Vitamin D in human reproduction: some answers and many more questions

We are in the midst of what has been deemed a worldwide vitamin D deficiency epidemic. Vitamin D is a secosteroid and its metabolites play a major role in the regulation of calcium and phosphate homeostasis. Although vitamin D is well known to contribute to bone growth and remodeling, it has been implemented in other imperative processes such as cell division, neuromuscular and immune function, and inflammation reduction. Its involvement in reproductive physiology is a topic currently undergoing much research. Many women using infertility treatment and assisted reproduction opt to supplement with vitamin D, but whether repletion ultimately improves outcomes remains unclear.

Ozkan et al. (1) was one of the first groups to look at assisted reproductive technology (ART) outcomes with respect to vitamin D status and investigate whether replete vitamin D stores predicted clinical pregnancy after in vitro fertilization (IVF). They found that serum and follicular fluid 25(OH)D levels were highly correlated, and patients who achieved clinical pregnancy had significantly higher 25(OH)D levels. Rudick et al. (2) used donor-recipient IVF cycles to further delineate vitamin D's impact on the oocyte vs. the endometrium, and found recipients with nonreplete 25(OH)D levels had lower pregnancy rates. These findings are in contrast to Franasiak et al. (3) who demonstrated vitamin D status was unrelated to pregnancy outcomes in the setting of an optimized endometrium controlling for embryo and endometrial synchrony. Measurement of 25(OH)D levels did not predict the likelihood of implantation after euploid frozen embryo transfers. In the recent publication by Cai et al. (4), similarly they were unable to demonstrate a significant association between IVF pregnancy outcomes and vitamin D status. An examination of the methods for vitamin D assessment as well as a comparison of the differences in the IVF cycle characteristics among these studies may help to explain discrepant findings in the literature.

One of the major differences that sets the study by Cai et al. (4) apart from the available literature is their approach to measurement of free 25(OH)D in addition to total 25(OH)D. Current clinical recommendations for vitamin D deficiency are based on assays that measure total 25(OH)D. This measurement includes 25(OH)D that is bound to vitamin D binding protein (VDBP) (approximately 85%–90%), 25(OH)D that is bound to albumin (10%–15%), and 25(OH)D that is unbound (<1%). Although free 25(OH)D may be a better indicator of vitamin D status than total 25(OH)D, this is likely dependent on the end organ where vitamin D is acting as well as the characteristics of the population in which it is being measured. Genotypic differences in VDBP exist that result in varying concentrations as well as fluctuations due to comorbidities and even estrogen levels. Different polymorphisms of VDBP result in differing affinities for binding to vitamin D, which ultimately affects the total amount measured. Measurement of free 25(OH)D has not been evaluated in the ART population. The findings of Cai et al. (4) that serum levels of free 25(OH)D and total 25(OH)D highly correlated in IVF patients are important to our understanding of vitamin D physiology in this unique population.

The majority of laboratories use automated immunoassays to measure 25(OH)D, which unfortunately have many intrinsic analytic issues. Immunoassays are not very selective and to measure properly total 25(OH)D they must measure 25(OH)D2 and 25(OH)D3 in an equimolar fashion without detecting other similar metabolites. Additionally, as previously discussed, vitamin D circulates bound to VDBP and albumin, which must be released prior to measurement. Immunoassays cannot use strong solvents to release binding proteins making efficient dissociation very difficult. Immunoassays also are subject to interference in measurements, for example, heterophilic antibodies, which can lead to either positive or negative interference. Liquid chromatography-mass spectrometry (LC-MS/MS) remains the gold standard for measurement of vitamin D levels, however, LC-MS/MS requires expensive equipment and well trained staff. Also, LC-MS/MS has less sample variation within and between laboratories compared with immunoassay-based testing, but some disparities still exist. The shortcomings of assay-based testing compared with LC-MS/MS may help to explain why Cai et al. (4) and others have presented varying results. Cai et al. (4) used immunoassay testing via enzyme-linked immunosorbent assay. Furthermore, samples were drawn the day before embryo transfer and kept frozen at -80°C until measurement. Recently, Lara-Molina et al. (5) demonstrated that 25(OH)D degrades significantly in frozen stored samples of serum and follicular fluid across time. Samples that were quantified via LC-MS/MS at baseline were compared with levels after 7 months of storage and levels were significantly lower after storage. Cai et al. (4) did not specify how long samples were stored until processing, but it is possible these samples similarly degraded over time. Only a small minority of patients included in this study were classified as having adequate vitamin D levels. If the time of storage was lengthy, this phenomenon of degradation over time could help explain why measured levels were low throughout most of the population.

When interpreting data collected from an infertile population undergoing ART, it is prudent to take into consideration the characteristics of the IVF cycles. The study by Cai et al. (4) investigated pregnancy outcomes after fresh embryo transfers. During the process of a fresh embryo transfer the embryos are placed back into the uterus while estradiol levels remain very high. Supraphysiologic estradiol levels likely affect vitamin D's bioavailability due to influences on binding protein concentrations. Therefore, it is difficult to ascertain if these findings are applicable to frozen embryo transfer cycles where estrogen levels tend to be much lower. Additionally, vitamin D levels may have been significantly different during the earlier course of the IVF stimulation. This study only drew samples on the day prior to embryo transfer, and it cannot be determined what level of vitamin D may be impactful with regard to oocyte quality and resultant embryo competency. Furthermore, the nature of fresh embryo transfers precludes the use of preimplantation genetic testing for aneuploidy.

There is some emerging data that vitamin D levels may be associated with aneuploidy. Furthermore, vitamin D concentrations in serum and follicular fluid also have been shown to correlate with telomere length in cumulus cells of mature follicles. Although Cai et al. (4) did not perform preimplantation genetic testing for aneuploidy cycles and, therefore, the ploidy status of embryos transferred remains unknown, they did provide data that the quality of embryos transferred (good vs. fair) was not significantly different between the quintiles of vitamin D levels.

One aspect of the study by Cai et al. (4) that limits its generalizability is the homogeneity of the population studied. Not only were all of the patients included in this study of the same ethnicity, but they were all young, thin, good responders with relatively good prognoses. This homogeneity also may explain why the vast majority of patients had inadequate or insufficient vitamin D levels according to their predetermined cutoffs. Only 0.03% of the population in this study had adequate vitamin D levels (>30 ng/mL). It is possible if they were to study a population that had more variation in vitamin D levels and included more patients classified as vitamin D replete, a significant difference in pregnancy outcomes may arise.

The study by Cai et al. (4) adds valuable data to an area of reproductive medicine that is becoming increasingly relevant. The role vitamin D plays in folliculogenesis and embryonic blastulation vs. implantation at the level of the endometrium has not been defined clearly and more studies are needed. Nevertheless, using current measurement techniques, a global epidemic of vitamin D deficiency exists and many reproductive-aged women are taking vitamin D supplements. Future studies should aim to assess accurately 25(OH)D levels and identify women with adequate and deficient vitamin D levels

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