Article details: 2012-0032	
Title	Effect of vitamin D status on clinical pregnancy rates following in vitro fertilization
Authors	Kimberley Garbedian, Miranda Boggild, Joel Moody, Kimberly E Liu
Reviewer 1	Arthur Leader
Institution	Department of Obstetrics and Gynecology, University of Ottawa, Ottawa, Ont.
General comments	Pages 3 and 4 cites multiple definitions of deficiency, insufficiency and sufficiency yet the criteria for the study are sufficiency and insufficiency. The`study is too heterogeneous as it has too many variables between too many groups to attribute success or failure to the vit D levels alone. Would suggest a study in women with same protocol in women under 36 yrs of age. There was an equal incidence of sufficiency and insufficiency but a low percentage of deficiency contrary to the authors conclusion.
Reviewer 2	Elizabeth Bertone Johnson
Institution	Public Health, University of Massachusetts
General comments	This article presents results from a prospective analysis of vitamin D status and outcomes following IVF. There is ample evidence supporting a role for vitamin D in reproductive outcomes and pregnancy success following IVF, but relatively few studies have directly addressed these relations in clinical populations. This study thus contributes substantially to knowledge of this important issue. The study is well designed and the paper is well written. My main comment concerns the categorization of 25OHD levels and the importance of Figure 1. (First, as a side note- I am surprised by the range of percentages shown in Figure 1, which don't seem to coincide with those in Table 2. Mean clinical pregnancy rates in Table 2 are approx. 35% vs. 53% between groups. However, in Figure 1, clinical pregnancy rates are approx. 14% vs. 15% vs. 32% between groups. Why are these so much lower across the board than in Table 2?) Assuming that the relations shown in Figure 1 are accurate- While the results presented in Table 2 show significant differences in IVF outcomes between women with 25OHD levels of <75 nmol/L vs. ≥75nmol/L, the results presented in Figure 1 suggest that this differences is actually driven by even higher 25OHD levels. Figure 1 suggests that there may be a threshold effect, as there is no difference in success rates of women in the 1st and 2nd tertile, but significant higher rates among women in the 3rd tertile. The 25OHD levels in this group are substantially higher than the ≥75nmol/L cutoff used in the other analyses. This is a potentially important clinical finding, as it suggests that the target serum 25OHD level for IVF populations may be considerably higher than merely "sufficiency." This finding isn't entirely consistent with the main interpretation presented by the authors in their abstract and discussion, and should be explored in greater detail. In particular, it would be beneficial for the authors to use the tertile categorization of 25OHD in their other analyses also. If there is a threshold effect rather than a linear relation, then the use of continuous 25OHD levels in the multivariable regression analyses (Table 3) will not accurately capture the association of vitamin D on pregnancy success. My other comments are minimal: • Tables should be titled and include more explanatory footnoting, especially Table 3. It is currently unclear that all variables are included in the adjusted models. • Given that most previous studies of this topic have evaluated follicular fluid 25OHD levels, and there has been considerable inconsistency in their findings, additional discussion of the relation of serum and follicular fluid 25OHD levels is warranted. Are levels highly correlated in these tissues? Are follicular fluid levels likely more physiologically relevant for reproductive outcomes than serum levels? Please expand discussion of this issue.
Author response	**Response to Reviewer 1** Vitamin D tertiles First, I would like to address reviewer one's concerns regarding the different clinical pregnancy values shown in Figure 1 and listed in Table 2. Simply they are different because they are reflecting different things. Specifically, figure one reflects vitamin D cut off values as tertiles of serum vitamin D (lowest 34.2-58.0, middle 64.7-77.1, and Highest 84.1-118.1 nmol/L). Whereas, clinical pregnancy rates in Table 2 reflect the Canadian guideline cut off values for serum vitamin D (deficiency <25 nmol/L,

	insufficiency 25-74 nmol/L, and sufficiency (> or = 75 nmol/L) (5). Second, reviewer one made an excellent observation that the significant differences seen in clinical pregnancy rates may not be simply explained by vitamin D sufficiency vs. insufficiency, but there may be a threshold effect of vitamin D. However, our group feels that representing our data as both tertiles and tables may be confusing to the reader. The tables present the most clear and clinically relevant information ie. the cut-off references for vitamin D sufficiency, insufficiency, and deficiency. Therefore, we have removed the tertile information and figure from the manuscript. Follicular Fluid vs. Serum Vitamin D levels The Ozkan et al. study measured vitamin D levels in both serum and follicular fluid. The levels were highly correlated ($r=0.94$, $p<0.001$) and levels of 25 OH-D are well known to be reliable reflectors of body stores of the vitamin. Thus, there does not appear to be an advantage to measuring follicular fluid vitamin D over serum levels. We chose to use measure serum vitamin D levels for a number of reasons. First, cut off values for vitamin D deficiency, insufficiency, and sufficiency in the literature were determined via serum vitamin D levels. In addition, serum vitamin D levels are easy to obtain. Follicular fluid levels of vitamin D can only be obtained at the time of oocyte transfer and can only be obtained in fertility patients undergoing IVF. Thus, studies on vitamin D and fertility should focus on serum levels, as they can be obtained and monitored in all fertility patients. In addition, serum vitamin D levels can be obtained prior to IVF oocyte retrieval and in infertility patients not pursuing IVF and therefore have more clinical utility than follicular fluid levels. *the limitations section has been modified to include this explanation. **Response to Reviewer 2** Please refer to the above comments regarding vitamin D cut offs. Infertility populations are heterogeneous, as there are numerous indications for IVF (ovulatatory, tubal, uterine, male factor). Our exclusion criteria excluded IVF indications that are known in the literature to significantly impact implantation such as uncorrected mullerian anomalies and hydrosalpinx. For a study on infertility to be generalizable it should include all reproductive age women (ie. women 18-42 years) with diverse infertility etiologies. A large portion of IVF patients are over the age of 35 years, as less aggressive forms of IVF are more likely to be successful in women <35 years. Table 1 demonstrates that the patient characteristics, specifically the age and indication for IVF, were not statistically different between study groups. In addition, multivariate regression analysis confirmed that vitamin D status was an independent predictor of clinical pregnancy. Contrary to reviewer #2 comments, we did not anticipate a large number of vitamin D deficient patients. Our study population includes reproductive age healthy women that are taking prenatal vitamins containing a minimum of 400 IU of vitamin D. Our findings are in line with other Canadian population based vitamin D prevalence studies cited in the manuscript. The patients in our study with vitamin D deficiency had several risk factors including darker skin and higher BMI. In closing, I would like to thank the review committee for reading this lengthy appraisal response. As reviewer one alluded to there is longstanding support of the role of vitamin D in reproduction in the basic science and animal literature. However, there are very few studies looking at vitamin D status in fertility population and clinical populations. Therefore, pilot prospective studies such as this one are important to expanding the initial knowledge and prompting larger randomized controlled trials in this area.