnancy loss (RR 0.88, 95% CI: 0.77, 0.99 per 25 nmol/L; Table 4). Women with sufficient concentrations of 25(OH)D had increased fecundability in unadjusted models, but the association was attenuated after adjustment.

On average, 25(OH)D concentrations were similar between preconception and 8 weeks' gestation among the 624 women with both measures (paired t-test average difference 0.97 nmol/L, SD 27.36, $p=0.37$ for 8 week minus preconception). 69% of women ($n=428/624$) remained in the same vitamin D status category at both time points, whereas 13% ($n=82/624$) went from sufficient to insufficient levels and 18% ($n=114/624$) from insufficient to sufficient. As expected, this change was associated with changing season from study enrollment to becoming pregnant. Specifically, the mean difference in concentrations from preconception to 8 weeks gestation was 7.26 nmol/L (95% CI 3.29, 11.23) for those randomized in the spring (and who were more likely to conceive during the summer), compared to -4.87 (95% CI -8.91, -0.82) for those randomized in the fall (and more likely to conceive in the winter). We observed that 25(OH)D concentrations in early pregnancy were not associated with pregnancy losses after 8 weeks (RR 0.98, 95% CI: 0.95, 1.01 per 25 nmol/L).

DISCUSSION

Overall, preconception vitamin D sufficiency was associated with increased live birth and pregnancy rates among healthy women with a history of prior pregnancy losses and no diagnosis of infertility. Higher preconception 25(OH)D concentrations were associated with reduced risk of pregnancy loss, though early pregnancy concentrations were not associated with pregnancy loss after 8 weeks gestation. These results highlight the potential benefit of preconception vitamin D status in achieving and maintaining a healthy pregnancy among women who previously experienced pregnancy losses.

There is evidence that maternal vitamin D is associated with pregnancy outcomes, though results are mixed and several supplementation trials fail to show effects. Our results are consistent with many findings regarding vitamin D and reproductive outcomes among couples undergoing fertility treatment. For example, higher serum vitamin D concentrations predicted clinical pregnancy and implantation among infertile women undergoing IVF,³ and vitamin D deficiency (<50 nmol/L) was associated with lower pregnancy rates among non-Hispanic whites in a study of infertile women.¹⁷ A systematic review indicated that five of

eight studies examined reported improved ART outcomes after vitamin D screening and repletion using supplementation, whereas two reported no association and one reported a negative relationship.¹⁸ Of note is the wide range in supplement dosing and the variability in whether vitamin D was measured prior to supplementation. Among healthy women, one small study reported that women were less likely to conceive with inadequate or deficient vitamin D levels,⁷ and another study indicated no association.⁸ Our study joins a growing body of evidence that supports the importance of preconception vitamin D, and notably extends prior research in this area to include a large population of fertile couples attempting spontaneous conception.

Regarding pregnancy loss, our findings with preconception-measured 25(OH)D, but not with measures in early pregnancy, are consistent with a prospective cohort study of 1683 pregnant women, which found that women with 25(OH)D concentrations <50 nmol/L before 22 weeks' gestation had an increased hazard ratio for first trimester loss compared with concentrations ≥ 50 nmol/L.¹⁹ Additionally, one retrospective cross-sectional study found that a high proportion of women with recurrent pregnancy loss had vitamin D deficiency.²⁰ A similar lower level of 25(OH)D was observed among healthy women with a loss after 10 weeks, compared to women who did not have a loss.⁸ Indeed, we also observed that mean preconception vitamin D concentrations were lower among women with any loss occurring during the study, in addition to having prior losses. Moreover, our data indicate a reduced risk of pregnancy loss for increases in preconception, but not early pregnancy vitamin D concentrations, highlighting a potential beneficial role of preconception vitamin D status which may be useful for preconception counseling and family planning. This finding mirrors results from a recent clinical trial showing that supplementation during pregnancy was not associated with reduced risk of preeclampsia, though women with sufficient levels in early and late pregnancy were less likely to develop preeclampsia.²¹ Authors noted that initiating supplementation in early pregnancy may be too late and that screening should occur before pregnancy, especially in deficient populations. Thus, for outcomes such as pregnancy loss and preeclampsia, preconception supplementation may be needed. Studies show that prenatal vitamins containing 400 IU/d are not likely to be effective, and supplementation of 4000 IU/d is needed to achieve sufficient levels.²² Importantly, supplementation is also dependent on preconception weight, sunlight exposure, baseline serum levels, among other factors, and should be individualized for optimal outcomes.

Our findings of a null relationship between preconception vitamin D and TTP are consistent with prior work. A recent study among two preconception cohorts found no association, though was limited by evaluating vitamin D status using food frequency questionnaires, as opposed to direct serum measurement.²³ A case-control study reported no associations, though was based on retrospective report of TTP among pregnant women and only evaluated long (12-24 months) versus short (<12 months) TTP.²⁴ One study among healthy women noted a nonsignificant potential association with TTP.⁷ Thus, our data relating preconception vitamin D concentrations and TTP which was observed prospectively with careful, early observation are novel. Though our unadjusted results suggest a shorter TTP in women with sufficient concentrations, these findings were attenuated after adjustment. Given the paucity of data in this area and the positive trend observed, the relationship with fecundability

warrants further investigation to understand whether vitamin D may be more related to improvements in implantation and pregnancy incidence rather than ovulation or TTP.

The biological rationale for the role of vitamin D in influencing pregnancy outcomes is likely multifactorial. As fetal survival depends on immune tolerance by the mother, the immune modulating function of both 25(OH)D and the biologically active form 1,25(OH)₂D may play an important role in pregnancy through the maternal-fetal immunologic response.^{5,6} IVF studies suggest that vitamin D may improve endometrial receptivity and thus improve implantation, providing a potential biological explanation for the associations we observed. Specifically, 1,25(OH)₂D is thought to have anti-inflammatory properties at the interface of maternal and fetal tissue and has also been shown to regulate HOXA10, which is necessary for embryo implantation.²⁵ Importantly, vitamin D receptors have been identified in every organ involved in the hypothalamic-pituitary-ovarian (HPO) axis and the uterus, and has been positively associated with HPO axis function.¹ Though biological mechanisms regarding the effects of vitamin D on pregnancy are complex, evidence collectively points to an importance of preconception vitamin D status on improved pregnancy outcomes.

Our study had several strengths and limitations. First, the prospective design allowed us to explore the effects of preconception vitamin D concentrations on outcomes throughout pregnancy, including very early losses. However, measurement of 1,25(OH)₂D, as well as known genetic factors related to vitamin D status,²⁶ were unavailable, though such factors may shed further light on these findings and the potential causal relationships between vitamin D concentrations and pregnancy outcomes. The proven fecundity of our population with a history of one or two prior pregnancy losses also improves generalizability to reproductively healthy women seeking natural conception. The low numbers of pregnancy losses in this study were a limitation, but loss rates were similar to other reports,^{27,28} and given the close preconception follow up, we were able to include very early pregnancy losses that often go undetected. Vitamin D status of the male partners was not available, which may be of interest given studies showing associations between male vitamin D and live birth rates.²⁹ Additionally, while our study population was not specifically supplemented with vitamin D, 91% of participants (n=1089/1191) were taking a multivitamin and rates of insufficiency were similar between users and non-users (though the specific content of the multivitamins taken was unknown, most over the counter multivitamins include 400 IU of vitamin D³⁰). Lastly, it is important to note that our findings are only applicable to women with 25(OH)D concentrations within the range studied in our population (mostly 50 to 112 nmol/L), and as such we are limited in making comparisons with vitamin D deficient populations, as well as determining the optimal level for fertility. We also relied on sufficiency cutpoints from the Endocrine Society, and additional studies are needed to determine the most appropriate cutpoint for reproductive outcomes.

In conclusion, we found that higher vitamin D concentrations were associated with increases in live birth, clinical pregnancy, and hCG pregnancy rates, as well as decreased rates of pregnancy loss, highlighting the potential protective role of vitamin D in promoting pregnancy. Further research should investigate the possible effects of preconception vitamin D supplementation on chances of conceiving and risk of pregnancy loss among couples seeking natural conception.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Research in context

Evidence before this study

Vitamin D deficiency during pregnancy has been associated with adverse pregnancy outcomes. To establish the current evidence base for the association between vitamin D and live birth, pregnancy loss, and fecundability, we performed a literature review in February 2018 using the search terms “vitamin D” or “25-hydroxyvitamin D,” and “preconception,” “live birth,” “pregnancy loss,” “fecundity,” “fecundability,” “fertility,” “miscarriage,” or “time to pregnancy”. We identified several studies of preconception vitamin D and pregnancy outcomes in women undergoing *in vitro* fertilization (IVF). These studies typically show that higher preconception serum vitamin D concentrations are predictive of clinical pregnancy and implantation rates in women undergoing IVF, and a systematic review of vitamin D supplementation demonstrated mixed results. Two small preconception studies indicate mixed results on pregnancy and loss. With respect to pregnancy loss, previous studies have assessed vitamin D concentrations during early pregnancy, with no prior studies evaluating the role of preconception concentrations outside of IVF populations. Results from these studies suggest a potential role of vitamin D on loss. Specifically, a cross-sectional study found that a high proportion of women with recurrent pregnancy loss were vitamin D deficient, and a prospective cohort study in Denmark found that women with low serum concentrations of vitamin D had an increased risk of miscarriage in the first, but not second trimester.

Added value of this study

Though non-IVF populations have suggested a beneficial role of vitamin D, these studies typically recruit women during early pregnancy and are limited in their ability to record and evaluate early pregnancy losses and also fail to consider the importance of preconception health. Using data from a prospective cohort of women set in the EAGeR trial, we demonstrated that preconception vitamin D sufficiency is associated with higher live birth and pregnancy rates among healthy women with a history of prior pregnancy losses and no diagnosis of infertility. In addition, higher preconception vitamin D concentrations were associated with reduced risk of pregnancy loss, though concentrations at 8 weeks gestation were not associated with loss highlighting the importance of the preconception period. To our knowledge, this is the largest study to investigate associations between preconception vitamin D levels and fecundability, pregnancy loss, and live birth in fecund women with a prospective and rigorous outcome assessment. These results add valuable insight into the potential impacts of vitamin D during the preconception period for fertile or subfertile couples attempting spontaneous conception.

Implications of all the available evidence

Our findings suggest a protective role for vitamin D in promoting pregnancy, which could have clinical implications for fertile couples attempting to conceive. Further research is needed to investigate the possible effects of interventions with preconception vitamin D supplementation on chances of attaining pregnancy and pregnancy loss risk among

couples seeking natural conception, as well as the optimal levels of intake for reproductive outcomes.

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EAGeR CONSORT Flow Diagram

Figure 1. EAGeR trial CONSORT flow diagram

*The trial included 2 pre-specified eligibility strata: original (women with 1 loss at less than 20 weeks' gestation during the previous year) and expanded (women with 1-2 previous losses, with no restrictions on gestational age or time of loss).

Table 1

Participant characteristics by preconception vitamin D status in the EAGeR Trial, 2007-2011.

	Total Cohort	Insufficient Preconception Vitamin D <75 nmol/L*	Sufficient Preconception Vitamin D ≥75 nmol/L	P-value
N	1191	636 (53)	555 (47)	
Preconception 25(OH)D, nmol/L	76.9 ± 30.5	56.7 ± 12.2	99.8 ± 28.5	
Age, years	28.7 ± 4.8	28.6 ± 4.8	28.9 ± 4.8	0.36
BMI, kg/m ²	26.3 ± 6.6	27.8 ± 7.3	24.5 ± 5.1	<0.0001
Race/ethnicity				
White	1128 (95)	586 (92)	542 (98)	<0.0001
Non-white	63 (5)	50 (8)	13 (2)	
Education				
> High School	1033 (87)	542 (85)	491 (89)	0.10
High school	158 (13)	94 (15)	64 (12)	
Season of blood collection				
Winter (Dec-Feb)	268 (23)	147 (23)	121 (22)	0.40
Spring (Mar-May)	342 (29)	192 (30)	150 (27)	
Summer (Jun-Aug)	270 (23)	134 (21)	136 (25)	
Fall (Sep-Nov)	311 (26)	163 (26)	148 (27)	
Vitamin Use				
No folic acid - no vitamins	86 (7)	43 (7)	43 (8)	0.073
No folic acid - take vitamins	143 (12)	64 (10)	79 (14)	
Yes	946 (81)	517 (83)	429 (78)	
Smoking in past year				
Never	1033 (88)	551 (87)	482 (87)	0.53
<6 times/week	85 (7)	42 (7)	43 (8)	
Daily	63 (5)	37 (6)	26 (5)	
Household income (annual)				
\$100,000	470 (40)	261 (41)	209 (38)	0.0004
\$75,000-\$99,999	147 (12)	63 (10)	84 (15)	
\$40,000-\$74,999	174 (15)	75 (12)	99 (18)	
\$20,000-\$39,999	307 (26)	179 (28)	128 (23)	
\$19,999	92 (8)	57 (9)	35 (6)	
Employed				
Yes	871 (73)	448 (70)	423 (76)	0.017
No	278 (23)	158 (25)	120 (22)	
Missing	42 (4)	30 (5)	12 (2)	
Time from last loss to randomization (months)				
4	631 (54)	326 (52)	305 (56)	0.10
5-8	214 (18)	107 (17)	107 (20)	
9-12	98 (8)	59 (9)	39 (7)	
>12	229 (20)	135 (22)	94 (17)	

	Total Cohort	Insufficient Preconception Vitamin D <75 nmol/L*	Sufficient Preconception Vitamin D ≥75 nmol/L	P-value
Number of previous pregnancies, not including losses				
0	506 (43)	259 (41)	247 (45)	0.30
1	423 (36)	224 (35)	199 (36)	
2	241 (20)	141 (22)	100 (18)	
3	21 (2)	12 (2)	9 (2)	
Number of previous live births				
0	550 (46)	279 (44)	271 (49)	0.053
1	433 (36)	231 (36)	202 (36)	
2	208 (18)	126 (20)	82 (15)	
Alcohol consumption in past year				
Often	26 (2)	14 (2)	12 (2)	0.0017
Sometimes	368 (31)	168 (27)	200 (36)	
Never	782 (67)	445 (71)	337 (61)	
Physical activity				
Low	310 (26)	183 (29)	127 (23)	0.041
Moderate	491 (41)	245 (39)	246 (44)	
High	390 (33)	208 (33)	182 (33)	
Low-dose aspirin treatment arm	598 (50)	313 (49)	285 (51)	0.46

BMI, body mass index

* Vitamin D concentrations ranged from 12.5 to 358.4 nmol/L.

Table 2

Frequencies of outcomes by preconception vitamin D status in the EAGeR Trial, 2007-2011.

Vitamin D Status	Insufficient Preconception (<75 nmol/L) N (%)	Sufficient Preconception (≥75 nmol/L) N (%)	Difference Mean (95% CI)	P-value
hCG pregnancy	382 (60)	392 (71)	-11% (-16%, -5%)	0.0001
Clinical pregnancy	345 (54)	364 (66)	-11% (-17%, -6%)	<0.0001
Any pregnancy loss	97 (15)	88 (16)	-0.6% (-5%, 4%)	0.77
Live birth	280 (44)	298 (54)	-10% (-15%, -4%)	0.0009

Table 3

Mean preconception vitamin D concentrations (nmol/L) by pregnancy outcome status among women in the EAGeR Trial, 2007-2011.

	Pregnancy Outcome Absent	Pregnancy Outcome Present	Difference	
	Mean $\pm$ SE	Mean $\pm$ SE	Mean (95% CI)	p-value
hCG pregnancy	73.6 $\pm$ 31.0	78.4 $\pm$ 30.0	-4.7 (-8.5, -1.2)	0.008
Clinical pregnancy	73.6 $\pm$ 30.2	78.9 $\pm$ 30.2	-5.2 (-8.7, -1.7)	0.003
Any pregnancy loss *	79.6 $\pm$ 31.7	74.6 $\pm$ 23.0	5.2 (0.2, 10.2)	0.04
Live birth	73.9 $\pm$ 28.7	79.9 $\pm$ 31.7	-5.7 (-9.2, -2.5)	0.0009

* among women with an hCG pregnancy

Table 4

Associations between preconception and early pregnancy vitamin D and hCG pregnancy, clinically confirmed pregnancy, time to pregnancy, any pregnancy loss, and live birth.

			Sufficient 75 vs Insufficient <75 nmol/L	Continuous (per 25 nmol/L)
hCG pregnancy (preconception 25(OH)D)	Unadjusted	RR (95% CI)	1·10 (1·01, 1·19)	1·02 (0·99, 1·05)
	Adjusted ¹	RR (95% CI)	1·05 (0·99, 1·10)	1·01 (0·99, 1·03)
Clinically confirmed pregnancy (preconception 25(OH)D)	Unadjusted	RR (95% CI)	1·14 (1·04, 1·25)	1·03 (1·00, 1·07)
	Adjusted ¹	RR (95% CI)	1·10 (1·01, 1·20)	1·02 (0·99, 1·05)
Time to pregnancy ² (preconception 25(OH)D)	Unadjusted	FOR (95% CI)	1·25 (1·06, 1·48)	1·05 (0·98, 1·13)
	Adjusted ¹	FOR (95% CI)	1·13 (0·95, 1·34)	1·00 (0·93, 1·08)
Any pregnancy loss ³ (preconception 25(OH)D)	Unadjusted	RR (95% CI)	0·90 (0·69, 1·16)	0·88 (0·77, 0·99)
	Adjusted ¹	RR (95% CI)	0·91 (0·70, 1·18)	0·88 (0·77, 0·99)
(8 weeks gestation 25(OH)D) ⁴	Unadjusted	RR (95% CI)	0·83 (0·54, 1·30)	0·98 (0·95, 1·00)
	Adjusted ¹	RR (95% CI)	0·98 (0·95, 1·01)	0·98 (0·95, 1·01)
Live birth (preconception 25(OH)D)	Unadjusted	RR (95% CI)	1·16 (1·03, 1·30)	1·06 (1·02, 1·10)
	Adjusted ¹	RR (95% CI)	1·15 (1·02, 1·29)	1·05 (1·01, 1·10)

CI, confidence interval; FOR, fecundability odds ratio; RR, risk ratio

¹ Models adjusted for age, body mass index, race, physical activity, season of blood draw, treatment assignment, vitamin use, number of prior losses, and number of prior live births

² Models for time to pregnancy estimate FOR and 95% CIs

³ Models for pregnancy loss are weighted to control for potential selection bias introduced by restricting to women with any hCG detected pregnancy as pregnancy loss is conditional upon becoming pregnant.

⁴ Models for evaluating 25(OH)D at 8 weeks gestation and pregnancy loss are weighted to control for potential selection bias introduced by restricting to women with an hCG detected pregnancy that lasted at least 8 weeks.