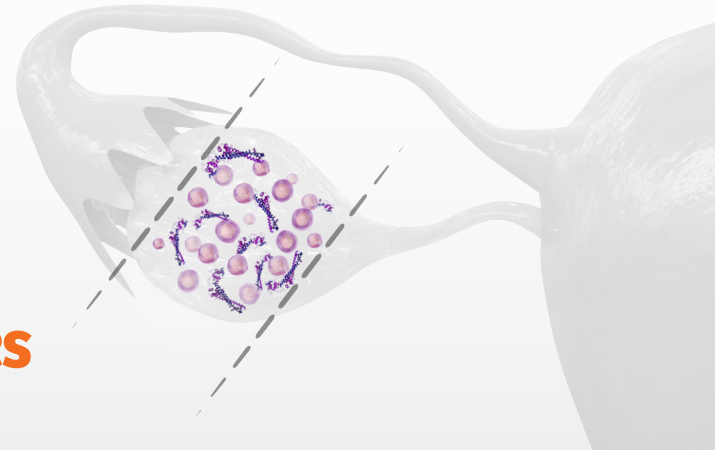

Anti-Müllerian hormone (AMH)

AMH testing in fertility assessment and assisted reproductive technologies

Introduction

Deferred childbearing has been a prominent trend in recent decades, driven in part by lifestyle choices and socioeconomic factors.[1,2] Delayed pregnancies are contributing to the growing number of women seeking assisted reproductive technologies (ART), along with those experiencing trouble conceiving. In-vitro fertilization (IVF) is the leading methodology, accounting for millions of births worldwide, including an estimated 1-to-5 percent of yearly births in the U.S. and Europe.[3,4,5]

For women considering ART, assessment of ovarian reserve (OR) can be essential to help identify those more likely to benefit from fertility treatments. Testing options for OR include biochemical (immunoassays) and transvaginal ultrasound (imaging of the ovaries).

AMH is the preferred hormonal assay for OR assessment, and multiple studies indicate equivalency with imaging.[6-9] While both methods are recommended by the American Society for Reproductive Medicine (ASRM), they note that AMH testing is simpler to administer, and, if imaging is used, it should occur at an "experienced" site.[8] AMH is non-invasive and widely accessible, and is considered the earliest and most sensitive indicator.[7] Testing for AMH is frequently used in the initial evaluation for ART support.

Testing for anti-Müllerian hormone (AMH) plays a critical role in fertility assessment, aiding identification of women with higher versus diminished ovarian reserve (OR).

"Reproductive and ovarian senescence occurs with depletion of the number of oocytes, ovarian reserve."

Source: Testing and interpreting measures of ovarian reserve: a committee opinion.[8]

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What is IVF?

IVF is a medical procedure involving treatment (ovarian stimulation) to induce production of multiple mature oocytes (eggs) that are then harvested (removed) from the ovaries. Fertilization by sperm occurs in vitro, and the developing embryo is implanted into the womb for gestation.[4]

Initiation of IVF requires that sufficient eggs capable of maturing remain in a woman's ovaries (ovarian reserve or OR) as multiple mature oocytes are required for the procedure. Testing for OR is typically done prior to a decision to initiate IVF. For women, IVF is a complex process involving multiple steps, commonly requiring several weeks to recruit and prepare the eggs in vivo for retrieval and fertilization ex vivo.[10-13]

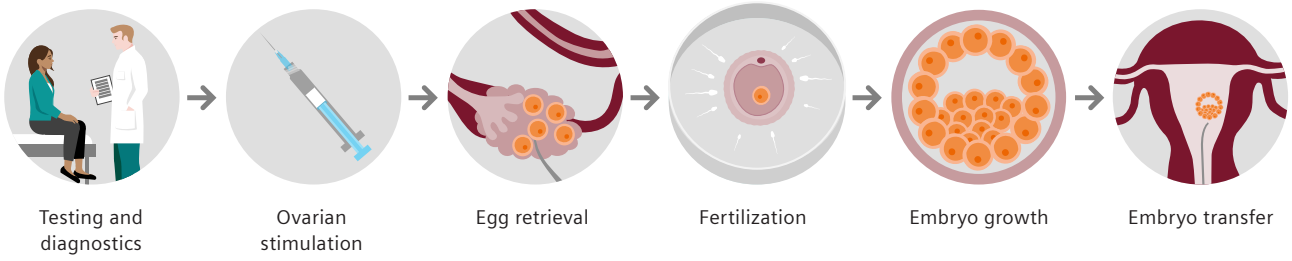

Figure 1. The IVF process.[13]

Ovarian reserve (OR), age, and IVF

Ovarian reserve is, in part, defined by the number of viable oocytes in the ovaries that remain capable of producing a mature egg.[6,7] Peak fertility levels are observed principally for females in their twenties, declining with time.[6,9] (Figure 2)

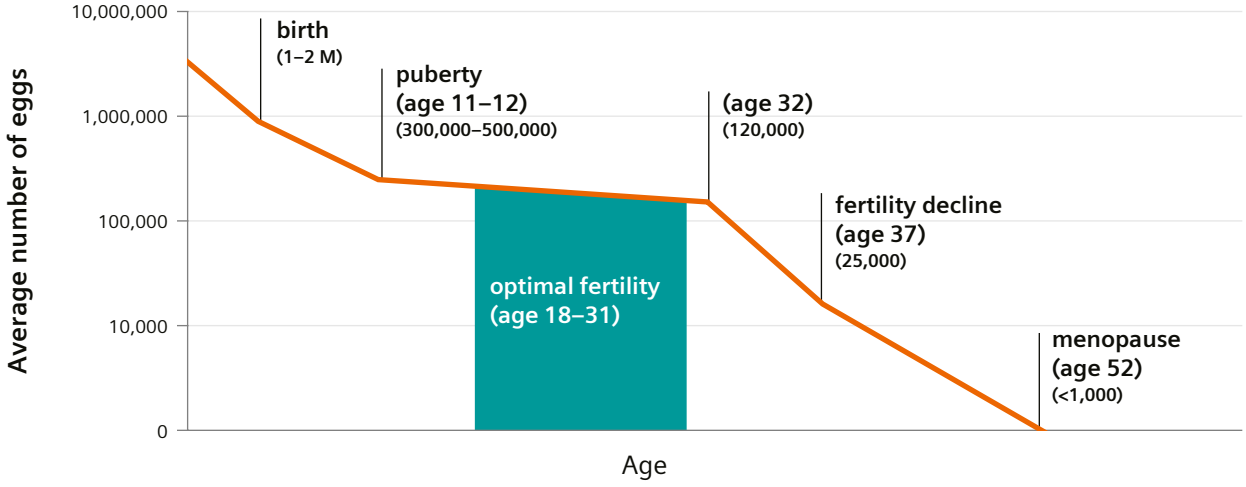

Figure 2. Average number of available eggs decreases with age.

While limited data support OR as predictive of overall fertility, OR can be highly predictive in identifying women who are good candidates for IVF.[6-9] Egg quality also begins to diminish with age—often by the early 30s—and can impact the likelihood of a healthy embryo. OR reflects egg count, but not egg quality.[6,8]

Age and other factors can impact fertility and treatment decisions

Assessment for OR is an important consideration, as IVF success rates (as defined by live births) can decline dramatically with increasing age (Figure 3).[9,14]

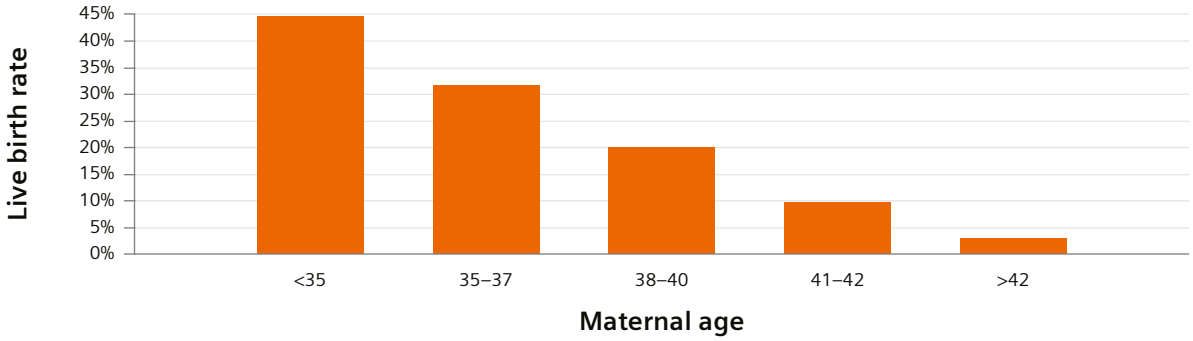

Figure 3. Live births per intended egg retrieval in the U.S.

Knowledge of a low OR may support a woman's decision to avoid the cost, time, and risk of attempting IVF. However, per the ASRM, a low OR should not be a reason for refusing treatment.[8] Other factors that may impact egg reserve or quality include prior ovarian surgery, chemotherapy, radiation therapy, severe endometriosis, smoking, pelvic infection, or a strong family history of early menopause.[9]

Background: follicles, hormones, and egg maturation

Women are born with a finite number of immature oocytes contained within primordial follicles in the ovaries. Numbers peak prior to birth (around 20 weeks) and then begin to decline with time, with exhaustion typically occurring around menopause (Figure 2).[6-9]

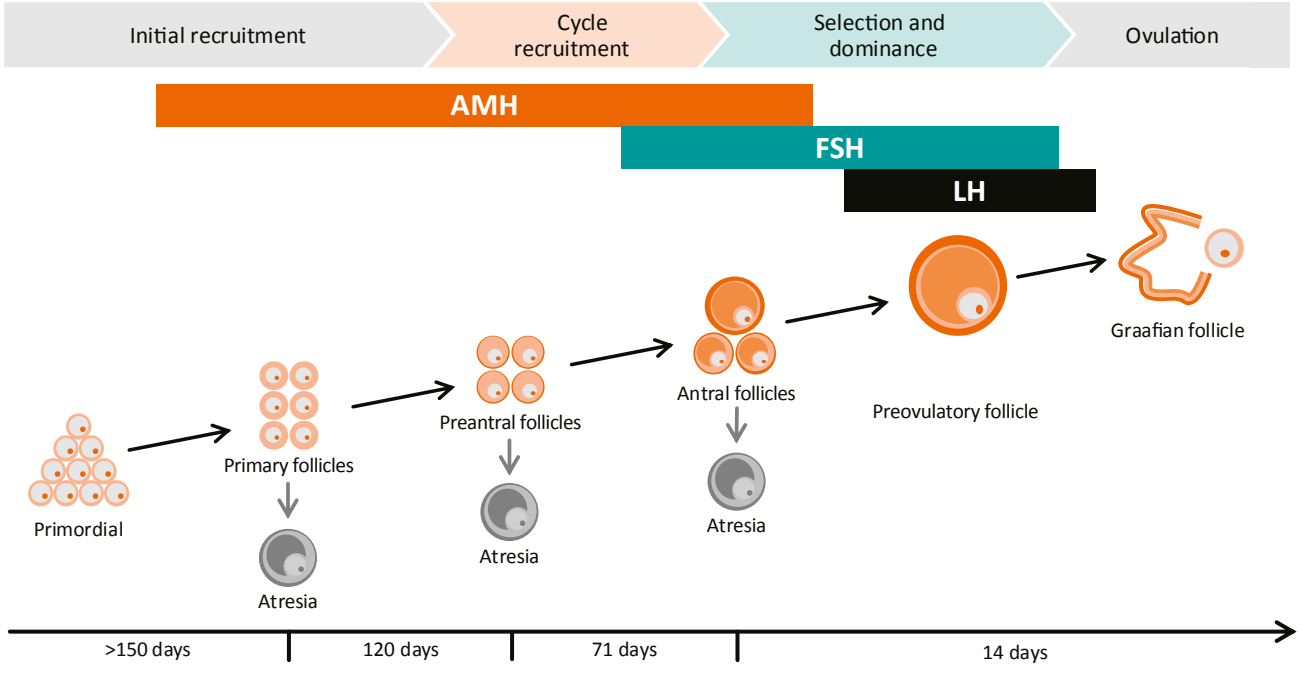

Figure 4. Follicular maturation in the normal reproductive cycle.[7]

"True" ovarian reserve

The resting, nongrowing primordial follicle population constitutes the true ovarian reserve. As no method can currently measure the resting primordial follicles, assessment of growing follicles has been established as a good surrogate.[15,16]

Primordial egg-containing follicles mature via a process called folliculogenesis that drives growth and differentiation (Figure 4). Most will not survive and are removed by atresia, a physiologic process resulting in follicular degeneration and egg loss.[17]

FSH and LH in follicular maturation

Growing follicles develop into preantral follicles (the antrum is a fluid-filled sac containing the egg).[7] Continued growth results in larger antral follicles expressing receptors for follicle stimulating hormone (FSH) and luteinizing hormone (LH), necessary for the next steps in maturation (the gonadotropin: responsive phase)[7,16,18] (Figure 4).

Maturation and ovulation in the normal reproductive cycle

The follicular phase of each menstrual cycle is characterized by elevated levels of FSH that stimulate development of multiple follicles in the ovaries. In a natural monthly cycle, only one (the dominant follicle) typically completes the process, producing a Graafian follicle containing the selected mature egg.[16,18] As levels of FSH decline and LH surges, the mature egg is released (ovulation) and becomes available for fertilization and subsequent pregnancy (Figure 4).

Superovulation in IVF

In IVF, levels of FSH can remain elevated with treatment (controlled ovarian stimulation or COS).[19-21] This helps stimulate the continued maturation of more than one follicle simultaneously (superovulation), with the goal of producing multiple Graafian follicles. The mature eggs are retrieved prior to ovulation via follicular puncture for in-vitro fertilization (Figure 5).

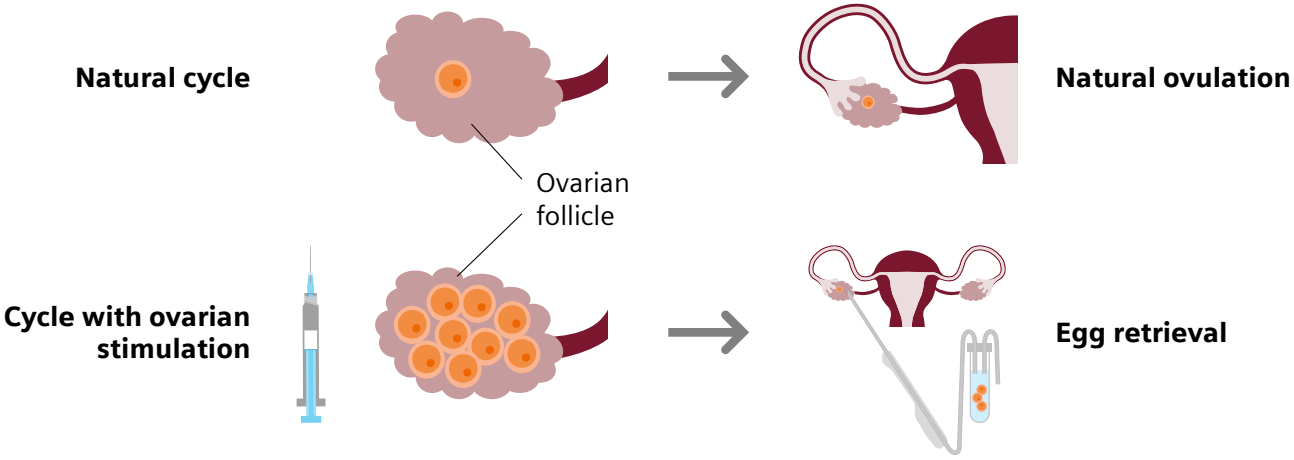

Figure 5. Egg retrieval following COS.

OR and a decision to pursue IVF or alternatives

Knowledge of OR helps inform decision making for both patient and provider to determine if the woman is a good candidate for IVF. Women with a diminished OR are more likely to exhibit a poor response to COS and a related lower likelihood of IVF success.[22-24]

For women with poor reserve and other relevant findings that lower her odds of successfully harvesting multiple mature oocytes, alternate options, such as using an egg donor, may be considered. This can help avoid a lengthy (and costly) COS process with a low likelihood of success.

Women with a high OR are more likely to experience IVF success. Therefore, estimating a woman's OR is an important consideration if contemplating ART. While no test evaluates the true ovarian reserve, both AFC and AMH are accepted as good surrogate markers that reflect the number of growing follicles.[7,8,14,25]

Methods to determine OR

- **Antral Follicular Count (AFC) by ultrasonography:**[7,8,14,24] Transvaginal ultrasound is used to visualize and count antral follicles typically in the range of 2–10 mm in diameter. A higher AFC reflects a good potential, while low counts are predictive of reduced fertility. [8,23,24]
  - However, the procedure is invasive, requires a trained operator, and is subject to inter-operator variability.[8,14,15]
  - Ideally it should be performed early in the menstrual cycle for maximum accuracy (as it reduces the likelihood of the presence of an ovarian cyst or a corpus luteum, which may mask some antral follicles) and can vary with the equipment used.[7,14,15]
  - The ASRM recommends AFC be performed at a site well-experienced with the procedure.[8]
- **Clomiphene citrate challenge test (CCCT):** CCCT involves measurements of serum FSH before (cycle day 3) and after (cycle day 10) treatment with clomiphene citrate (CCC).[8] While an elevated FSH following CCC suggests diminished OR, the ASRM has suggested disuse of CCCT due to inferiority to other methods.[8]
- **Hormone levels: FSH, estradiol, and inhibin B**
  - Historically, measurements of these hormones during the follicular phase have been used to aid the assessment of OR as they are produced by antral follicles.[7,8]
  - However, they perform more poorly compared to either AFC or AMH as predictors of OR, especially if measured independently (versus collectively).[7,8,25]
  - Timing of testing is important, and utility can be challenged by variability between assays and the influence of reproductive aging.[7,26]
  - FSH alone measured on day three of the menstrual cycle has been used to assess ovarian reserve, but levels can be inconsistent due to inter- and intra-cyclic fluctuations.[23,27,28]
- **Anti-Müllerian hormone (AMH)**
  - AMH has now largely supplanted older hormonal measurements for the estimation of OR and is currently recommended.[6-8,14,23,25,28,29]
  - AMH is produced principally by primary and growing antral follicles, and quantitative levels can be determined in the blood (serum, plasma) by immunoassay.[8,14,16,23, 30,31]
  - As growth of the preantral follicles expressing AMH is continuous rather than cyclical, AMH exhibits less inter- and intra-cycle variability (although some variation has been observed).[6-8,28,32] This means testing is not limited to timing of the menstrual cycle (i.e., such as day three of menstruation).[7,8,32]
  - Since AMH levels reflect the pool of growing follicles (Figure 6A), values are predictive of the OR. AMH is highly associated with AFC and so provides an effective (and typically more convenient) alternative. Use of either method (AMH or AFC) is recommended in guidelines.[8,33] AMH levels can differ significantly between women, reflecting the variable number of growing follicles even with subjects of similar age.[34] (Figure 6B).
  - As a simple blood test that can be drawn at any point during the cycle, AMH has operational advantages over AFC and offers greater accessibility.[7,8]
  - AMH assays currently lack international standardization so quantitative values can vary between assays.[32,33,35,36]

"AMH testing is simpler to administer. AFC and AMH have been shown in multiple studies to be equivalent."

Source: Practice Committee of the American Society for Reproductive Medicine[8]

Factors other than age that may influence AMH levels

Multiple factors have been identified that may influence a woman's AMH levels (in addition to age).[36-40] These include obesity, genetics, lifestyle (including smoking and diet), dysmenorrhea, ovarian surgery, chemotherapy, vitamin D status, ethnicity, and a family history or early menopause. AMH results should be evaluated within the overall clinical context as a part of the decision to pursue IVF.

AMH and pregnancy prediction

While AMH levels are predicative of mature oocyte yield and highly utilized in ART, it does not, like other methods, measure egg quality or the probability of a successful pregnancy.[7,8,28,32] Multiple variables, including age, sperm quality, embryo development, stimulation protocols, and procedural and laboratory techniques are thought to play significant roles for pregnancy and live birth rates.

AMH and additional hormonal testing

According to the ASRM (and other guidance), AMH has largely replaced basal FSH and related hormonal testing for OR.[8] They note that AMH tends to decline before FSH rises, so may reflect a more subtle decline in OR. However, they comment that additional testing such as basal FSH and estradiol (E2) testing may provide added insight in women with very low AMH levels.

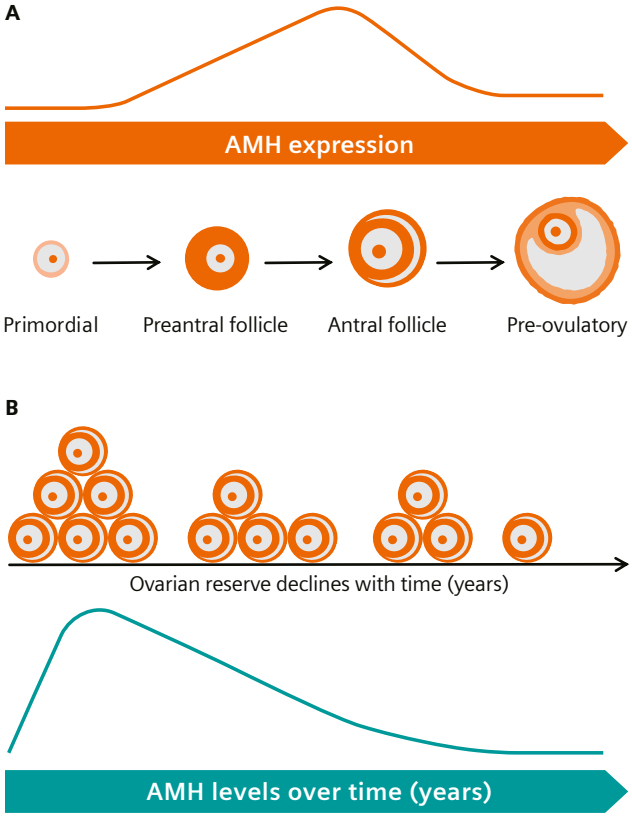

Figure 6A. AMH is produced by growing follicles.

Figure 6B. AMH levels decline with reductions in the follicular pool.

Automated quantitative testing for AMH

Testing options for AMH include manual to fully automated. The AMH Assay from Siemens Healthineers is a fully automated assay available on the Centaur XP/XPT and the Atellica IM and CI—routine immunoassay analyzers widely used in labs worldwide.

Analyte targeted by the AMH Assay from Siemens Healthineers[41]

AMH is synthesized as a larger precursor protein (140 kDa), that undergoes post-translational processing to generate identical 70 kDa disulfide-linked subunits. Further processing results in the active (hormonal) fragment and an inactive fragment, with both biologically active and inactive AMH isoforms present in the blood. Two mouse monoclonal anti-AMH antibodies are used in the assay, which is designed to detect total AMH. Either serum or plasma can be used.[41]

AMH on the Atellica IM, Atellica CI, and ADVIA Centaur XP/XPT

The reagent formulations used on the Atellica IM and CI Analyzers are the same as those used on the ADVIA Centaur XP/XPT platforms.[41] Expected values were established using the ADVIA Centaur XP system and confirmed by assay comparison for the Atellica IM and CI. The clinical decision (cut point) value is the same across all analyzers, with high correlations reported.[41]

Clinical cut point when using the AMH Assay from Siemens Healthineers to predict OR[41]

A result of **>1.77* ng/ml** is associated with a higher than normal OR (Table 1). This value was determined by comparing AMH values in women with ultrasound findings of normal to diminished ovarian reserve (defined as AFC ≤15) and those with high ovarian reserve (AFC >15).

The sensitivity, specificity, PPV, and NPV are reported in Table 2 and support use of the 1.77 ng/mL as a medical decision value for predicting OR.

Table 1.[41] Concordance of Siemens Healthineers AMH results with AFC using the 1.77 ng/ml cut point.

		AFC, determined by ultrasound		
		>15	≤15	**Total**
ADVIA Centaur AMH result	>1.77 ng/mL	256	120	**376**
	≤1.77 ng/mL	27	130	**157**
	Total	**283**	**250**	**533**

Table 2.[41]* Performance of the 1.77 ng/ml cut point for the AMH assay from Siemens Healthineers.

Parameter	N[a]	Estimate	95% Confidence interval
Sensitivity	283	90.5% (256/283)	(86.47, 93.36)
Specificity	250	52.0% (130/250)	(45.82, 58.12)
PPV	376	68.1% (256/376)	(63.21, 72.59)
NPV	157	82.8% (130/157)	(76.13, 87.90)

[a] *Number of measurements.*

**Applies to both Atellica and ADVIA Centaur analyzers.*

Performance of the AMH Assay from Siemens Healthineers compared to other automated AMH assays[27,28]

Available AMH assays are not currently standardized using common reference material.[7,31-33,35,36,42] Until and if standardization can be achieved, it is important to use the assay-specific value and ideally utilize the same assay for comparisons within a given patient.

Despite lacking formal standardization, studies have investigated correlations between available automated assays. Two recent publications examined the AMH from Siemens Healthineers (run either on the ADVIA Centaur or Atellica analyzers which utilize the same set of reagents) to the assays available from Beckman and Roche.[31,35]

AMH on the ADVIA Centaur compared to Roche and Beckman AMH assays:[35]*

The analytical and clinical performance of the ADVIA Centaur AMH from Siemens Healthineers was compared to the Beckman and Roche assays. While all three assays demonstrated comparable clinical performance (according to the authors), correlation differences for quantitative values obtained were observed (Tables 3 and 4).

- A good quantitative correlation was noted between the mean for the assay from Siemens Healthineers and the Beckman assay associated with a high versus normal or diminished ovarian reserve (defined by AFC).
- A lower correlation was observed for the Roche assay compared to both Siemens Healthineers and Beckman.
- Substantial between-method bias was observed with the Roche assay when compared to results from either Beckman or AMH assays from Siemens Healthineers.

There was a strong correlation observed between the ADVIA Centaur and the Beckman Access assays with a high Kendall's tau correlation coefficient of 0.909, while a moderate correlation was observed with Roche Elecsys ($\tau = 0.777$).[27]

Similar sensitivities and NPVs were observed between the assay from Siemens Healthineers and the Beckman assay, but differing values reported for Roche when applying the cut point from Siemens Healthineers of 1.77 ng/mL (Table 3).

The authors noted that use of the 1.77 ng/mL for the assay from Siemens Healthineers provides a high probability (>90%) of identifying women with a high OR.

The authors commented that, in patients with borderline results, decision making could be informed by additional clinical factors and lab tests.

Table 3.[35] Mean individual assay values relative to AFC.

AFC data were divided into two groups: >15 (high OR) or ≤15 (normal to diminished OR) and the mean AMH value for the assay determined.

Mean AMH value	AFC ≤15	AFC >15
Centaur AMH	2.21	4.94
Beckman AMH	2.23	5.05
Roche AMH	1.42	3.35

Table 4.[35] Higher correlations observed for the Centaur and Beckman AMH assays vs. Roche for AFC and an AMH result of >1.77 ng/mL vs. ≤1.77 ng/mL.

	AMH result >1.77 ng/mL vs. ≤1.77 ng/ml for AFC >15 vs. ≤15		
	ADVIA Centaur	Beckman Access	Roche Elecsys
Sensitivity	90.2%	89.9%	77.3%
Specificity	51.8%	51.0%	71.0%
Positive predictive value	67.0%	66.6%	74.3%
Negative predictive value	83.0%	82.3%	74.2%

**Study was supported by Siemens Healthineers.*

Atellica IM AMH compared to Beckman and Roche[31]*

An analytical comparison study investigating the performance of the Atellica IM AMH Assay to the assays from Beckman and Roche found that all methods demonstrated "excellent" correlations (R^2 values exceeding 0.95). While the assays showed correlation across the range of AMH concentrations, quantitative biases were observed (Table 5).

The authors noted that the Atellica and Beckman DxI AMH assays from Siemens Healthineers showed minimal bias (slope: 1.07). However, results from the Roche cobas Elecsys® e801 AMH assay were misaligned with those of Beckman and Siemens Healthineers (slope: 0.74 and 1.40, respectively).

These findings highlight the lack of standardization between assays, but some may align better than others. Use of the same assay is ideal in each patient, but if using alternate assays, an awareness of the potential relative bias in the reported value is vital.

Table 5.[31] Comparison of the Atellica IM, Elecsys e801, and DxI AMH assays.

AMH Assays compared	Slope	R^2	y-Intercept
Atellica IM and Beckman DxI	1.07	0.9881	0
Beckman DxI and Roche cobas Elecsys e801 assay	0.74	0.9696	+0.27
Atellica IM and Roche cobas Elecsys e801 assay	1.40	0.9768	-0.38

Conclusion

Use of AMH supports important decision-making for women considering IVF by aiding assessment of ovarian reserve. Data indicate superior performance and greater flexibility in timing of sample collection for AMH compared to early-follicular-phase hormone levels. Despite AMH assays lacking current international standardization, the assay from Siemens Healthineers shows a high degree of correlation to the Beckman AMH, while both show a moderate correlation to the Roche assay. The AMH from Siemens Healthineers offers proven clinical performance comparable to the AMH assays available on alternate platforms.

For additional information, please contact your local Siemens Healthineers representative about AMH or related products or assays.

Glossary of Terms

AFC	Antral follicular count	**FSH**	Follicle stimulating hormone
AMH	Anti-Müllerian hormone	**IVF**	In-vitro fertilization
ART	Assisted reproductive technologies	**LH**	Luteinizing hormone
COS	Controlled ovarian stimulation	**OR**	Ovarian reserve

Study was supported by Siemens Healthineers.

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Published by Siemens Healthcare Diagnostics Inc. · CLS-25-4097-76 · QR700023848 · 06-2025 · ©Siemens Healthcare Diagnostics Inc., 2025