Serum levels of 25-hydroxyvitamin D and time to natural pregnancy

Running title: Vitamin D and fertility

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Key words: fecundity / pregnancy / vitamin D

Abstract

Aim: Aim of the present study was to evaluate whether natural fertility is related to serum 25-hydroxyvitamin D levels.

Methods: A nested case-control study was designed from a prospective cohort of pregnant women undergoing first trimester screening for aneuploidies. Cases were women seeking pregnancy for 12-24 months. Controls were the subsequent age-matched women conceiving in less than one year. We excluded women aged ≥ 40 years or < 18 years, those assuming supplementary products that included vitamin D before or during pregnancy, those with irregular menstrual cycles or known causes of subfertility, those conceiving through assisted reproductive techniques or requiring ovarian stimulation and those who were overweight or obese. A quantitative detection of serum 25-hydroxyvitamin D and patients' interview were performed.

Results: Seventy-three cases and 73 matched controls were selected. The mean ± SD serum 25-hydroxyvitamin D was 21.2 ± 6.8 and 19.7 ± 7.3 ng/ml, respectively (p=0.16). The number (%) of women with serum levels < 20 ng/ml (vitamin D insufficiency) was 34 (47%) and 37 (51%), respectively (p=0.73). The adjusted OR of longer time to pregnancy in women with vitamin D insufficiency was 0.84 (95%CI: 0.42-1.66).

Conclusions: Our study does not support a crucial role of 25-OH-vitamin D in natural fertility.

Introduction

There is growing evidence that vitamin D influences reproductive mechanisms in human [1-3]. In rodents, animals knock-out for the Vitamin D receptor and those fed with vitamin D deficient diet have a reduced fertility [4]. However, up to now, data in human is not univocal. Some authors reported worse oocyte competence and embryo implantation in women with deficient serum levels of vitamin D undergoing IVF [5-8] but others did not [9-12]. Interventional studies testing the potential benefits of vitamin D supplementation prior to IVF are ongoing but yet unavailable [13].

IVF is a valuable and largely used model to investigate factors affecting fertility in women. It provides reliable data on oocyte quality and embryo implantation. However, IVF may not properly reveal all the potential detrimental effects of vitamin D deficiency on natural fertility. Of potential relevance here is the impact on the cervix function, the sperm transport (and possibly function), the tubal function and the endocrinological feedbacks. For this reason, we deemed valuable to investigate the impact of serum levels of vitamin D on natural fecundity. To elucidate this aspect, we evaluated whether serum levels of 25-hydroxyvitamin D (25-OH-vitamin D), the form of vitamin D reflecting the store of the vitamin, may affect time to natural pregnancy. To this aim, we recruited natural pregnant women referring for routine first trimester screening for aneuploidies and compared serum levels of 25-OH-Vitamin D in those who achieved pregnancy in less or more than one year.

Materials and methods

Women referring to our Institution for routine first trimester screening for aneuploidies (11^{+0}-12^{+6} weeks of gestation) since January 2012 were invited to participate to a large prospective study ultimately aimed at assessing the impact of vitamin D levels on fertility and obstetrical complications. The program of aneuploidies screening is open to any woman age in our Institution but, for this nested case-control study, we excluded women aged ≥ 40 years (to reduce the

confounding effect of older age) or < 18 years. Moreover, we excluded those assuming supplementary products that included vitamin D before or during pregnancy, those with irregular menstrual cycles or known causes of subfertility (male factor, tubal factor or endometriosis), those conceiving through assisted reproductive techniques or requiring ovarian stimulation and those who were overweight or obese (BMI > 25 Kg/m^2). Cases were women who sought pregnancy for 12-24 months. Controls corresponded to the following age-matched women who become pregnant in less than one year. A matched design and analysis was decided to overcome the important impact of age and seasonality. Women agreeing to participate provided a blood sample. Blood samples were allowed to clot at room temperature and then centrifuged at 2,000 g for 10 minutes. The resulting serum was stored at -20 °C until assayed. The assessments were performed in three distinct experiments thawing a similar number of matched case-controls. The quantitative detection of total 25-OH-vitamin D levels was performed using a commercially available kit based on a chemiluminescence technology (DiaSorin). The intra- and inter-assay coefficients of variations were below 10% and 15%, respectively. Vitamin D insufficiency and deficiency corresponded to serum levels of 25-OH-vitamin D <20 ng/ml and <10 ng/ml, respectively [14].

Statistical analyses were performed using SPSS 18.0 software (Chicago, IL.). Data was compared using unpaired or paired Student-t test, non-parametric Wilcoxon test, Fisher Exact test or McNemar test, as appropriate. P values below 0.05 were considered statistically significant. A logistic regression analysis including variables found to differ at univariate analysis was used to calculate the adjusted Odds Ratio (OR). The sample size was calculated based on a paired study design, setting type I and II errors at 0.05 and 0.20, respectively, assuming as clinically relevant a 15% reduction in serum 25-OH-vitamin D in cases (thus from the expected 20 ng/ml to 17 ng/ml) and referring to an expected SD of the difference when matching for study period of 9 ng/ml, as calculated on our own preliminary data [15]. On these bases, we calculated that 73 cases and 73

controls were necessary. The adequate number of women to be recruited was fulfilled within June 2013.

Results

Baseline characteristics of the 146 included women are shown in Table 1. As a consequence of not reassuring/positive results of the screening test, invasive prenatal diagnosis was requested by 6 (8%) and 4 (5%) women among cases and controls, respectively (p=0.75). One woman aged 35 years belonging to the group of controls had a definite diagnosis of aneuploidy and a spontaneous abortion (p=1.00). The rate of obstetrics complications in cases (n=73) and controls (n=72) were mostly similar. Preterm birth occurred in 8 (11%) and 8 (11%) women (p=1.00), hypertensive disorders in 6 (8%) and 8 (11%) women (p=0.59), gestational diabetes mellitus in 6 (8%) and 5 (7%) women (p=1.00) and cesarean delivery was required in 27 (37%) and 26 (36%) women (p=1.00), respectively. Excluding the three twin pregnancies, the newborn weights resulted also similar (3,248 ± 593 g and 3,304 ± 348 g, respectively, p=0.49).

Serum 25-OH-vitamin D does not differ between cases and controls (Figure 1). The mean ± SD was 21.2 ± 6.8 and 19.7 ± 7.3 ng/ml, respectively (paired *t*-test, p=0.16; unpaired *t*-test p=0.20). The mean (95%CI) difference was + 1.5 (-0.6 / +3.6) ng/ml for cases compared to controls. The number (%) of women with serum levels < 20 ng/ml was 34 (47%) and 37 (51%), respectively (McNemar test, p=0.73; Fisher Exact test, p=0.74). The OR of longer time to pregnancy in women with vitamin D insufficiency was 0.85 (95%CI: 0.44-1.62). The OR adjusted for men age and parity was 0.84 (95%CI: 0.42-1.66). The number (%) of women with serum levels <10 ng/ml was 3 (4%) and 6 (8%), respectively (McNemar test, p=0.45; Fisher Exact test, p=0.49). The crude and adjusted ORs of subfertility in women with vitamin D deficiency was 0.48 (95%CI: 0.12-1.99) and 0.70 (95%CI: 0.15-2.95), respectively. Correlation for the whole population (n=146) between time to pregnancy and serum 25-OH-vitamin D was unremarkable (Pearson R^2=2%, p=0.07). The analyses were

repeated excluding the woman who had a spontaneous abortion (and her matched case) but results were extremely similar (data not shown)

Discussion

Our results contrast with the available emerging evidence suggesting a detrimental effect of vitamin D deficiency on woman fertility. They are however in agreement with a recent study comparing women who did or did not conceive naturally within six months [16]. Our data is more robust in this regard considering that a threshold of 12 months is generally considered more appropriate to define a condition of difficulty in conception [17]. Our study design may be at prima faces arguable because we recruited both cases and controls among pregnant women. In this regard, it has however to be highlighted that time to pregnancy is widely used in the literature to investigate factors that may influence fertility. This outcome was used in pivotal studies evaluating whether fertility varied over historical periods [18] and demonstrating the detrimental effect of several factors including women age, male age, obesity and smoking [19]. Noteworthy, time to pregnancy was also used to sort out the currently used WHO reference ranges for semen [20,21]. The main advantage of this study design is preventing confounders. Accordingly, in our study, recorded baseline characteristics of cases and controls were similar with the expected exceptions of men age and parity [19].

On the other hand, we believe that some caution is warranted prior to definitely conclude for a lack of an association between vitamin D insufficiency and infertility for the following reasons. Firstly, serum levels of 25-OH-vitamin D were generally low in both cases and controls. Only a small minority of women (7 cases and 7 controls, data not analyzed) have actually levels above the recommended optimal threshold of 30 ng/ml [22]. In other worlds, our population allowed us to assess whether very low levels could prolong time to pregnancy but did not consent us to assess whether appropriate serum 25-OH-vitamin D levels would shorten time to pregnancy. Secondly, 25-OH-vitamin D varies during the year. This should not have markedly affected our conclusions since

serum levels of the vitamin were shown to remain mainly stable over time relative to other members of a population. Large cohort studies that have monitored 25-OH-vitamin D indeed showed that those who are low (or high) at baseline tend to remain low (or high) during multiyear follow-up [23-25]. On the other hand, it has to be recognized that an optimal (but complex and very costly) study design would be to prospectively recruit women seeking pregnancy and to regularly monitor serum 25-OH-vitamin D. Thirdly, the pathogenetic mechanisms linking vitamin D to fertility have not yet been clarified. It cannot be excluded that adequate levels may be of relevance only in particular subgroups of women to compensate for other insufficiencies. If so, our study would be underpowered. Noteworthy, since women were recruited at the end of the first trimester, our data is not informative on the risk of miscarriage. Fourthly, one may claim that pregnancy-related modifications may have diluted the differences. Serum 25-OH-vitamin D may modify during initial pregnancy and women habits may also change. However, we do not estimate that this may have significantly impacted on our results. Indeed, available data did not show significant change of serum 25-OH-vitamin D with the advent of pregnancy [16]. Moreover, even if some dietary changes at the beginning of pregnancy are likely, there is no reason to hypothesize significant changes in sun exposure habits during initial pregnancy. Noteworthy, vitamin D reserve is for the most part due to sun exposure (90%) compared to dietary intake (10%) [26]. Finally, even if serum levels would be influenced by the onset of pregnancy, this confounder is expected to have a similar impact in the two study groups.

Overall, our study does not support a crucial role of 25-OH-vitamin D in natural fertility. Further studies and in particular interventional RCTs are however warranted to draw definite conclusions.

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Figure legend

Figure 1:   Serum levels of 25-OH-vitamin D in subfertile (black columns) and fertile (grey columns) women according to study period. None of the tested differences resulted statistically significant.

Table 1. General characteristics of women with subfertility (cases) and controls.

Characteristics	Cases n=73	Controls n=73	p
Woman age (years)	32.8 ± 3.7	32.7 ± 3.8	0.96
Partner age (years)	36.6 ± 4.9	35.0 ± 4.0	0.04
BMI (Kg/m^2)	22.1 ± 3.4	21.8 ± 3.4	0.62
Ethnicity			1.00
Caucasian	70 (96%)	71 (97%)	
Other	3 (4%)	2 (3%)	
Marital status			0.72
Married	51 (70%)	48 (66%)	
Unmarried	22 (30%)	23 (34%)	
Scholarity			0.42
Elementary-middle school	11 (15%)	6 (8%)	
High School	29 (40%)	33 (45%)	
College-university	31 (42%)	34 (47%)	
Previous pregnancies	31 (42%)	37 (51%)	0.41
Previous live births	13 (18%)	26 (36%)	0.02
Menstrual cycle lenght (days)	28 ± 2	29 ± 2	0.64
Smoking prior to pregnancy	16 (22%)	11 (15%)	0.39
Twin pregnancy	1 (1%)	2 (3%)	1.00
Gestational age at first trimester screening (weeks)	11.5 ± 0.5	11.6 ± 0.6	0.34

Data is presented as mean ± SD or Number (%) as appropriate.
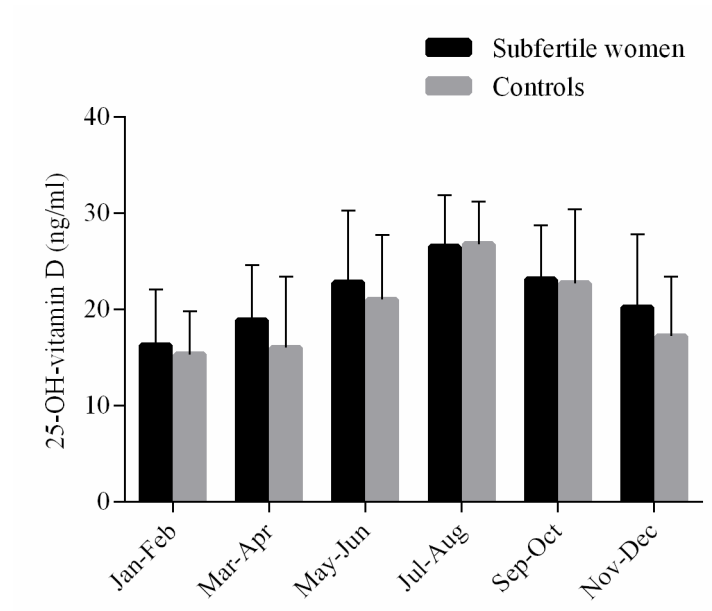
P values from unpaired comparisons are reported.

Figure 1

Figure 1