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Vitamin D and ovarian reserve: making clinical decisions

Sir,

We read with interest the commentary by McLennan and Pankhurst (2016), regarding the inadequate design of studies evaluating the role of anti-Mullerian hormone (AMH) in human reproduction, and especially in the field of vitamin D.

Provocatively speaking, according to the authors' comments, the negative results of all previous studies are false, mainly due to the inappropriate methodological approach. Nevertheless, we need to highlight that the conclusions driven by the authors are based on a single study showing a significant positive correlation ($r = 0.36$, P value = 0.004) between seasonal changes in AMH and vitamin D levels in 33 premenopausal women (Dennis *et al.*, 2012). These findings are a typical example of a misconception regarding correlation coefficients, suggesting that significant results (P value < 0.05) imply a strong association, regardless of the low r value (Bland and Altman, 1994). What is clearly established is that a regression coefficient of 0.36, as demonstrated in the above-mentioned study, does not signify a strong association at all. On the contrary, this only implies a weak correlation between the two variables, which of course is of a very limited clinical value.

This is clearly shown if we also consider the absolute numbers in the study by Dennis *et al.* (2012), in which an 18% decrease in seasonal AMH levels has been shown, by analyzing stored blood samples, randomly acquired irrespective of patients' menstrual cycle day. However, even in such conditions, an 18% decrease cannot be attributed solely to an effect of vitamin D on AMH levels, simply because the effect of inter- and intra-cycle variability was not taken into consideration and no adjustment has been made for. Thus, if we consider that previous well-conducted longitudinal studies, identified inter-individual AMH variability, secondary to individual fluctuations of AMH levels, which can be as high as 11% (Fanchin *et al.*, 2005; van Disseldorp *et al.*, 2010), it is really questionable whether this decrease in AMH levels, identified by Denis, is indeed evident, or may simply reflect (at least partially) the inter-/intra-cycle variability of AMH in the serum (La Marca *et al.*, 2013).

This may also be the reason behind the completely diverse results found by a recent prospective longitudinal study, not cited by the authors, which not only did not find a positive association between changes in AMH and vitamin D levels, but in fact demonstrated that vitamin D supplementation either significantly reduces (in polycystic ovary syndrome patients) or does not affect at all (in normoovulatory women) the serum AMH levels (Irani *et al.*, 2014).

Given that all but one of the available studies in the field did not demonstrate any significant association between AMH and vitamin D, there is clearly insufficient evidence to suggest routine assessment of vitamin D status and vitamin D supplementation of deficient patients in an attempt to delay ovarian reserve loss. Based on the available literature, it would be irrelevant to support an association between vitamin D and ovarian reserve markers, unless future studies replicate authors' findings (Ioannidis, 2005). Furthermore, we consider that the role of the cross-sectional studies should not be underestimated, especially in case of correct design and appropriate sample size (Drakopoulos *et al.*, 2016).

Vitamin D is indeed one of the 'talking points' of the last decade, attracting new studies, reviews and meta-analyses, not only restricted to our field. Nonetheless, it seems that in the end, firm universal conclusions about its benefits cannot be drawn (Theodoratou *et al.*, 2014).

References

Bland JM, Altman DG. Correlation, regression, and repeated data. *BMJ* 1994;**308**:896.

Dennis NA, Houghton LA, Jones GT, van Rij AM, Morgan K, McLennan IS. The level of serum anti-Mullerian hormone correlates with vitamin D status in men and women but not in boys. *J Clin Endocrinol Metab* 2012; **97**:2450–2455.

Drakopoulos P, van de Vijver A, Schutyser V, Milatovic S, Anckaert E, Schiettecatte J, Blockeel C, Camus M, Tournaye H, Polyzos NP. The effect of serum vitamin D levels on ovarian reserve markers: a prospective cross-sectional study. *Hum Reprod* 2016;**32**:208–214.

Fanchin R, Taieb J, Lozano DH, Ducot B, Frydman R, Bouyer J. High reproducibility of serum anti-Mullerian hormone measurements suggests a multi-staged follicular secretion and strengthens its role in the assessment of ovarian follicular status. *Hum Reprod* 2005;**20**: 923–927.

Ioannidis JP. Why most published research findings are false. *PLoS Med* 2005;**2**:e124.

Irani M, Minkoff H, Seifer DB, Merhi Z. Vitamin D increases serum levels of the soluble receptor for advanced glycation end products in women with PCOS. *J Clin Endocrinol Metab* 2014;**99**:E886–E890.

La Marca A, Grisendi V, Griesinger G. How much does AMH really vary in normal women? *Int J Endocrinol* 2013;**2013**:959487.

McLennan IS, Pankhurst MW. Is the understanding of AMH being confounded by study designs that do not adequately reflect that it is an atypical hormone? *Hum Reprod* 2016;**32**:14–17.

Theodoratou E, Tzoulaki I, Zgaga L, Ioannidis JP. Vitamin D and multiple health outcomes: umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials. *BMJ* 2014; **348**:g2035.

van Disseldorp J, Lambalk CB, Kwee J, Looman CW, Eijkemans MJ, Fauser BC, Broekmans FJ. Comparison of inter- and intra-cycle variability of anti-Mullerian hormone and antral follicle counts. *Hum Reprod* 2010;**25**: 221–227.

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Reply: Vitamin D and ovarian reserve—making clinical decisions

Sir,

We share the view of Drakopoulos *et al.* that there is a compelling need for further research into the relationship between vitamin D (VD) status and anti-Müllerian hormone (AMH) in women. However, we emphasize that our commentary (McLennan and Pankhurst, 2017) discusses the validity and efficiency of the methods used to analyse putative regulators of AMH. The letter by Drakopoulos *et al.* does not address our concerns about the limitations of certain methodologies. Our commentary does not review the literature relating to VD, and makes no conclusions relating to the VD regulation of AMH. We do not hold the views attributed to us by Drakopoulos *et al.*

The acute and chronic effects of VD on AMH levels are not necessarily identical, due to the breadth of physiological actions of VD. We agree that the 6-month period used by Dennis *et al.* (2012) is insufficient by itself to define the relationship between VD and AMH, in women. For this reason, we have undertaken an acute study to compliment and extend the 2012 study. Both studies by Dennis *et al.* are longitudinal investigations of women recruited from the community. They contribute to the understanding of normal reproductive physiology, but neither paper presumes that the VD regulation of the ovary is identical in fertile and subfertile women. The relevance of VD to the clinical management of women with limited fertility needs to be established by observation, using rigorous methodology.

References

Dennis NA, Houghton LA, Jones GT, van Rij AM, Morgan K, McLennan IS. The level of serum anti-Mullerian hormone correlates with Vitamin D status in men and women but not in boys. *J Clin Endocrinol Metab* 2012; **97**:2450–2455.

McLennan IS, Pankhurst MW. Is the understanding of AMH being confounded by study designs that do not adequately reflect that it is an atypical hormone? *Hum Reprod* 2017;**32**:14–17.

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