Ovarian reserve testing: a user's guide

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Ovarian reserve is a complex clinical phenomenon influenced by age, genetics, and environmental variables. Although it is challenging to predict the rate of an individual's ovarian reserve decline, clinicians are often asked for advice about fertility potential and/or recommendations regarding the pursuit of fertility treatment options. The purpose of this review is to summarize the state-of-the-art of ovarian reserve testing, providing a guide for the obstetrician/gynecologist generalist and reproductive endocrinologist. The ideal ovarian reserve test should be convenient, be reproducible, display little if any intracycle and intercycle variability, and demonstrate high specificity to minimize the risk of wrongly diagnosing women as having diminished ovarian reserve and accurately identify those at greatest risk of developing ovarian hyperstimulation prior to fertility treatment. Evaluation of ovarian reserve can help to identify patients who will have poor response or hyperresponse to ovarian stimulation for assisted reproductive technology. Ovarian reserve testing should allow individualization of treatment protocols to achieve optimal response while minimizing safety risks. Ovarian reserve testing may inform patients regarding their reproductive lifespan and menopausal timing as well as aid in the counselling and selection of treatment for female cancer patients of reproductive age who receive gonadotoxic therapy. In addition, it may aid in establishing the diagnosis of polycystic ovary syndrome and provide insight into its severity. While there is currently no perfect ovarian reserve test, both antral follicular count and antimüllerian hormone have good predictive value and are superior to day-3 follicle-stimulating hormone. The convenience of untimed sampling, age-specific values, availability of an automated platform, and potential standardization of antimüllerian hormone assay make this test the preferred biomarker for the evaluation of ovarian reserve in women.

Key words: antimüllerian hormone, antral follicular count, follicle-stimulating hormone, ovarian biomarkers, ovarian reserve, primordial follicles

Introduction

A woman is born with about 2 million primordial follicles, yet by the onset of menarche only about 400,000 follicles are left due to natural follicular atresia. As a woman reaches her mid-30s the pace of oocyte depletion begins to increase and by the time she reaches her late 30s, the number of follicles declines to approximately 25,000, concomitant with a significant increase in miscarriage rate. The term "ovarian reserve" has traditionally been used to describe a woman's reproductive potential—specifically, the number and quality of oocytes she possesses.[1] However, commonly used ovarian reserve markers serve as a proxy for oocyte quantity but are considered poor predictors of oocyte quality. Therefore, modern usage of the term pertains to the quantity of remaining oocytes rather than oocyte quality, for which age still remains the best predictor. Diminished ovarian reserve (DOR) describes women of reproductive age having menses whose response to ovarian stimulation or fecundity is reduced compared with women of comparable age.[1] It is distinct from menopause or premature ovarian insufficiency.

Ovarian reserve is a complex clinical phenomenon influenced by age, genetics, and environmental variables.[2] The decline in a woman's ovarian reserve with time is irreversible and the rate at which women lose primordial follicles varies considerably, with wide variation regarding the onset of sterility and timing of the menopausal transition. Although it is challenging to predict the rate of an individual's ovarian reserve decline, clinicians are often asked for advice about fertility potential and/or recommendations regarding the pursuit of fertility treatment options. Over the past few years, there have been several comprehensive reviews on ovarian reserve tests that focused mainly on ovarian response prediction in the context of assisted reproductive technology (ART).[3-6] The purpose of this review is to summarize the state-of-the-art of ovarian reserve testing, providing a practical guide for the obstetrician/gynecologist generalist and reproductive endocrinologist.

What makes a reliable ovarian reserve test?

Ovarian reserve tests started to emerge during the rise of ART in the late 1980s to predict both responsiveness to superovulation drugs and the odds of pregnancy with treatment. They include both biochemical basal and provocative tests and ultrasound imaging of the ovaries. The first test to be introduced was day-3 follicle-stimulating hormone (FSH) (1988), followed by clomiphene citrate challenge test (CCCT) (1989), gonadotropin releasing-hormone (GnRH) agonist (1989), inhibin B (1997),

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antral follicular count (AFC) (1997), and antimüllerian hormone (AMH) (2002). Most of these measures, however, have poor predictive value, often because they are indirect measures of ovarian reserve (eg, FSH, CCCT, GnRH agonist) or have substantial intracycle or intercycle variability (eg, FSH).[7-9] The provocative tests (CCCT and GnRH agonist) have been virtually abandoned due to expense and inconvenience as they require >1 timed patient visit. Today, the markers most often used in clinical practice are the basal tests—FSH, AFC, and AMH—which are discussed in detail below. The other biochemical markers that have proven to be less useful are reviewed in detail elsewhere.[10]

The purpose of using ovarian reserve testing as a screening test is to identify infertility patients at risk for DOR, who are more likely to have poor response to gonadotropin stimulation and less likely to achieve pregnancy with ART or ovulation induction. It can also contribute to making ovulation induction safer by identifying and potentially precluding those at greatest risk of developing ovarian hyperstimulation syndrome (OHSS), a life-threatening iatrogenic complication, prior to receiving such therapy. The ideal ovarian reserve test should be affordable, noninvasive, and rapidly interpretable. It should also be reproducible and display minimal variability within the menstrual cycle and between cycles. Moreover, it should be able to detect the decline in ovarian reserve at an early enough stage such that timely interventions could be pursued if desired. Lastly, it should have validity, ie, good sensitivity and specificity. From a clinical standpoint, the threshold for considering a test abnormal for DOR should have high specificity. This would minimize the number of patients with normal ovarian reserve wrongly categorized as DOR. Importantly, this would avoid unnecessary treatments in patients with normal ovarian reserve or recommendations for oocyte donation or adoption in those patients who should be able to conceive on their own. On the other hand, optimizing specificity sacrifices sensitivity and the ability to identify all women with DOR. Moreover, it is important to emphasize that ovarian reserve tests have limitations and should not be used as sole criteria to deny patients access to ART or other treatments. Evidence of DOR does not necessarily mean inability to conceive, only that it may be less likely. Furthermore, identification of those at greatest risk of OHSS prior to ovulation induction would allow for judicious selection and use of gonadotropins or oral fertility agents and the deployment of additional available strategies for OHSS prevention (GnRH agonist trigger, cabergoline, freeze all) as well as patient appropriate counseling to set realistic expectations prior to beginning treatment.

Early follicular follicle-stimulating hormone

The use of early follicular phase (basal) FSH as a marker of ovarian reserve was proposed almost 30 years ago, as a tool to predict ovarian response to in vitro fertilization (IVF).[11-13] This test is an indirect assessment of ovarian reserve and is based on the feedback inhibition of FSH pituitary secretion by ovarian factors. At the beginning of the menstrual cycle, estradiol and inhibin B levels reach a nadir, offering a glimpse to the unsuppressed hypothalamus-pituitary-ovarian axis before levels of these ovarian hormones rise and inhibit FSH secretion. Women with normal ovarian reserve have sufficient production of ovarian hormones at this early stage of the menstrual cycle to maintain FSH levels within normal range. In contrast, elevation of FSH at this stage of the menstrual cycle indicates poor production of ovarian hormones by diminishing the ovarian follicular pool consistent with DOR. However, basal FSH testing has several major limitations including significant intercycle and intracycle variability that limits its reliability,[7,8] it requires a functional hypothalamus-pituitary-ovarian axis, and it is not adequately sensitive for clinical utility—only elevations carrying significance. The latter limitation is the reason that basal FSH test must be combined with estradiol, which enhances the sensitivity of early follicular FSH testing. Combining basal FSH with estradiol is more meaningful since even normal-range FSH can imply DOR in the setting of elevated basal estradiol. In women with declining ovarian reserve, premature elevations in FSH in the early follicular phase drive estradiol levels higher, which, in turn, may lead to increased negative feedback on pituitary FSH production thus masking abnormal FSH elevation, which would otherwise reveal DOR. Measurement of both FSH and estradiol on cycle day 3 may therefore help decrease the incidence of false—negative testing. Despite its limitations, FSH is commonly used as an ovarian reserve test, and high values have been associated with both poor ovarian response and failure to achieve pregnancy.[14] FSH has particularly high specificity (45-100%) for predicting poor response to ovarian stimulation (usually defined as ≤4 retrieved oocytes) using multiple cut-off points >10 IU/L (10-20 IU/L), but its sensitivity is generally poor (11-86%) and decreases with increasing FSH cut-off points.[14,15] In terms of predicting failure to conceive, FSH testing is still specific (50-100%) but much less sensitive (3-65%) using similar cut-offs (Table 1).[14,15] This test is still clinically useful since an abnormally elevated FSH result is almost synonymous with late DOR (high positive predictive value), but the majority of women who are tested (including those with DOR) will have a normal test result (low negative predictive value). Moreover, a single abnormal FSH value in a woman <40 years of age may not predict a poor response to stimulation or failure to achieve pregnancy,[16] and should prompt repeat testing. In terms of OHSS, FSH has no predictive value for this complication.

Antimüllerian hormone

AMH is a glycoprotein that belongs to the transforming growth factor-β superfamily and is produced in the female exclusively by granulosa cells of small and large preantral and small antral follicles.[4] Although AMH was first noted to

TABLE 1
Comparison of ovarian reserve markers follicle-stimulating hormone, antral follicular count, and antimüllerian hormone

Test	Basal FSH	AFC	AMH
Year described	1988	1997	2002
Timing	Day 2–5 of menstrual cycle	Day 2–5 of menstrual cycle	Any day
Temporal change indicating ovarian aging	Latest	Early	Earliest
Intracycle variability	Clinically significant	Clinically significant	Minimal
Intercycle variability	Clinically significant	Minimal	Minimal
Methodology	Automated	Ultrasound	ELISA/automated
Cost, $	95–125	300–500	76–95
Advantages	Widespread use	Immediate results; good predictive value for stimulation ovarian response, including predicting OHSS	Reliable; high sensitivity; good predictive value for stimulation ovarian response, including predicting OHSS
Limitations	Reliability; low sensitivity; dependent on functional HPO axis; less precision due to intercycle and intracycle variability; does not predict OHSS	Interobserver variability (sonographer-dependent); requires cost of ultrasound technician and availability of ultrasound machine; significant intercycle variation in overweight and obese	Lack of international standardized assay; requires careful sample preparation and storage
Cut-offs used for determining sensitivities and specificities	10–20 IU/L	<3–4 follicles (total)	0.1–1.66[a] ng/mL or <0.1–<0.3[b] ng/mL
Sensitivity for poor response, %	11–86[15]	9–73[15]	44–97[4]
Specificity for poor response, %	45–100[15]	73–97[15]	41–100[4]
AUC for poor response	0.68 (95% CI 0.61–0.74)[42]	0.76 (95% CI 0.70–0.82)[42]	0.78 (95% CI 0.72–0.84)[42]
Sensitivity for nonpregnancy, %	3–65[15]	7–34[15]	19–66[32]
Specificity for nonpregnancy, %	50–100[15]	64–98[15]	55–89[32]

AFC, antral follicular count; *AMH*, antimüllerian hormone; *AUC*, area under the curve; *CI*, confidence interval; *ELISA*, enzyme-linked immunosorbent assay; *FSH*, follicle-stimulating hormone; *HPO*, hypothalamus-pituitary-ovarian; *OHSS*, ovarian hyperstimulation syndrome.

[a] Cut-offs used for calculating sensitivities and specificities for prediction of poor ovarian response; [b] Cut-offs used for calculating sensitivities and specificities for prediction of nonpregnancy.

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be present in follicular fluid in 1993,[17] its function was incompletely understood. It was later that its clinical utility as an ovarian reserve marker was first reported[18] following studies of AMH–deficient mice demonstrating accelerated atresia when the AMH gene was deficient.[19,20] While lack of AMH does not seem to affect fertility in female mice, increased recruitment of primordial follicles leads to early depletion in ovaries of AMH–deficient mice.[19,20] The ovary begins producing AMH in utero at about 36 weeks of gestation,[21] its levels rise in young women beginning in adolescence and peak at about 25 years of age, then gradually decline until reaching undetectable levels a few years prior to menopause. AMH acts as a leading negative paracrine regulator of early folliculogenesis as it inhibits recruitment of primary follicles from the primordial pool, prevents selection of follicles by FSH, and inhibits aromatase.[22-24] Since AMH is expressed during normal early folliculogenesis (secreted by early follicles up to 6 mm), it is relatively independent of gonadotropins circulating at physiologic levels and allows for testing anytime throughout the cycle. While several earlier studies suggested that AMH is relatively stable throughout the menstrual cycle in normoovulatory

women,[25-29] other studies noted significant fluctuations within 1 menstrual cycle.[30-33] While this issue remains highly debated, evidence suggests that if significant fluctuations in AMH levels do occur, they are limited to younger women[34] and those with high basal AMH levels,[31] and that in patients with low ovarian reserve (usually aged women) AMH fluctuations have little clinical relevance.[31,34] Moreover, the random and noncyclic fluctuations in AMH indicate that measuring the hormone on a fixed day of the menstrual cycle would not yield any advantage to random assessment.[31]

Sensitivity and specificity

Among all ovarian reserve tests, AMH is considered the earliest and most sensitive. It correlates strongly with the primordial follicle pool, has an inverse correlation with chronologic age,[35,36] reliably predicts ovarian response in ART,[37,38] and is predictive of the timing of the onset of menopause.[39-41] In a systematic review of studies in women undergoing controlled ovarian stimulation with gonadotropins, low AMH cut-off points (0.1-1.66 ng/mL) have been found to have sensitivities ranging between 44-97% and specificities ranging between 41-100% for prediction of poor ovarian response.[4] In a meta-analysis that included 28 studies, AMH was found to have good predictive ability for poor ovarian response, with an area under the curve (AUC) of 0.78.[42] Moreover, AMH has remarkable utility in predicting ovarian hyperstimulation to gonadotropin stimulation, with sensitivities ranging from 53-90.5% and specificities ranging from 70-94.9% when using cut-off values of 3.36-5.0 ng/mL.[4] However, despite its strong correlation with ovarian response to stimulation in ART, AMH is a poor predictor of nonpregnancy with sensitivities between 19-66%, and specificities between 55-89% when using cut-offs ranging from <0.1-1.66 ng/mL (Table 1).[3] Notably, a recent study of the Society for Assisted Reproductive Technology database found that while women with ultralow AMH (<0.16 ng/mL) had 54% cycle cancellation rate, the overall live birth rate per cycle start was 9.5%,[43] supporting the notion that denying infertility treatment solely on the basis of undetectable AMH is not advisable. Similarly, AMH is a poor predictor of pregnancy and live birth following ART, with 2 recent meta-analyses showing the AUC for AMH as predictor of clinical pregnancy and live birth to be 0.63 and 0.61, respectively.[44,45] The sensitivities and specificities for AMH in predicting clinical pregnancy ranged from 34.4-86.2% and 26-78.5%, respectively, with cut-offs ranging between 1.0-3.22 ng/mL (Table 1).[45] Moreover, AMH values were not associated with fecundability in unassisted conceptions in a cohort of fecund women with a history of 1 or 2 losses.[46] These data are consistent with AMH being a predictive marker of oocyte quantity but not quality.

Limitations

The main limitations of the AMH test relate to assay variability and lack of standardized international assay. Prior to 2010, 2 different assays were used; the European and US assays were developed independently with different antibodies and reported very different results, using different units. That problem was thought to be resolved by the manufacture of both enzyme-linked immunosorbent assays by the same company and the development of a new assay (Gen II, Beckman Coulter Inc, Brea, CA) that combines the best features of both.[47] However, several studies have demonstrated intraassay/interassay differences, between laboratory differences, and sample stability and storage issues related to the Gen II assay.[48] Automated AMH assay platforms offer improvement of greater precision (4-fold), faster turnaround time (18 minutes vs 6 hours), and greater sensitivity (10-fold) compared to current enzyme-linked immunosorbent assay based assays.[49,50] These new platforms are being actively used outside the United States in Europe and Asia. Most recently, one such automated platform received Food and Drug Administration clearance for determining ovarian reserve.[51]

Age-specific AMH values

Age-specific AMH values have been provided by several studies[52-58] and are informative for the population of women presenting to fertility clinics. Thus, the reference values are age appropriate and not referenced to a general population of women independent of age. Such values may be analogous to a Z score (compared to an age-matched cohort) for ovarian reserve. As a general guideline we consider the lower limit of age-appropriate serum AMH values for the following in 5-year age intervals to be approximately: 0.5 ng/mL for 45 years, 1 ng/mL for 40 years, 1.5 ng/mL for 35 years, 2.5 ng/mL for 30 years, and 3.0 ng/mL for 25 years. These are likely to be conservative estimates as recent iterations of the most widely used AMH assay, Gen II, report about 30-40% higher mean values compared to these guidelines. So, in practice, if a 35-year-old woman presents with an AMH of 1 ng/mL this may be of concern as one would have conservatively expected an AMH value of at least 1.5 ng/mL. An AMH of 1 ng/mL is what one might conservatively expect from a 40-year-old woman. Such a lower observed value in the context of the rest of a patient's history (ie, smoking or early onset of menopause of her mother) may point to taking a more expeditious approach to her fertility treatment depending on her expectations for conceiving >1 child in the future.

Factors affecting AMH results

In addition, when interpreting a patient's AMH test results it is important for the clinician to consider the effects of possible influencing factors to avoid inaccurate assessment of ovarian reserve. Table 2 summarizes the biological, reproductive, and environmental/lifestyle factors suggested to affect AMH levels. For example, polycystic ovary syndrome (PCOS) is associated with elevated AMH levels,[59,60] while ovarian suppression related to oral contraceptive pills or GnRH agonist administration can decrease AMH levels, with AMH levels generally returning to baseline within 3-4 months of oral contraceptive discontinuation.[61-67] Among

environmental/lifestyle factors, current smoking,[63,66,68-70] low vitamin-D levels,[71] and obesity have been associated with lower AMH levels, although the effect of obesity is inconsistent among studies.[61,63,66,72-78]

Antral follicular count

AFC is the sum of follicles in both ovaries as observed on ultrasound in the early follicular phase (day 2-4) of the menstrual cycle. Antral follicles are defined as those measuring 2-10 mm in largest mean diameter on 2-dimensional plane. AFC is easy to carry out, provides an immediate result, and has good intercycle reliability and good interobserver reliability when measured in experienced centers using a minimal number of sonographers. Its precision is compromised with overweight and obese individuals or when using multiple sonographers.[3,15] As suggested by a meta-analysis, a low AFC is associated with poor ovarian response to ovarian stimulation during IVF, but has poor predictability for pregnancy. Across general IVF study populations of patients at both low and high risk of DOR, low AFC cut-off points of 3-4 follicles (both ovaries combined) are highly specific (73-97%) for predicting poor ovarian response (<3-4 oocytes, cycle cancellation) but have low sensitivity (9-73%).[15] In another meta-analysis, the AUC for AFC in predicting poor ovarian response was 0.76.[42] In terms of nonpregnancy prediction, AFC is still specific (64-98%) but much less sensitive (7-34%).[15] There are several limitations to AFC measurement. It must be carried out at the beginning of a cycle due to intracycle variation. In addition, AFC has inherent variability related to technology and interobserver variability. Significant variation in AFC has been observed both between as well as within centers.[3,15] This variation may be caused by differences in operator training, number of sonographers, methodology as well as criteria for measuring antral follicles, and differences in ultrasound technology (eg, resolution of ultrasound, 2- vs 3-dimensional). In addition, AFC has a tendency to overestimate the number of FSH-sensitive follicles and oocytes retrieved since it inevitably also measures atretic follicles of the same size.[79,80] Moreover, greater intracycle and intercycle variation has been observed in overweight and obese women, limiting the predictive value of AFC in this subpopulation of women, which is ever increasing in industrial nations.[3,15] In terms of OHSS prediction, both AFC and AMH demonstrate strong predictive value for predicting those at greatest risk for OHSS.

TABLE 2
Effect of biological, reproductive, and environmental/lifestyle factors on antimüllerian hormone

Potential factor	Effect on AMH levels
Biological characteristics	
Race and ethnicity	White higher than black, Chinese, and Latina[78,120,121]
Systemic illness (eg, Crohn's, SLE)	Decrease[122,123]
BRCA1 carrier	Decrease[124,125]
FMR1 premutation	Decrease[126,127]
Reproductive factors	
Ovarian suppression (OCPs, GnRH agonists)	Decrease[61-67]
Polycystic ovarian syndrome	Increase[102-106]
Current pregnancy	Decrease[63]
Parity	Increase[63,78]
History of ovarian surgery	Decrease[128,129]
Endometriosis	Decrease[130,131]
Granulosa cell tumor	Increase[132,133]
Environmental/lifestyle	
Body mass index (obesity)	Inconsistent—decrease or no change[61,63,66,72-78]
Socioeconomic status	No effect[63]
Past smoking	No effect[63,28]
Current smoking	Decrease[63,66,68-70]
Chemotherapy	Decrease[97-99]
Low vitamin-D level	Decrease[71]
Alcohol use	No effect[63]
Physical exercise	No effect[63]

AMH, antimüllerian hormone; *BRCA-1*, breast cancer gene-1; *FMR1*, fragile X mental retardation 1; *GnRH*, gonadotropin releasing-hormone; *OCPs*, oral contraceptive pills; *SLE*, systemic lupus erythematosus.

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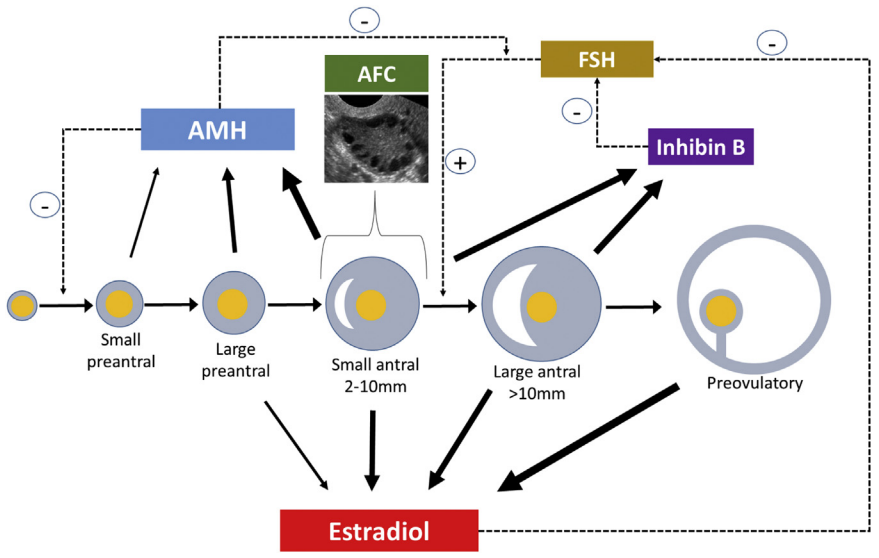
Which ovarian reserve test should I choose?

A large body of evidence has demonstrated greater clinical value of AMH and AFC compared to FSH.[5] AMH will decline years prior to a rise in FSH and thus, is an earlier, more sensitive real-time biomarker of ovarian reserve. It is now well recognized that AMH is a direct product of both cumulus and mural granulosa cells from preantral and small antral follicles during early folliculogenesis. AMH has a greater correlation with the primordial follicle pool (egg supply) compared to ovarian markers produced by follicles during late folliculogenesis (inhibin B and estradiol) and compared to indirect markers such as FSH. In comparison, AFC measures only antral follicles that can be visualized by ultrasound in

FIGURE
Follicular stages reflected by ovarian reserve tests



Estradiol production by follicle increases as follicle develops being highest at preovulatory stage. Inhibin B is produced by granulosa cells of small and large antral follicles. Both estradiol and inhibin B inhibit pituitary secretion of follicle-stimulating hormone (FSH). Antimüllerian hormone (AMH) is produced by granulosa cells of primary and secondary follicles, but mostly small antral follicles. In comparison, antral follicular count (AFC) pertains only to small antral follicles (2-10 mm) as measured by transvaginal ultrasound. No ovarian reserve test is directly reflective of primordial follicular pool. Arrow thickness indicates relative production of ovarian marker.

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contrast to AMH, which reflects an additional population of preantral follicles, thus serving as a better proxy of the primordial follicle pool. This can be more easily understood by referring to the Figure, which characterizes the stage of follicles reflected by the different ovarian reserve markers. Comparisons of AFC and AMH level have generally yielded similar predictive value for ovarian response and outcome in 3 meta-analyses.[3,15,42] However, in marked contrast to these reports, 4 recent large, prospective, multicenter trials in IVF/intracytoplasmic sperm injection patients consistently concluded that AMH was a better predictor of the number of oocytes retrieved as well as categorization of low and high responders than AFC.[81-84] Due to the limitations of AFC in terms of sonographer-dependent variability and technical aspects of ultrasound equipment[79] and the increasing advantages of AMH testing in terms of patient convenience and assay robustness, AMH is more and more being recognized as the preferred biomarker of ovarian response to controlled ovarian stimulation. A comparison among the 3 ovarian reserve tests is summarized in Table 1.

What to do in case of discordance between ovarian reserve tests?

Since information about >1 ovarian reserve test is often available for infertility patients, it is not uncommon to have discordant test results that may complicate patient counseling and decision-making regarding the most appropriate treatment. In a large study of 5354 women that examined discordance between AMH and FSH results obtained by a single reference laboratory, 1 in 5 women were found to have discordant AMH and FSH values defined as AMH <0.8 ng/mL (concerning) with FSH <10 IU/L (reassuring) or AMH ≥0.8 ng/mL (reassuring) with FSH ≥10 IU/L (concerning).[85] Of the women with reassuring FSH values (n = 4469), the concerning AMH values were found in 1 in 5 women in a highly age-dependent fashion with increasing frequency as women were ≥38 years old. This is not surprising, since AMH is more sensitive than FSH is in diagnosing DOR and would normally reach abnormal levels before FSH levels become abnormal. This type of discordance may therefore be viewed as the natural history of DOR. On the other hand, of the women with reassuring AMH values (n = 3742), 1 in 18 had concerning FSH values, a frequency that did not vary significantly by age.[85] Another study investigated AMH and FSH values in 366 infertility patients with DOR undergoing their first IVF cycle, finding discordance between age-specific values in 38.6% of women.[86] Interestingly, the authors found that at age 34-42 years, normal AMH carried better prognosis than normal FSH in terms of oocyte yield, while at age >42 years normal FSH was associated with better prognosis than normal AMH. For both age ranges, the group with both normal AMH and FSH always had the best prognosis while the one with both values abnormal had the worst prognosis.[86]

A different study assessing discordance between AMH and AFC in patients undergoing IVF found that 32.3% of women had discordant results.[87] The discordant groups with low to normal ovarian reserve had ovarian responsiveness intermediate between those with concordantly low and concordantly normal AMH and AFC. Likewise the discordant groups with normal to high ovarian reserve had ovarian responsiveness intermediate between those with concordantly normal and concordantly high AMH and AFC. In addition, within each AFC category, those with higher AMH had significantly higher number of oocytes retrieved.[87]

Therefore, when ovarian reserve tests fall into discordant categories, it would be reasonable to consider the ovarian

reserve to be an intermediate between the 2 tests. For purpose of IVF stimulation dosing, for example, this would mean choosing an intermediate dose of gonadotropin as compared to those with concordant AMH and AFC categories on either end.

Who should get ovarian reserve testing and how to use it in clinical practice?

Historically, ovarian reserve testing was used for prediction of ovarian response to controlled ovarian stimulation in ART, helping to identify patients who are more likely to have a poor response or hyperresponse to gonadotropins. Over the past decade, since the discovery of AMH as an ovarian reserve marker, it has been increasingly recognized that AMH testing may have utility for a variety of other clinical applications in reproductive medicine (Table 3).[48,88]

TABLE 3
Indications for ovarian reserve testing

Women undergoing infertility evaluation/treatment
Individualization of assisted reproductive technology ovarian stimulation protocol and dosing
History of premature ovarian failure (insufficiency) or early menopause
Polycystic ovarian syndrome
Women considering elective (social egg) freezing
Oocyte donors
Fertility preservation before and after gonadotoxic treatment
Preoperative prior to ovarian surgery in reproductive-age women
Diagnosis and recurrence surveillance for granulosa cell tumors
Perimenopause
Women with BRCA-1 or FMR1 premutation

BRCA-1, breast cancer gene-1; *FMR1*, fragile X mental retardation 1.

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Assisted reproductive technology

The usefulness of AMH in predicting ovarian response to controlled ovarian stimulation in ART has led to AMH-based pretreatment counselling and individualization of ART stimulation protocols including choice of regimens and dose adjustments.[38,56,89,90] The following general guidelines are useful for clinical practice:

- AMH <0.5 ng/mL predicts poor ovarian response in IVF with yield of ≤4 oocytes.[4,91] In such cases, a discussion with the patient about the short window of opportunity to conceive seems warranted.[5] Ovarian stimulation protocols in these patients should include the ones reserved for the most challenging patients (ie, using microdose GnRH agonist flare with high starting dose or late luteal estradiol priming with or without late luteal presuppression antagonist with high starting dose of gonadotropins).
- AMH level ≥1.0 ng/mL but ≤3.5 ng/mL if age appropriate is consistent with normal ovarian response to ovarian stimulation. IVF protocols for these patients should include standard GnRH agonist or antagonist, with adjustments based on age-specific values.[51-58]
- AMH >3.5 ng/mL predicts hyperresponse to ovarian stimulation and is associated with significantly higher risk of OHSS.[4] Such patients would benefit from mild lower dose regimens, and protocols that minimize OHSS risk (eg, GnRH antagonist with GnRH agonist trigger, possible addition of metformin).[92,93]

Menopause

Several studies have suggested that AMH in conjunction with age may be able to predict the timing of menopause with reasonable accuracy in late reproductive-age women.[39-41] A recent report from the Study of Women's Health Across the Nation provided data on the use of AMH in predicting the final menstrual period (FMP) using the ultra-sensitive picoAMH assay, enabling especially accurate measurements at the lower end of detection.[94] At baseline, women were aged 42-52 years and blood was collected serially until the first visit after FMP. AMH was measured in a total of 1560 women. Women whose AMH values were 5-10 pg/mL had a 75% chance of having their FMP within 24 ± 3 months, while women with AMH levels of 25-≥30 pg/mL had <2.5% chance of having their FMP within 2 years.[94] However, in a Dutch cohort study that included women of younger reproductive age (range 21-46 years), while AMH was predictive for age at natural menopause, the prediction intervals were broad and extreme ages at menopause could not be predicted.[95] The limited predictive ability of AMH for menopause in the general population may make this marker currently unsuitable for individualized counselling of women regarding their reproductive lifespan. Research is ongoing in pursuit of developing more accurate biomarker-based formulas for prediction of menopause timing.

Fertility preservation

With rising numbers of young girls and women being successfully cured by life-saving, but often gonadotoxic treatments, subsequent reproductive health has become a major quality-of-life issue, and the ability to predict which patients may lose their fertility or ovarian function as a result of treatment has become of increasing importance. AMH testing in young cancer patients both pretreatment and posttreatment has become a useful tool for assessment of iatrogenic damage to the ovarian follicular reserve inflicted by gonadotoxic

chemotherapy agents or pelvic irradiation, and may help in fertility preservation counselling and strategies. Chemotherapy and radiotherapy can both have deleterious effects on ovarian function and consequently AMH levels, with the extent of injury dependent on the patient's age, type of treatment, and treatment dose.[96] Studies have demonstrated that women with higher pretreatment AMH levels have higher post-chemotherapy levels and display faster recovery rate in AMH levels once treatment had been completed.[97,98] Importantly, 2 studies involving breast cancer patients provided prognostic tools by which clinicians can inform patients more precisely regarding ovarian function after chemotherapy using pretreatment AMH levels, with other important variables.[99,100] The first prognostic tool demonstrated that all women, regardless of age, with AMH levels of <0.54 ng/mL will have amenorrhea posttreatment.[99] The second tool is a prognostic scoring system based on a patient's age, pretreatment AMH levels, and body mass index with 1 point awarded for each of age <40 years, AMH level >0.7 ng/mL, and body mass index >25 kg/m^2.[100] The likelihood of, and time to, recovery of ovarian function after completion of chemotherapy improves with increasing number of points. For example, menses returned in 75% of women with 3 points by 160 days whereas only 25% of women with 0 points had a return of ovarian function by 221 days.[100] Since both of these prognostic systems were developed in breast cancer patients, and the systemic effects of differing forms of cancer and their treatment vary, it is not possible to generalize the results above to all cancers. Therefore, further research into different types of malignancies and treatment regimens is required to build up a panel of prognostic tools. Moreover, it should be emphasized that since the primary outcome of the research thus far has been posttreatment amenorrhea/premature ovarian insufficiency, there is not yet direct evidence that pretreatment AMH levels can predict subsequent fertility. This important outcome should be the focus of future investigations in this field.

Polycystic ovarian syndrome

It has been suggested that AMH plays an important role in the pathogenesis of PCOS.[101] Substantial evidence indicates that AMH correlates strongly with the severity of various hallmarks of PCOS, including polycystic ovarian morphology, hyperandrogenism, and oligo/anovulation.[102-107] Moreover, elevated serum AMH concentrations are predictive of poor response to various treatments of PCOS including weight loss, ovulation induction, and laparoscopic ovarian drilling, while improvement in various clinical parameters following treatment is associated with serum AMH decline, further supporting an important role for AMH in the pathophysiology of this syndrome.[101] A cut-off of AMH >5.0 ng/mL has been shown to be highly diagnostic of PCOS (AUC 0.973, sensitivity 92%, specificity 97%), and suggested to be incorporated as a diagnostic criterion for this syndrome.[59,60] Thus, AMH testing could be considered in the routine workup of PCOS patients and could aid in establishing the diagnosis, provide insight to the severity and potential treatment resistance, as well as alerting physicians to the increased risk of OHSS if ovulation induction is being considered.

Table 3 summarizes the current indications for AMH testing.

Ovarian reserve screening for the general reproductive-age female population?

It is estimated that approximately 10% of the general female population will undergo accelerated loss of ovarian reserve leading to loss of fertility from their mid-30s and early menopause by age 45 years.[108,109] In the past, this was of minimal concern from a public health standpoint since most women had completed their family plans by their mid−30s. However, over the past 2 decades the average age of a mother at her first birth has steadily increased and is currently 30 years of age in the Western world, with a further 1 in 5 women not having commenced pregnancy attempt by 35 years of age.[110,111] While some of these women have made a conscious decision not to have children, the majority still do want children but have simply not begun trying for a baby, often due to gaps of knowledge about age-related fertility decline.[112,113] Importantly, recent studies have shown that information about one's ovarian reserve would lead individuals to modify life choices.[112,114] One study showed that among health care workers, if testing of the individual or individual's partner indicated DOR, 48% would try to have a child sooner, 21% would opt for oocyte cryopreservation, 7% would try to find a partner sooner, 7% would pursue adoption, and 3% would select embryo cryopreservation. Only 14% would not actively pursue treatment or make lifestyle changes.[114]

Despite the consensus that AMH is an excellent marker of ovarian reserve, there is currently no agreement on the use of AMH to screen for ovarian reserve in the general, noninfertile population.[115,116] Concerns raised by opponents of such testing are that a poor result does not definitively mean diminished chances of natural conception,[115,116] while an abnormally low value may also lead to substantial anxiety with multiple potential negative consequences (eg, premature termination of education and career development to have children, seeking motherhood outside of a stable relationship). However, contrary to this argument, a recent study showed that bankers and non-bankers of oocytes have a surprising congruent relational status and reproductive choices, indicating that freezing oocytes does not appear to influence the life choices of the women. The study provides insights into the important psychological aspect of reassurance associated with preventive oocyte banking, expressed by high satisfaction after banking in combination with a decreased intention of ever using the eggs.[117] An important ethical

consideration, however, is that oocyte banking is costly (~$10,000) and may not be affordable to many women identified as having low ovarian reserve by AMH screening.

Proponents of ovarian reserve screening of the general population of reproductive-age women argue that it may offer several advantages.[118] First, women identified as having low ovarian reserve are thought to be at increased risk of early loss of fertility potential in the longer term. However, evidence in support of this argument is conflicting as 1 study found that women with low AMH (<0.7 ng/mL) have significantly decreased fecundability after adjusting for age,[119] while a more recent study reported that lower (<1.0 ng/mL) and higher (>3.5 ng/mL) AMH values were not associated with fecundability in unassisted conceptions in a cohort of fecund women with a history of 1 or 2 losses.[46] Second, women often disregard generic advice to avoid delaying conception >30 years of age, yet studies suggest that personalized risk assessment tools such as ovarian reserve testing can actually affect individual's family planning.[112,114] Finally, it is reasonable to argue that women have a right, based on the ethical concept of autonomy, to be made aware of ovarian reserve screening, so that they themselves can determine if ovarian reserve testing is useful in assisting them with reproductive life planning.[118] However, before firm recommendations can be made, large longitudinal studies are needed to determine whether such screening strategies may be beneficial from a population health perspective.

Conclusions and recommendations

Evaluation of ovarian reserve can help identify patients who will have poor response or hyperresponse to ovarian stimulation for ART and individualize treatment protocols to achieve optimal response while minimizing safety risks. It may inform patients regarding their reproductive lifespan and menopausal timing, and also aid in counseling and treatment strategy planning of young female cancer patients receiving gonadotoxic therapy. In addition, it may aid in establishing the diagnosis of PCOS and provide insight into disease severity. Finally, ovarian reserve testing may be considered as a screening tool in selected populations of women for assisting in their reproductive life planning. The ideal ovarian reserve test should be convenient, be reproducible, display little if any intracycle and intercycle variability, and demonstrate high specificity to minimize the risk of wrongly diagnosing women as having DOR and accurately identify those at greatest risk of developing OHSS prior to fertility treatment. While there is currently no perfect ovarian reserve test, both AFC and AMH level have good predictive value and are superior to day-3 FSH. The convenience of untimed sampling, age-specific values, availability of an automated platform, and potential standardization of AMH assay make this test the preferred biomarker for the evaluation of ovarian reserve in most women. It is important to consider age-specific values when interpreting results and to remember that conditions such as PCOS and hormonal suppression can affect the values obtained. ■

REFERENCES

1. Practice Committee of the American Society for Reproductive Medicine. Testing and interpreting measures of ovarian reserve: a committee opinion. Fertil Steril 2015;103:e9-17.
2. Tal R, Seifer DB. Potential mechanisms for racial and ethnic differences in antimüllerian hormone and ovarian reserve. Int J Endocrinol 2013;2013:818912.
3. Broer SL, Mol BW, Hendriks D, Broekmans FJ. The role of antimüllerian hormone in prediction of outcome after IVF: comparison with the antral follicle count. Fertil Steril 2009;91:705-14.
4. La Marca A, Sighinolfi G, Radi D, et al. Antimullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). Hum Reprod Update 2010;16:113-30.
5. Toner JP, Seifer DB. Why we may abandon basal follicle-stimulating hormone testing: a sea change in determining ovarian reserve using antimüllerian hormone. Fertil Steril 2013;99:1825-30.
6. Iliodromiti S, Nelson SM. Ovarian response biomarkers: physiology and performance. Curr Opin Obstet Gynecol 2015;27:182-6.
7. Scott RT Jr, Hofmann GE, Oehninger S, Muasher SJ. Intercycle variability of day 3 follicle-stimulating hormone levels and its effect on stimulation quality in in vitro fertilization. Fertil Steril 1990;54:297-302.
8. Kwee J, Schats R, McDonnell J, Lambalk CB, Schoemaker J. Intercycle variability of ovarian reserve tests: results of a prospective randomized study. Hum Reprod 2004;19:590-5.
9. Jayaprakasan K, Campbell B, Hopkisson J, Clewes J, Johnson I, Raine-Fenning N. Establishing the intercycle variability of three-dimensional ultrasonographic predictors of ovarian reserve. Fertil Steril 2008;90:2126-32.
10. Sharara FI, Scott RT Jr, Seifer DB. The detection of diminished ovarian reserve in infertile women. Am J Obstet Gynecol 1998;179:804-12.
11. Muasher SJ, Oehninger S, Simonetti S, et al. The value of basal and/or stimulated serum gonadotropin levels in prediction of stimulation response and in vitro fertilization outcome. Fertil Steril 1988;50:298-307.
12. Scott RT, Toner JP, Muasher SJ, Oehninger S, Robinson S, Rosenwaks Z. Follicle-stimulating hormone levels on cycle day 3 are predictive of in vitro fertilization outcome. Fertil Steril 1989;51:651-4.
13. Toner JP, Philput CB, Jones GS, Muasher SJ. Basal follicle-stimulating hormone level is a better predictor of in vitro fertilization performance than age. Fertil Steril 1991;55:784-91.
14. Esposito MA, Coutifaris C, Barnhart KT. A moderately elevated day 3 FSH concentration has limited predictive value, especially in younger women. Hum Reprod 2002;17:118-23.
15. Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. Hum Reprod Update 2006;12:685-718.
16. Roberts JE, Spandorfer S, Fasouliotis SJ, Kashyap S, Rosenwaks Z. Taking a basal follicle-stimulating hormone history is essential before initiating in vitro fertilization. Fertil Steril 2005;83:37-41.
17. Seifer DB, MacLaughlin DT, Penzias AS, et al. Gonadotropin-releasing hormone agonist-induced differences in granulosa cell cycle kinetics are associated with alterations in follicular fluid mullerian-inhibiting substance and androgen content. J Clin Endocrinol Metab 1993;76:711-4.
18. Seifer DB, MacLaughlin DT, Christian BP, Feng B, Shelden RM. Early follicular serum mullerian-inhibiting substance levels are associated with ovarian response during assisted reproductive technology cycles. Fertil Steril 2002;77:468-71.
19. Behringer RR, Finegold MJ, Cate RL. Mullerian-inhibiting substance function during mammalian sexual development. Cell 1994;79:415-25.
20. Durlinger AL, Kramer P, Karels B, et al. Control of primordial follicle recruitment by anti-mullerian hormone in the mouse ovary. Endocrinology 1999;140:5789-96.
21. Rajpert-De Meyts E, Jorgensen N, Graem N, Muller J, Cate RL, Skakkebaek NE. Expression of anti-mullerian hormone during

normal and pathological gonadal development: association with differentiation of Sertoli and granulosa cells. J Clin Endocrinol Metab 1999;84:3836-44.
22. Garcia-Velasco JA, Moreno L, Pacheco A, et al. The aromatase inhibitor letrozole increases the concentration of intraovarian androgens and improves in vitro fertilization outcome in low responder patients: a pilot study. Fertil Steril 2005;84:82-7.
23. Grossman MP, Nakajima ST, Fallat ME, Siow Y. Mullerian-inhibiting substance inhibits cytochrome P450 aromatase activity in human granulosa lutein cell culture. Fertil Steril 2008;89(Suppl):1364-70.
24. Durlinger AL, Gruijters MJ, Kramer P, et al. Anti-mullerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology 2001;142:4891-9.
25. 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;20:923-7.
26. Hehenkamp WJ, Looman CW, Themmen AP, de Jong FH, Te Velde ER, Broekmans FJ. Anti-mullerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. J Clin Endocrinol Metab 2006;91:4057-63.
27. La Marca A, Stabile G, Artenisio AC, Volpe A. Serum anti-mullerian hormone throughout the human menstrual cycle. Hum Reprod 2006;21:3103-7.
28. Tsepelidis S, Devreker F, Demeestere I, Flahaut A, Gervy C, Englert Y. Stable serum levels of anti-mullerian hormone during the menstrual cycle: a prospective study in normo-ovulatory women. Hum Reprod 2007;22:1837-40.
29. van Disseldorp J, Lambalk CB, Kwee J, et al. Comparison of inter- and intra-cycle variability of anti-mullerian hormone and antral follicle counts. Hum Reprod 2010;25:221-7.
30. Wunder DM, Bersinger NA, Yared M, Kretschmer R, Birkhauser MH. Statistically significant changes of antimüllerian hormone and inhibin levels during the physiologic menstrual cycle in reproductive age women. Fertil Steril 2008;89:927-33.
31. Sowers M, McConnell D, Gast K, et al. Anti-mullerian hormone and inhibin B variability during normal menstrual cycles. Fertil Steril 2010;94:1482-6.
32. Hadlow N, Longhurst K, McClements A, Natalwala J, Brown SJ, Matson PL. Variation in antimüllerian hormone concentration during the menstrual cycle may change the clinical classification of the ovarian response. Fertil Steril 2013;99:1791-7.
33. Kissell KA, Danaher MR, Schisterman EF, et al. Biological variability in serum anti-mullerian hormone throughout the menstrual cycle in ovulatory and sporadic anovulatory cycles in eumenorrheic women. Hum Reprod 2014;29:1764-72.
34. Overbeek A, Broekmans FJ, Hehenkamp WJ, et al. Intra-cycle fluctuations of anti-mullerian hormone in normal women with a regular cycle: a re-analysis. Reprod Biomed Online 2012;24:664-9.
35. Hansen KR, Hodnett GM, Knowlton N, Craig LB. Correlation of ovarian reserve tests with histologically determined primordial follicle number. Fertil Steril 2011;95:170-5.
36. Kelsey TW, Anderson RA, Wright P, Nelson SM, Wallace WH. Data-driven assessment of the human ovarian reserve. Mol Hum Reprod 2012;18:79-87.
37. Majumder K, Gelbaya TA, Laing I, Nardo LG. The use of anti-mullerian hormone and antral follicle count to predict the potential of oocytes and embryos. Eur J Obstet Gynecol Reprod Biol 2010;150:166-70.
38. Yates AP, Rustamov O, Roberts SA, et al. Anti-mullerian hormone-tailored stimulation protocols improve outcomes whilst reducing adverse effects and costs of IVF. Hum Reprod 2011;26:2353-62.
39. Broer SL, Eijkemans MJ, Scheffer GJ, et al. Anti-mullerian hormone predicts menopause: a long-term follow-up study in normoovulatory women. J Clin Endocrinol Metab 2011;96:2532-9.
40. Freeman EW, Sammel MD, Lin H, Boorman DW, Gracia CR. Contribution of the rate of change of antimüllerian hormone in estimating time to menopause for late reproductive-age women. Fertil Steril 2012;98:1254-9. e1-2.
41. Freeman EW, Sammel MD, Lin H, Gracia CR. Anti-mullerian hormone as a predictor of time to menopause in late reproductive age women. J Clin Endocrinol Metab 2012;97:1673-80.
42. Broer SL, van Disseldorp J, Broeze KA, et al. Added value of ovarian reserve testing on patient characteristics in the prediction of ovarian response and ongoing pregnancy: an individual patient data approach. Hum Reprod Update 2013;19:26-36.
43. Seifer DB, Tal O, Wantman E, Edul P, Baker VL. Prognostic indicators of assisted reproduction technology outcomes of cycles with ultralow serum antimüllerian hormone: a multivariate analysis of over 5,000 autologous cycles from the Society for Assisted Reproductive Technology Clinic Outcome Reporting System database for 2012-2013. Fertil Steril 2016;105:385-93.e3.
44. Iliodromiti S, Kelsey TW, Wu O, Anderson RA, Nelson SM. The predictive accuracy of anti-mullerian hormone for live birth after assisted conception: a systematic review and meta-analysis of the literature. Hum Reprod Update 2014;20:560-70.
45. Tal R, Tal O, Seifer BJ, Seifer DB. Anti-müllerian hormone as predictor of implantation and clinical pregnancy after assisted conception: a systematic review and meta-analysis. Fertil Steril 2015;103:119-30.e3.
46. Zarek SM, Mitchell EM, Sjaarda LA, et al. Is anti-mullerian hormone associated with fecundability? Findings from the EAGeR trial. J Clin Endocrinol Metab 2015;100:4215-21.
47. Nelson SM, La Marca A. The journey from the old to the new AMH assay: how to avoid getting lost in the values. Reprod Biomed Online 2011;23:411-20.
48. Broer SL, Broekmans FJ, Laven JS, Fauser BC. Anti-mullerian hormone: ovarian reserve testing and its potential clinical implications. Hum Reprod Update 2014;20:688-701.
49. Nelson SM, Pastuszek E, Kloss G, et al. Two new automated, compared with two enzyme-linked immunosorbent, antimüllerian hormone assays. Fertil Steril 2015;104:1016-21.e6.
50. van Helden J, Weiskirchen R. Performance of the two new fully automated anti-mullerian hormone immunoassays compared with the clinical standard assay. Hum Reprod 2015;30:1918-26.
51. FDA News. FDA Grants Clearance to Roche's Anti-Mullerian Hormone Test Elecsys. Available at: http://www.fdanews.com/articles/179932-fda-grants-clearance-to-roches-anti-mullerian-hormone-test-elecsys. Accessed March 27, 2017.
52. La Marca A, Sighinolfi G, Giulini S, et al. Normal serum concentrations of anti-mullerian hormone in women with regular menstrual cycles. Reprod Biomed Online 2010;21:463-9.
53. Almog B, Shehata F, Suissa S, et al. Age-related normograms of serum antimüllerian hormone levels in a population of infertile women: a multicenter study. Fertil Steril 2011;95:2359-63.e1.
54. Barad DH, Weghofer A, Gleicher N. Utility of age-specific serum anti-mullerian hormone concentrations. Reprod Biomed Online 2011;22:284-91.
55. Seifer DB, Baker VL, Leader B. Age-specific serum anti-mullerian hormone values for 17,120 women presenting to fertility centers within the United States. Fertil Steril 2011;95:747-50.
56. La Marca A, Papaleo E, Grisendi V, Argento C, Giulini S, Volpe A. Development of a nomogram based on markers of ovarian reserve for the individualization of the follicle-stimulating hormone starting dose in in vitro fertilization cycles. BJOG 2012;119:1171-9.
57. Lee JY, Jee BC, Lee JR, et al. Age-related distributions of anti-mullerian hormone level and anti-mullerian hormone models. Acta Obstet Gynecol Scand 2012;91:970-5.
58. Lie Fong S, Visser JA, Welt CK, et al. Serum anti-mullerian hormone levels in healthy females: a nomogram ranging from infancy to adulthood. J Clin Endocrinol Metab 2012;97:4650-5.
59. Pigny P, Jonard S, Robert Y, Dewailly D. Serum anti-mullerian hormone as a surrogate for antral follicle count for definition of the polycystic ovary syndrome. J Clin Endocrinol Metab 2006;91:941-5.
60. Dewailly D, Gronier H, Poncelet E, et al. Diagnosis of polycystic ovary syndrome (PCOS): revisiting the threshold values of follicle count on ultrasound and of the serum AMH level for the

definition of polycystic ovaries. Hum Reprod 2011;26:3123-9.
61. Shaw CM, Stanczyk FZ, Egleston BL, et al. Serum antimüllerian hormone in healthy premenopausal women. Fertil Steril 2011;95:2718-21.
62. Bentzen JG, Forman JL, Pinborg A, et al. Ovarian reserve parameters: a comparison between users and non-users of hormonal contraception. Reprod Biomed Online 2012;25:612-9.
63. Dolleman M, Verschuren WM, Eijkemans MJ, et al. Reproductive and lifestyle determinants of anti-mullerian hormone in a large population-based study. J Clin Endocrinol Metab 2013;98:2106-15.
64. Kallio S, Puurunen J, Ruokonen A, Vaskivuo T, Piltonen T, Tapanainen JS. Antimüllerian hormone levels decrease in women using combined contraception independently of administration route. Fertil Steril 2013;99:1305-10.
65. Johnson LN, Sammel MD, Dillon KE, Lechtenberg L, Schanne A, Gracia CR. Antimüllerian hormone and antral follicle count are lower in female cancer survivors and healthy women taking hormonal contraception. Fertil Steril 2014;102:774-81.e3.
66. Iliodromiti S, Anderson RA, Nelson SM. Technical and performance characteristics of anti-mullerian hormone and antral follicle count as biomarkers of ovarian response. Hum Reprod Update 2015;21:698-710.
67. Hagen CP, Aksglaede L, Sorensen K, et al. Individual serum levels of anti-mullerian hormone in healthy girls persist through childhood and adolescence: a longitudinal cohort study. Hum Reprod 2012;27:861-6.
68. Freour T, Masson D, Mirallie S, et al. Active smoking compromises IVF outcome and affects ovarian reserve. Reprod Biomed Online 2008;16:96-102.
69. Plante BJ, Cooper GS, Baird DD, Steiner AZ. The impact of smoking on antimüllerian hormone levels in women aged 38 to 50 years. Menopause 2010;17:571-6.
70. Waylen AL, Jones GL, Ledger WL. Effect of cigarette smoking upon reproductive hormones in women of reproductive age: a retrospective analysis. Reprod Biomed Online 2010;20:861-5.
71. 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;97:2450-5.
72. Sahmay S, Usta T, Erel CT, et al. Is there any correlation between AMH and obesity in premenopausal women? Arch Gynecol Obstet 2012;286:661-5.
73. Halawaty S, ElKattan E, Azab H, ElGhamry N, Al-Inany H. Effect of obesity on parameters of ovarian reserve in premenopausal women. J Obstet Gynaecol Can 2010;32:687-90.
74. Steiner AZ, Stanczyk FZ, Patel S, Edelman A. Antimüllerian hormone and obesity: insights in oral contraceptive users. Contraception 2010;81:245-8.
75. Buyuk E, Seifer DB, Illions E, Grazi RV, Lieman H. Elevated body mass index is associated with lower serum anti-mullerian hormone levels in infertile women with diminished ovarian reserve but not with normal ovarian reserve. Fertil Steril 2011;95:2364-8.
76. Freeman EW, Gracia CR, Sammel MD, Lin H, Lim LC, Strauss JF III. Association of anti-mullerian hormone levels with obesity in late reproductive-age women. Fertil Steril 2007;87:101-6.
77. Su HI, Sammel MD, Freeman EW, Lin H, DeBlasis T, Gracia CR. Body size affects measures of ovarian reserve in late reproductive age women. Menopause 2008;15:857-61.
78. Bleil ME, Gregorich SE, Adler NE, Sternfeld B, Rosen MP, Cedars MI. Race/ethnic disparities in reproductive age: an examination of ovarian reserve estimates across four race/ethnic groups of healthy, regularly cycling women. Fertil Steril 2014;101:199-207.
79. Broekmans FJ, de Ziegler D, Howles CM, Gougeon A, Trew G, Olivennes F. The antral follicle count: practical recommendations for better standardization. Fertil Steril 2010;94:1044-51.
80. Fleming R, Seifer DB, Frattarelli JL, Ruman J. Assessing ovarian response: antral follicle count versus anti-Mullerian hormone. Reproductive Biomedicine Online 2015;31:486-96.
81. Andersen AN, Witjes H, Gordon K, Mannaerts B. Xpect Investigators. Predictive factors of ovarian response and clinical outcome after IVF/ICSI following a rFSH/GnRH antagonist protocol with or without oral contraceptive pretreatment. Hum Reprod 2011;26:3413-23.
82. Arce JC, La Marca A, Mirner Klein B, Nyboe Andersen A, Fleming R. Antimüllerian hormone in gonadotropin releasing-hormone antagonist cycles: prediction of ovarian response and cumulative treatment outcome in good-prognosis patients. Fertil Steril 2013;99:1644-53.
83. Polyzos NP, Tournaye H, Guzman L, Camus M, Nelson SM. Predictors of ovarian response in women treated with corifollitropin alfa for in vitro fertilization/intracytoplasmic sperm injection. Fertil Steril 2013;100:430-7.
84. Nelson SM, Klein BM, Arce JC. Comparison of antimüllerian hormone levels and antral follicle count as predictor of ovarian response to controlled ovarian stimulation in good-prognosis patients at individual fertility clinics in two multicenter trials. Fertil Steril 2015;103:923-30.e1.
85. Leader B, Hegde A, Baca Q, et al. High frequency of discordance between antimüllerian hormone and follicle-stimulating hormone levels in serum from estradiol-confirmed days 2 to 4 of the menstrual cycle from 5,354 women in US fertility centers. Fertil Steril 2012;98:1037-42.
86. Gleicher N, Weghofer A, Barad DH. Discordances between follicle stimulating hormone (FSH) and anti-mullerian hormone (AMH) in female infertility. Reprod Biol Endocrinol 2010;8:64.
87. Li HW, Lee VC, Lau EY, Yeung WS, Ho PC, Ng EH. Ovarian response and cumulative live birth rate of women undergoing in-vitro fertilization who had discordant anti-mullerian hormone and antral follicle count measurements: a retrospective study. PLoS One 2014;9:e108493.
88. Seifer DB, Tal R, eds. Antimüllerian hormone: biology, role in ovarian function and clinical significance. New York: Nova Science Publishers; 2016.
89. Arce JC, Nyboe Andersen A, Fernandez-Sanchez M, et al. Ovarian response to recombinant human follicle-stimulating hormone: a randomized, antimüllerian hormone-stratified, dose-response trial in women undergoing in vitro fertilization/intracytoplasmic sperm injection. Fertil Steril 2014;102:1633-40.e5.
90. Nelson SM, Yates RW, Lyall H, et al. Anti-mullerian hormone-based approach to controlled ovarian stimulation for assisted conception. Hum Reprod 2009;24:867-75.
91. Gnoth C, Schuring AN, Friol K, Tigges J, Mallmann P, Godehardt E. Relevance of anti-mullerian hormone measurement in a routine IVF program. Hum Reprod 2008;23:1359-65.
92. Huang X, Wang P, Tal R, Lv F, Li Y, Zhang X. A systematic review and meta-analysis of metformin among patients with polycystic ovary syndrome undergoing assisted reproductive technology procedures. Int J Gynaecol Obstet 2015;131:111-6.
93. Youssef MA, Van der Veen F, Al-Inany HG, et al. Gonadotropin-releasing hormone agonist versus HCG for oocyte triggering in antagonist-assisted reproductive technology. Cochrane Database Syst Rev 2014;10:CD008046.
94. Finkelstein JS LH, Burnett-Bowie S-AM, Santoro N, et al. Utility of anti-mullerian hormone for predicting the time to final menstrual period: the Study of Women's Health Across the Nation (SWAN). Endocrine Society Meeting 2016.
95. Depmann M, Eijkemans MJ, Broer SL, et al. Does anti-mullerian hormone predict menopause in the general population? Results of a prospective ongoing cohort study. Hum Reprod 2016;31:1579-87.
96. Anderson RA, Wallace WH. Antimüllerian hormone, the assessment of the ovarian reserve, and the reproductive outcome of the young patient with cancer. Fertil Steril 2013;99:1469-75.
97. Rosendahl M, Andersen CY, la Cour Freiesleben N, Juul A, Lossl K, Andersen AN. Dynamics and mechanisms of chemotherapy-induced ovarian follicular depletion in women of fertile age. Fertil Steril 2010;94:156-66.
98. Dillon KE, Sammel MD, Prewitt M, et al. Pretreatment antimüllerian hormone levels determine rate of posttherapy ovarian reserve recovery: acute changes in ovarian reserve during and after chemotherapy. Fertil Steril 2013;99:477-83.
99. Anderson RA, Rosendahl M, Kelsey TW, Cameron DA. Pretreatment anti-mullerian hormone predicts for loss of ovarian function after chemotherapy for early breast cancer. Eur J Cancer 2013;49:3404-11.

100. Su HC, Haunschild C, Chung K, et al. Prechemotherapy antimüllerian hormone, age, and body size predict timing of return of ovarian function in young breast cancer patients. Cancer 2014;120:3691-8.
101. Garg D, Tal R. The role of AMH in the pathophysiology of polycystic ovarian syndrome. Reprod Biomed Online 2016;33:15-28.
102. Tal R, Seifer DB, Khanimov M, Malter HE, Grazi RV, Leader B. Characterization of women with elevated antimüllerian hormone levels (AMH): correlation of AMH with polycystic ovarian syndrome phenotypes and assisted reproductive technology outcomes. Am J Obstet Gynecol 2014;211:59.e1-8.
103. La Marca A, Orvieto R, Giulini S, Jasonni VM, Volpe A, De Leo V. Mullerian-inhibiting substance in women with polycystic ovary syndrome: relationship with hormonal and metabolic characteristics. Fertil Steril 2004;82:970-2.
104. Piouka A, Farmakiotis D, Katsikis I, Macut D, Gerou S, Panidis D. Anti-mullerian hormone levels reflect severity of PCOS but are negatively influenced by obesity: relationship with increased luteinizing hormone levels. Am J Physiol Endocrinol Metab 2009;296:E238-43.
105. Nardo LG, Yates AP, Roberts SA, Pemberton P, Laing I. The relationships between AMH, androgens, insulin resistance and basal ovarian follicular status in non-obese subfertile women with and without polycystic ovary syndrome. Hum Reprod 2009;24:2917-23.
106. Lin YH, Chiu WC, Wu CH, Tzeng CR, Hsu CS, Hsu MI. Antimüllerian hormone and polycystic ovary syndrome. Fertil Steril 2011;96:230-5.
107. Sjaarda LA, Mumford SL, Kissell K, et al. Increased androgen, anti-mullerian hormone, and sporadic anovulation in healthy, eumenorrheic women: a mild PCOS-like phenotype? J Clin Endocrinol Metab 2014;99:2208-16.
108. Tietze C. Reproductive span and rate of reproduction among Hutterite women. Fertil Steril 1957;8:89-97.
109. te Velde ER, Pearson PL. The variability of female reproductive aging. Hum Reprod Update 2002;8:141-54.
110. Schmidt L, Sobotka T, Bentzen JG, Nyboe Andersen A. ESHRE Reproduction and Society Task Force. Demographic and medical consequences of the postponement of parenthood. Hum Reprod Update 2012;18:29-43.
111. Martin JA, Hamilton BE, Osterman MJ, Curtin SC, Matthews TJ. Births: final data for 2012. Natl Vital Stat Rep 2013;62:1-68.
112. Bavan B, Porzig E, Baker VL. An assessment of female university students' attitudes toward screening technologies for ovarian reserve. Fertil Steril 2011;96:1195-9.
113. Mac Dougall K, Beyene Y, Nachtigall RD. Age shock: misperceptions of the impact of age on fertility before and after IVF in women who conceived after age 40. Hum Reprod 2013;28:350-6.
114. Azhar E, Seifer DB, Melzer K, Ahmed A, Weedon J, Minkoff H. Knowledge of ovarian reserve and reproductive choices. J Assist Reprod Genet 2015;32:409-15.
115. Loh JS, Maheshwari A. Anti-mullerian hormone—is it a crystal ball for predicting ovarian aging? Hum Reprod 2011;26:2925-32.
116. Nelson SM, Anderson RA, Broekmans FJ, Raine-Fenning N, Fleming R, La Marca A. Anti-mullerian hormone: clairvoyance or crystal clear? Hum Reprod 2012;27:631-6.
117. Stoop D, Maes E, Polyzos NP, Verheyen G, Tournaye H, Nekkebroeck J. Does oocyte banking for anticipated gamete exhaustion influence future relational and reproductive choices? A follow-up of bankers and non-bankers. Hum Reprod 2015;30:338-44.
118. Tremellen K, Savulescu J. Ovarian reserve screening: a scientific and ethical analysis. Hum Reprod 2014;29:2606-14.
119. Steiner AZ, Herring AH, Kesner JS, et al. Antimüllerian hormone as a predictor of natural fecundability in women aged 30-42 years. Obstet Gynecol 2011;117:798-804.
120. Butts SF, Seifer DB. Racial and ethnic differences in reproductive potential across the life cycle. Fertil Steril 2010;93:681-90.
121. Seifer DB, Golub ET, Lambert-Messerlian G, et al. Variations in serum mullerian inhibiting substance between white, black, and Hispanic women. Fertil Steril 2009;92:1674-8.
122. Lawrenz B, Henes J, Henes M, et al. Impact of systemic lupus erythematosus on ovarian reserve in premenopausal women: evaluation by using anti-muellerian hormone. Lupus 2011;20:1193-7.
123. Senates E, Colak Y, Erdem ED, et al. Serum anti-mullerian hormone levels are lower in reproductive-age women with Crohn's disease compared to healthy control women. J Crohns Colitis 2013;7:e29-34.
124. Wang ET, Pisarska MD, Bresee C, et al. BRCA1 germline mutations may be associated with reduced ovarian reserve. Fertil Steril 2014;102:1723-8.
125. Phillips KA, Collins IM, Milne RL, et al. Anti-mullerian hormone serum concentrations of women with germline BRCA1 or BRCA2 mutations. Hum Reprod 2016;31:1126-32.
126. Gleicher N, Weghofer A, Barad DH. A pilot study of premature ovarian senescence: I. Correlation of triple CGG repeats on the FMR1 gene to ovarian reserve parameters FSH and anti-mullerian hormone. Fertil Steril 2009;91:1700-6.
127. Pastore LM, McMurry TL, Williams CD, Baker VL, Young SL. AMH in women with diminished ovarian reserve: potential differences by FMR1 CGG repeat level. J Assist Reprod Genet 2014;31:1295-301.
128. Iwase A, Hirokawa W, Goto M, et al. Serum anti-mullerian hormone level is a useful marker for evaluating the impact of laparoscopic cystectomy on ovarian reserve. Fertil Steril 2010;94:2846-9.
129. Chang HJ, Han SH, Lee JR, et al. Impact of laparoscopic cystectomy on ovarian reserve: serial changes of serum anti-mullerian hormone levels. Fertil Steril 2010;94:343-9.
130. Shebl O, Ebner T, Sommergruber M, Sir A, Tews G. Anti muellerian hormone serum levels in women with endometriosis: a case-control study. Gynecol Endocrinol 2009;25:713-6.
131. Pacchiarotti A, Frati P, Milazzo GN, Catalano A, Gentile V, Moscarini M. Evaluation of serum anti-mullerian hormone levels to assess the ovarian reserve in women with severe endometriosis. Eur J Obstet Gynecol Reprod Biol 2014;172:62-4.
132. Rey RA, Lhomme C, Marcillac I, et al. Antimüllerian hormone as a serum marker of granulosa cell tumors of the ovary: comparative study with serum alpha-inhibin and estradiol. Am J Obstet Gynecol 1996;174:958-65.
133. Rey R, Sabourin JC, Venara M, et al. Anti-mullerian hormone is a specific marker of sertoli- and granulosa-cell origin in gonadal tumors. Hum Pathol 2000;31:1202-8.