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The role of anti-Müllerian hormone testing for fertility prognosis

The use of anti-Müllerian hormone testing to measure ovarian reserve has advantages and disadvantages; therefore, it is vital to consider the clinical question being asked.

ABSTRACT: There is a growing demand for fertility care in British Columbia and an associated interest in ovarian reserve testing. Anti-Müllerian hormone (AMH) testing is a validated marker and one of the few direct measurements of ovarian reserve; AMH levels are stable throughout the menstrual cycle, and it is a readily accessible biochemical test in BC. Despite these advantages, providers must acknowledge that although AMH levels can be used to estimate the quantity of oocytes remaining, they cannot be used to estimate their quality. Furthermore, AMH levels are artificially lowered in women who are taking combined oral contraceptive pills, and this effect may be seen for up to 2 months after discontinuation of such pills. Clinical scenarios in which AMH testing is a helpful tool include predicting response to controlled ovarian stimulation, titrating gonadotropin dosing in controlled ovarian stimulation, and supporting a diagnosis of polycystic ovary syndrome in adults. However, AMH testing should not be used to predict natural fertility, exclude patients from assisted reproductive technology, or predict age of menopause. It is, therefore, important to carefully consider the clinical question being asked when ordering AMH testing.

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In recent years, demand for fertility care has increased as women choose to delay childbearing. Between 2000 and 2022, the mean age of the mother at the time of delivery in British Columbia increased from 29.3 to 32.4 years.[1] During the same time frame, the age-specific fertility rate for females aged 40 to 44 years nearly doubled, from 7.0% to 13.7%.[2] Therefore, it has become increasingly important to develop and implement reliable tools to appropriately counsel women who are seeking fertility care and assisted reproductive technology.

It is well established that primary contributing factors to age-related infertility include decreased ovarian reserve and a rise in chromosomally abnormal embryos with advancing age.[3] In controlled ovarian stimulation for assisted reproduction, subcutaneous injections of gonadotropins (follicle-stimulating hormone and luteinizing hormone) stimulate ovaries to grow follicles. Controlled ovarian stimulation followed by oocyte retrieval improves the probability of pregnancy in women with infertility. Individual responses to controlled ovarian stimulation vary, which can impact oocyte yield at the time of retrieval. However, tools exist to help predict response to controlled ovarian stimulation and successful in vitro fertilization outcomes.

I review the role of ovarian reserve testing, with a focus on anti-Müllerian hormone (AMH) testing. My goal is to provide a practical guide to interpreting AMH testing results and the implications for patients seeking fertility care.

Defining ovarian reserve testing

Human oocyte numbers peak around 20 weeks' gestation, undergo atresia or ovulation, and do not regenerate.[4] It is estimated that females have 500 000 to 1 000 000 oocytes at birth, but the number declines to approximately 400 000 at the time of puberty and 1000 at the time of menopause.[5,6] Ovarian reserve refers to both the quantity and the quality of the remaining ovarian primordial follicular pool.

Though it remains difficult to predict oocyte quality, ovarian reserve tests were designed to estimate the quantity of oocytes remaining and thereby predict which patients will have a poor response, hyper-response, or adequate response to controlled ovarian stimulation. A number of tests have been developed over the years, including biochemical, biophysical, and histological tests of ovarian reserve.[7]

The most applicable evidence-based tests are serum AMH concentration and ultrasonographic antral follicle count. I focus on serum AMH concentration, which peaks around age 25 and declines at a steady rate until age 40, at which time the rate of decline becomes steeper until the age of menopause.[8] This was demonstrated

in Kelsey and colleagues' 2011 validated model,[8] which strongly supports the use of AMH as an ovarian reserve test.

AMH testing origins

AMH was first discovered by French endocrinologist Dr Alfred Jost, who was famous for his research on the physiology of somatic sex differentiation. In 1947, Dr Jost published his finding that AMH was responsible for Müllerian duct regression, which suppressed the uterine and tubal structures during male sexual development.[9] It was not until the 1980s, however, that the role of AMH at the level of the ovary was well characterized. In 1981, Hutson and colleagues published the first evidence of ovarian expression of AMH in chicken gonads.[10] This persisted even after Müllerian duct regression, which suggested other implications of AMH in reproductive physiology. In 1999, Durlinger and colleagues published evidence that AMH controls primordial follicle recruitment in mice.[11] This was the first publication of its kind on the function of AMH in the ovary.

Several commercial AMH assays have been developed since then, starting with two commercial AMH enzyme-linked immunosorbent assays (ELISAs) manufactured by Diagnostic Systems Laboratories, Inc. and Immunotec, respectively, in the early 2000s.[12] After Beckman Coulter, Inc. acquired both companies, AMH Gen II ELISA was developed and distributed in 2010. More recently, the ultrasensitive human MIS/AMH ELISA kit, the automated Access AMH assay, and the Elecsys AMH immunoassay have been introduced. Despite this progress, according to the World Health Organization international standards, no reference reagent has been established that addresses the heterogeneity in AMH assay kits.

Advantages of AMH testing

AMH is produced by granulosa cells of early follicles once they differentiate from the primordial to the primary stage at the time of puberty. The number of early follicles is related to the size of the primordial follicle pool; therefore, serum AMH concentration is one of the few direct measurements of ovarian reserve.

Also, serum AMH concentration is gonadotropin independent, which makes it cycle-day independent as well.[13] Other common biochemical tests of ovarian reserve, such as follicle-stimulating hormone, estradiol, and inhibin B, fluctuate throughout the menstrual cycle, which makes them more difficult to interpret.

It is recommended that the use of combined oral contraceptive pills be discontinued a minimum of 2 months before measuring serum AMH concentration for accurate results.

Finally, serum AMH concentration is a simple biochemical test that is offered widely. This is very different from the other reliable ovarian reserve test—ultrasonographic antral follicle count—which requires a trained provider and access to an endovaginal ultrasound machine and probe. This test is offered only by fertility clinics and select radiology departments and clinics, which limits accessibility compared with AMH testing.

Disadvantages of AMH testing

Though serum AMH concentration is one of the most reliable ovarian reserve tests, it is not perfect. AMH does not predict oocyte quality, which is an important component of ovarian reserve. Additionally, there is heterogeneity in AMH assay kits, which makes it difficult to establish a standard on a national or international basis.

In addition, serum AMH concentration is currently an uninsured test. The cost is quoted at $78 on the LifeLabs website.[14] If patients are unable to access this test, informed treatment planning becomes more challenging for the fertility provider and perpetuates inequities in care.

Finally, though one of the strengths of AMH testing is its consistency throughout the menstrual cycle, serum AMH concentrations are lowered in women who are on combined oral contraceptive pills and should be interpreted with caution in this patient population.[15] The theory is that the use of combined oral contraceptive pills causes prolonged suppression of follicle-stimulating hormone, which then prevents pre-antral and small antral follicle formation. As a result, the cohort of cells that produce AMH is smaller. It is recommended that the use of combined oral contraceptive pills be discontinued a minimum of 2 months before measuring serum AMH concentration for accurate results, because follicle development is believed to take 2 months.[16]

Reporting of AMH concentrations in BC

AMH concentration may be reported in either ng/mL (Immunotec assay and AMH Gen II ELISA assay) or pmol/L (Diagnostic Systems Laboratories, Inc. assay). Both units may be encountered by BC physicians, though LifeLabs British Columbia reports AMH concentration in pmol/L. The conversion factor from pmol/L to ng/mL is 0.14.

The normal levels for AMH, according to LifeLabs British Columbia, are as follows (oral communication with medical/scientific staff, LifeLabs British Columbia, 15 July 2024):

- Ages 20–24: 8.7–83.6 pmol/L
- Ages 25–29: 6.4–70.3 pmol/L
- Ages 30–34: 4.1–58.0 pmol/L
- Ages 35–39: 1.1–53.5 pmol/L
- Ages 40–44: 0.2–39.1 pmol/L
- Ages 45–100: < 19.4 pmol/L

Because these normal levels include a broad range, results may be difficult to interpret. However, in general, an AMH concentration of less than 10 pmol/L is a concern for decreased ovarian reserve.

AMH testing as a clinical tool

AMH testing is a helpful clinical tool in the following scenarios:

- Predicting response to controlled ovarian stimulation. The main role of AMH testing is its ability to predict response to controlled ovarian stimulation. Serum AMH concentration and number of follicles obtained during maximal ovarian stimulation have a positive association.[17] This is significant because the greater the number of follicles obtained, the greater the likelihood of creating a euploid embryo for transfer. As a result, this helps when counseling patients who are considering egg freezing or embryo freezing regarding the urgency of moving forward with treatment.
- Titrating gonadotropin dosing in controlled ovarian stimulation. AMH testing results are helpful when deciding on gonadotropin dosing in controlled ovarian stimulation. Dosing is critical, because a poor response could lead to cycle cancellation, and a hyper-response could lead to ovarian hyperstimulation syndrome, which can be life-threatening. Broer and colleagues showed that AMH has a good discriminatory capacity to separate normal and excessive responders to controlled ovarian stimulation.[18] Multiple dosage algorithms have been proposed based on AMH results, with or without consideration of other factors, such as age or body mass index.[19] These are widely adopted and implemented by fertility clinics locally.
- Supporting a diagnosis of polycystic ovary syndrome in adults. Serum AMH concentration has been explored as a diagnostic test for polycystic ovary syndrome. Iliodromiti and colleagues showed a specificity and sensitivity of AMH in diagnosing polycystic ovary syndrome in symptomatic women of 79.4% and 82.8%, respectively, for a cutoff AMH value of 33.6 pmol/L.[20] However, serum AMH concentrations change over a woman's reproductive life, which suggests a need for age-specific thresholds. One study suggested cutoff levels for the prediction of polycystic ovary syndrome as follows: 20 to 27 years: 40.7 pmol/L, 27 to 35 years: 32.5 pmol/L, and 35 to 40 years: 26.4 pmol/L.[21] Nonetheless, the absence of a standardized AMH assay kit makes it difficult to adopt as a diagnostic test. As a result, elevated AMH levels are currently best used to support a diagnosis of polycystic ovary syndrome in patients who meet standard diagnostic criteria, such as the Rotterdam criteria.[22]

Serum AMH concentration does not predict natural fertility, a common misconception among patients.

When AMH testing is not a helpful clinical tool

AMH testing is not a helpful clinical tool in the following scenarios:

- Predicting natural fertility. No studies have shown that serum AMH concentration predicts fecundability (the probability of conceiving in a given menstrual cycle), probability of pregnancy, or infertility.[23] The Time to Conceive study published in 2017 showed that women aged 30 to 44 years who had no known history of or risk factors for infertility but had low AMH levels had similar cumulative pregnancy rates as women with normal AMH levels.[24] As a result, AMH should not be used to predict natural fertility in patients.
- Excluding patients from assisted reproductive technology. AMH levels should not be used to exclude patients from assisted reproductive technology. No studies have shown an AMH level below which no pregnancies occurred with assisted reproductive technology, because AMH does not reflect oocyte quality or chances of conception.[25] There is always a possibility of pregnancy after controlled ovarian stimulation and in vitro fertilization; age is still the primary predictor of success rates with assisted reproductive technology.
- Predicting age of menopause. Many studies have shown that the predictive power of AMH for menopause is poor.[26] Though a low AMH level at a young age may be a risk factor for early menopause, it cannot be used to predict age of menopause due to the variable rate of decline in AMH concentration, as well as the multifactorial nature of menopausal transition.

Summary

Serum AMH concentration is a useful tool in the workup of infertility. It informs counseling of patients who are seeking assisted reproductive technology and helps fertility providers tailor their approach to controlled ovarian stimulation to optimize patient safety and outcomes. However, serum AMH concentration does not predict natural fertility, a common misconception among patients. As a result, choosing the appropriate clinical scenario and patient counseling are vital when ordering ovarian reserve testing. Finally, there may be a role for serum AMH concentration in supporting the diagnosis of select medical conditions, including polycystic ovary syndrome and menopause, but research has not yet supported its role as a diagnostic test or predictive tool. This is a dynamic area of research, with academic interest worldwide. Locally, BC physicians will be challenged to remain current on ovarian reserve testing recommendations as more patients seek fertility care in light of the publicly funded in vitro fertilization program starting in April 2025. This will be an exciting chapter for BC physicians and patients alike. ■

Competing interests

None declared.

References

1. Statistics Canada. Mean age of mother at time of delivery (live births). 2023. Accessed 14 February 2024. www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=1310041701&cubeTimeFrame.startYear=2000&cubeTimeFrame.endYear=2022&referencePeriods=20000101%2C20220101.

2. Statistics Canada. Crude birth rate, age-specific fertility rates and total fertility rate (live births). 2023. Accessed 14 February 2024. www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=1310041801&pickMembers%5B0%5D=1.11&cubeTimeFrame.startYear=2000&cubeTimeFrame.endYear=2022&referencePeriods=20000101%2C20220101.
3. Broekmans FJ, Kwee J, Hendriks DJ, et al. A systematic review of tests predicting ovarian reserve and IVF outcome. Hum Reprod Update 2006;12:685-718.
4. Telfer EE, Grosbois J, Odey YL, et al. Making a good egg: Human oocyte health, aging, and in vitro development. Physiol Rev 2023;103:2623-2677.
5. Faddy MJ, Gosden RG, Gougeon A, et al. Accelerated disappearance of ovarian follicles in mid-life: Implications for forecasting menopause. Hum Reprod 1992;7:1342-1346.
6. Yatsenko SA, Rajkovic A. Genetics of human female infertility. Biol Reprod 2019;101:549-566.
7. Deadmond A, Koch CA, Parry JP. Ovarian reserve testing. Endotext [Internet]. Updated 21 December 2022. Accessed 8 August 2024. www.ncbi.nlm.nih.gov/books/NBK279058/.
8. Kelsey TW, Wright P, Nelson SM, et al. A validated model of serum anti-Müllerian hormone from conception to menopause. PLoS One 2011;6:e22024.
9. Josso N. Professor Alfred Jost: The builder of modern sex differentiation. Sex Dev 2008;2:55-63.
10. Hutson J, Ikawa H, Donahoe PK. The ontogeny of Mullerian inhibiting substance in the gonads of the chicken. J Pediatr Surg 1981;16:822-827.
11. Durlinger AL, Kramer P, Karels B, et al. Control of primordial follicle recruitment by anti-Müllerian hormone in the mouse ovary. Endocrinology 1999;140:5789-5796.
12. Li HWR, Robertson DM, Burns C, Ledger WL. Challenges in measuring AMH in the clinical setting. Front Endocrinol (Lausanne) 2021;12:691432.
13. Cook CL, Siow Y, Taylor S, Fallat ME. Serum Müllerian-inhibiting substance levels during normal menstrual cycles. Fertil Steril 2000;73:859-861.
14. LifeLabs. Fertility test: Anti-Müllerian hormone (AMH). Accessed 17 February 2024. www.lifelabs.com/test/anti-mullerian-hormone-amh/.
15. van den Berg MH, van Dulmen-den Broeder E, Overbeek A, et al. Comparison of ovarian function markers in users of hormonal contraceptives during the hormone-free interval and subsequent natural early follicular phases. Hum Reprod 2010;25:1520-1527.
16. Landersoe SK, Birch Petersen K, Sørensen AL, et al. Ovarian reserve markers after discontinuing long-term use of combined oral contraceptives. Reprod Biomed Online 2020;40:176-186.
17. Kwee J, Schats R, McDonnell J, et al. Evaluation of anti-Müllerian hormone as a test for the prediction of ovarian reserve. Fertil Steril 2008;90:737-743.
18. Broer SL, Dólleman M, Opmeer BC, et al. AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: A meta-analysis. Hum Reprod Update 2011;17:46-54.
19. Pilsgaard F, Grynnerup AG-A, Løssl K, et al. The use of anti-Müllerian hormone for controlled ovarian stimulation in assisted reproductive technology, fertility assessment and counseling. Acta Obstet Gynecol Scand 2018;97:1105-1113.
20. Iliodromiti S, Kelsey TW, Anderson RA, Nelson SM. Can anti-Müllerian hormone predict the diagnosis of polycystic ovary syndrome? A systematic review and meta-analysis of extracted data. J Clin Endocrinol Metab 2013;98:3332-3340.
21. Ramezani Tehrani F, Rahmati M, Mahboobifard F, et al. Age-specific cut-off levels of anti-Müllerian hormone can be used as diagnostic markers for polycystic ovary syndrome. Reprod Biol Endocrinol 2021;19:76.
22. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19:41-47.
23. Practice Committee of the American Society for Reproductive Medicine. Testing and interpreting measures of ovarian reserve: A committee opinion. Fertil Steril 2020;114:1151-1157.
24. Steiner AZ, Pritchard D, Stanczyk FZ, et al. Association between biomarkers of ovarian reserve and infertility among older women of reproductive age. JAMA 2017;318:1367-1376.
25. Cedars MI. Evaluation of female fertility—AMH and ovarian reserve testing. J Clin Endocrinol Metab 2022;107:1510-1519.
26. Depmann M, Eijkemans MJC, Broer SL, et al. Does AMH relate to timing of menopause? Results of an individual patient data meta-analysis. J Clin Endocrinol Metab 2018;103:3593-3600.