Serum levels of antimüllerian hormone: more than meets the eye

The serum levels of antimüllerian hormone (AMH), produced by the granulosa cells of preantral and small antral ovarian follicles, has gained widespread use in clinical practice as a valuable serum marker for the evaluation of ovarian reserve and the prediction of response during ovarian stimulation (1).

Although a positive correlation is typically observed between AMH and antral follicle count, there are instances where they may conflict, and discrepancies can occur, introducing complexity to the management of such cases (2). In these cases, where a marker is unexpectedly high or low compared with the other, the conventional understanding of ovarian reserve evaluation is challenged, and further investigation may be warranted.

In this issue of *F&S Reports*, Melado et al. (3) present such a case—a patient initially, but mistakenly, diagnosed with primary ovarian insufficiency because of low-serum AMH levels. However, subsequent genetic analysis revealed a novel homozygous missense variant in exon 1 of the AMH gene, not previously reported in the literature or population databases. This variant, classified as of uncertain significance, appears to severely impair circulating AMH immunodetection by different immunoassays.

Granulosa cells release AMH primarily as a nonactive prohormone, containing a covalently linked N-terminal proregion and a small C-terminal mature domain (covalent form). The active complex ($AMH_{N,C}$) is the result of the association between the proregion and mature domain after obligatory cleavage (noncovalent form) (1, 4). Circulating AMH comprises prohormone, vital in synthesis and transport, and $AMH_{N,C}$, the bioactive form (1, 4). In recent years, both manual and automated assays have been developed to measure the serum AMH levels, using antibodies against specific AMH regions (1). The total AMH level in circulation depends on the presence of specific isoforms and the antibodies' ability to detect them and may be affected because of new antigenic sites after processing, contributing to interassay variability (1).

This is important to understand because the AMH levels exhibit significant variation among women, with genetic factors believed to play a crucial role. Different AMH coding mutations have been previously described, including some affecting protein processing and bioactivity (4, 5). Rare AMH mutations have been identified, causing reduced AMH signaling and subsequent impairment of AMH immunoactivity (5). Additionally, polymorphisms in AMH or its receptor gene, AMHR2, have been associated with outcomes in ovarian stimulation, infertility, follicle recruitment, primary ovarian insufficiency, and polycystic ovary syndrome in candidate gene studies (1).

By integrating the clinical, laboratory, and genetic data gathered during their assessment, the investigators calibrated their clinical acuity and formulated a reasonable ovarian stimulation protocol for a patient with polycystic ovary syndrome, notwithstanding consistently diminished AMH levels. The investigators subsequently emphasize a nuanced approach to ovarian reserve assessment, advocating for a comprehensive consideration of various clinical and laboratory parameters during stimulation planning and not relying exclusively on the serum AMH levels, which reinforces the broader call for meticulous evaluation and individualized considerations, acknowledging the interpretation of clinical and biochemical data in tandem, in the context of ovarian stimulation protocols.

By contrast, the absence of a functional analysis for the identified mutation variant in the AMH gene, which has been previously performed in similar cases, such as was demonstrated in a study by Hoyos et al. (4), introduces a critical gap in the comprehensive evaluation of its clinical significance and the potential functional alterations induced by the mutation (4). Specifically, Hoyos et al. (4) conducted a functional analysis to assess the impact of the genetic variant p.(Ala515Val) on AMH, aiming to understand how it affects expression, processing, and bioactivity (4). Their analysis revealed that although the variant showed reduced immunoactivity in the pico-AMH enzyme-linked immunosorbent assay, its bioactivity remained normal in vitro, suggesting minimal alteration in protein function (4). Therefore, functional analyses are pivotal in understanding the biologic implications of AMH genetic variants (5). Without such an investigation, a nuanced understanding of how the AMH mutation may affect its physiological role and impact clinical outcomes is hindered. This information is crucial for tailoring treatment strategies, providing precise genetic counseling, and advancing our understanding of the genetic basis of fertility disorders (5). Recognizing this analytic gap underscores the need for further investigative efforts to characterize the functional ramifications of the identified AMH gene variant. Addressing this gap not only benefits immediate clinical considerations but also contributes significantly to the ongoing research in reproductive medicine.

Although the information provided in this case report may not catalyze a revolutionary change in and of itself, it serves as a shining example that AMH, although valuable, is an imperfect proxy for assessing ovarian reserve and should be used in conjunction with other markers for a comprehensive evaluation. Similar to any other gene, AMH is prone to mutations that may affect its immunoactivity and/or bioactivity; the former may cause it to "hide in plain sight". However, we need to understand that our inability to measure AMH does not mean it is not there and properly functioning, particularly when the clinical picture suggests so.

CRediT Authorship Contribution Statement

Kyara Marquez: Writing – original draft, Writing – review & editing. **Luis R. Hoyos:** Writing – review & editing.

Declaration of Interests

K.M. has nothing to disclose. L.R.H. has nothing to disclose.

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