Towards improving analysis and interpretation of antimüllerian hormone in women

Interest in the clinical utility of the measurement of circulating antimüllerian hormone (AMH) concentrations has grown very steeply over the two decades since the first indications that it showed a positive relationship with the number of oocytes that could be obtained after ovarian stimulation, that it declined with age, and that it was a marker, albeit indirect, of the ovarian reserve. Measurement of AMH before assisted reproduction has become established in clinical practice, predicting both excessive and poor response, and it has potential for diagnostic use in polycystic ovary syndrome, premature ovarian insufficiency and in the assessment of the remaining reproductive lifespan (1). Further applications include use as a tumor marker in granulosa cell tumors and in disorders of sexual development, the latter relating to the initial studies in the 1950s showing that AMH is produced in large quantities by the fetal Sertoli cell and that it is the key hormone that determines Müllerian duct regression in the male. In recent years, issues over the reliability of the assays used to determine serum AMH have clouded confidence in interpretation. It is likely that the new automated assays will herald a new era of more reliable and exact analysis, although the ongoing absence of an international standard and the resultant differences in calibration between the assays available continue to be significant issues. While the clinical utility of AMH has thus advanced dramatically, our knowledge of its biological function and regulation within the ovary, particularly in nonrodent species, has fallen rather behind.

Two key related areas are understanding of how follicular production of AMH translates into serum concentrations and what factors regulate this. This might translate into variability in serum concentrations in individual women, and these issues are addressed by two papers published in the current issue of *Fertility and Sterility* (2, 3). The two key intraovarian biological functions suggested for AMH that have been explored in animal models are suppression of the rate of primordial follicle activation and modulation of the sensitivity of early antral follicles to FSH, particularly around the time when the dominant follicle is selected. Human data relating to either of these are very limited, although evidence from the sheep as a useful mono-ovulatory large mammalian model is supportive of a role for AMH in limiting follicle selection as are very recent data from a nonhuman primate (reviewed in [4]). One of the key advantages attributed to AMH in clinical practice is its relative stability both within and between menstrual cycles, particularly compared with FSH. This has led to the general perception in clinical practice that AMH can be measured reliably at any stage of the cycle. However, the dramatic changes in AMH production by follicles as they go through the early antral stages (5), at which time they are clearly gonadotropin regulated, would per se indicate there are likely to be subtle changes in circulating AMH over the menstrual cycle. This, may be of value in understanding the biology of AMH within the ovary as well as causing us to reflect critically on the limitations of single serum measurements of the hormone.

In keeping with most biologically active peptides, AMH is produced in a larger precursor form, and there are known to be two forms of AMH present in the circulation, the biologically inactive precursor (proAMH) and the receptor binding form, identified as $AMH_{N,C}$ (2). Importantly, commercially available immunoassays are unable to distinguish between these two forms, measuring them both equally. Thus apparent serum AMH is the sum of these two forms, although the inactive precursor appears to be circulating at lower concentrations. The question of whether there is a variation in the relative abundance of these two forms across the menstrual cycle has been addressed by Pankhurst and Chong, whose group has over recent years developed assays able to distinguish them. This was used to analyze changes in serum AMH in a group of relatively young women with regular menstrual cycles, with the key finding being that the proportion of proAMH fell shortly after ovulation, although it was stable across the rest of the cycle. As the proAMH form predominates in follicular fluid but not in the blood, the authors interpret this to indicate that the LH surge may change the activity of the two relevant cleavage enzymes, but they also raise the possibility that coincident changes in the hierarchy of follicle development could also account for this, presumably by changes in the relative rate of growth of the different stages of ovarian follicles, each with their own characteristic pattern of production of AMH. These changes were relatively modest and may not have marked immediate clinical relevance, but they do serve to highlight that there are changes in AMH across the menstrual cycle that may not be as marked as other hormones used in this context (such as FSH) but may be of importance when specific cutoffs, for example, to determine the use of different gonadotropin stimulation regimens in the context of assisted reproduction (or potentially in diagnostic tests in the future), are chosen on the basis of single AMH measurements.

This is further explored in the publication from Hadlow and colleagues in Western Australia who have reassessed intracycle, and to a lesser extent intercycle, variation in AMH in women with regular menstrual cycles. Although the number of women is relatively modest, they highlight that the biological variation in serum AMH is much larger than the analytic variation, with low analytic variation being a key aspect of quality control for clinically useful immunoassays. In this study they used the plate-based Beckman Coulter Gen II assay, and thus analytic variation may fall further with the automated assays now increasingly being used. The authors addressed the issue of how often the biological variation would mean that an individual woman would be reclassified according to various cutoffs, indicating low, reduced, or normal ovarian reserve and risk of hyperstimulation. Previous studies investigating intracycle variation in AMH have tended to highlight a greater variability in women with high

AMH, but in contrast this study suggests that it is those with the lower AMH concentrations that are more likely to be reclassified on resampling. The number of women investigated in this study is limited, but this analysis provides a useful caution against overinterpretation of single timepoint samples. For clinicians, it is all too easy to see values produced by a laboratory as cast iron and not to consider sufficiently the underlying sources of error and variation that produce the lab report in front of us. The apparently high intraindividual variation at lower AMH concentrations in these women with regular cycles (but whose age is not specified) presumably indicates relatively high follicle cohort growth variability in these women with a reduced ovarian reserve: if you have only very few follicles growing, then a couple more or less becoming atretic may make a proportionally larger difference. This is in keeping with the clinical outcomes seen especially in younger women with "poor ovarian reserve," which has limited prediction of response in a subsequent cycle of ovarian stimulation. There is also now an increasing number of studies highlighting the effects of a whole range of factors on serum AMH, including intercurrent illnesses, altered gonadotropins such as in pregnancy or contraceptive use, and, indeed, seasonal changes.

Most clinicians measuring AMH before an IVF cycle do so on a single occasion and may base their gonadotropin stimulation regimen on that, and that will continue to be a valuable strategy. Whether repeated measurement, with associated increased cost and inconvenience, will result in improved outcomes for patients remains to be determined, but meanwhile it is useful for us to be reminded that biological variables do not respect sharp cutoffs that are introduced for clinical convenience. We should be cautious of overinterpreting single measurements of AMH; this should be in the light of understanding the biological and clinical context as good clinical practice always requires.

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