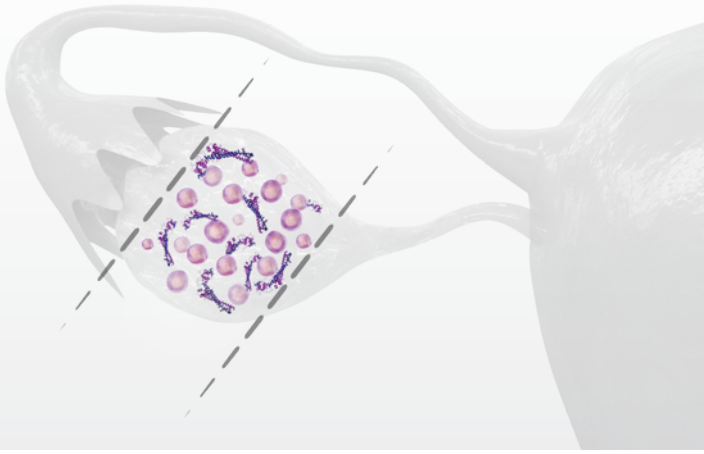
Anti-Müllerian hormone (AMH)

Expanded utility for AMH testing in fertility supports assessment of treatment response



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), with in-vitro fertilization (IVF) as the principal methodology.[3] IVF accounts for millions of births worldwide, including an estimated 1–5 percent of yearly births in the U.S. and Europe.[4,5]

Testing for anti-Müllerian hormone (AMH) plays a critical role in ART, supporting initial assessment of fertility and in determining response to ovarian stimulation protocols.

What is IVF?

IVF is a medical procedure where mature oocytes (eggs) are harvested from the ovaries and fertilized by a sperm outside of the womb. The resulting 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 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 requiring multiple steps, typically performed over several weeks to recruit and prepare the eggs in-vivo for retrieval and fertilization ex-vivo.[6-8]

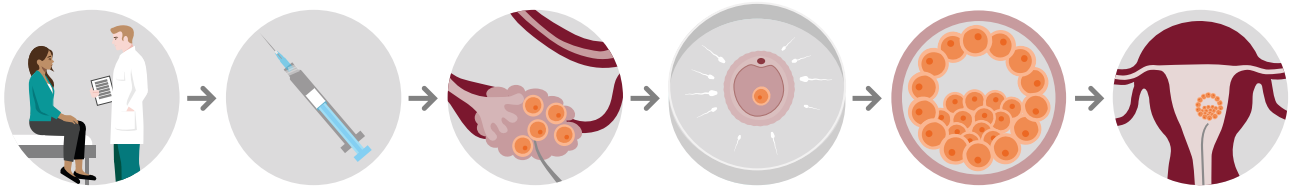


Figure 1. The IVF process.[9]

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Controlled ovarian stimulation (COS) in IVF

As not all retrieved eggs will produce a viable embryo, multiple mature oocytes are used for the fertilization step to increase the likelihood of high-quality embryos.[10] Normally, only a single mature "dominant" egg is generated by one ovary during the regular reproductive cycle.[10] COS uses fertility drugs to stimulate the ovaries to produce several mature eggs simultaneously that can then be harvested.

Higher egg retrieval following COS (5 to >15 mature oocytes per round of treatment) improves the success rate for fertlization.[11,12] A poor response (≤4 oocytes) is predictive of reduced chances of successful fertilization.

Ovarian reserve and the response to COS can be assessed with AMH testing or can be determined using a transvaginal ultrasound with visual counting.[13]

NEW!

In addition to assessment of ovarian reserve, the Atellica IM Anti-Müllerian Hormone (AMH) Assay from Siemens Healthineers can now be used for the prediction of a poor ovarian response (≤4 oocytes retrieved) to COS.

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.[14,15] Peak fertility levels are observed principally for females in their twenties, declining with time (Figure 2). Fertility is significantly associated with OR and can vary considerably between woman even of the same age.

Egg quality also begins to diminish with age, often by the early 30s, and can impact the likelihood of healthy embryo.[16]

Assessment for OR is important for ART, as IVF success rates can decline dramatically with increasing age. Knowledge of a low OR may support a decision to avoid the cost, time, and risk of attempting IVF. 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.[17]

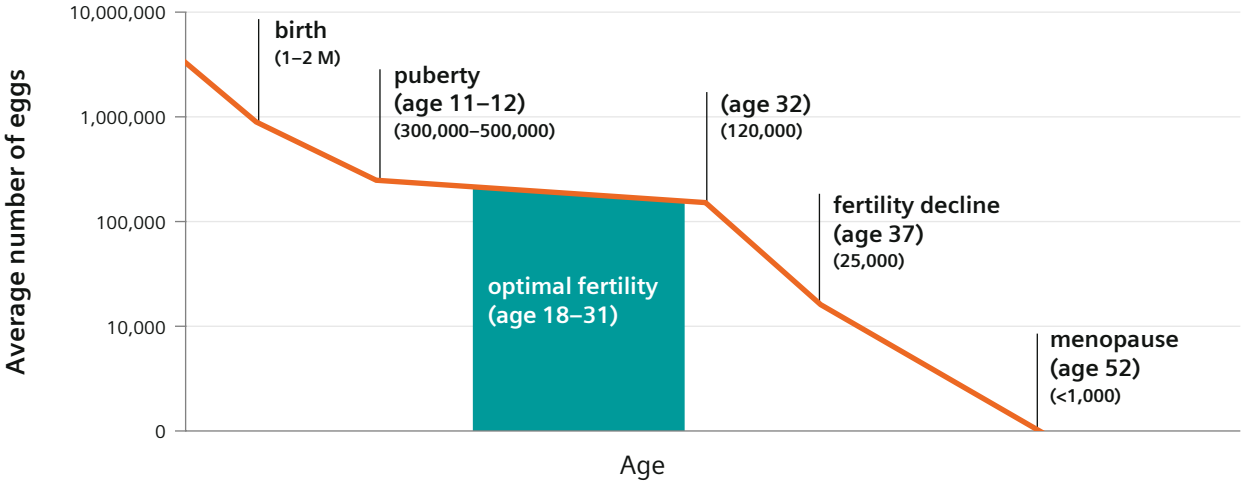


Figure 2. Average number of eggs a woman has by age.

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).[14-18]

The resting, nongrowing primordial follicle population constitutes the true ovarian reserve. As no method can currently measure the primordial follicles, assessment of growing follicles has been established as a good surrogate (Figure 3).[19,20]

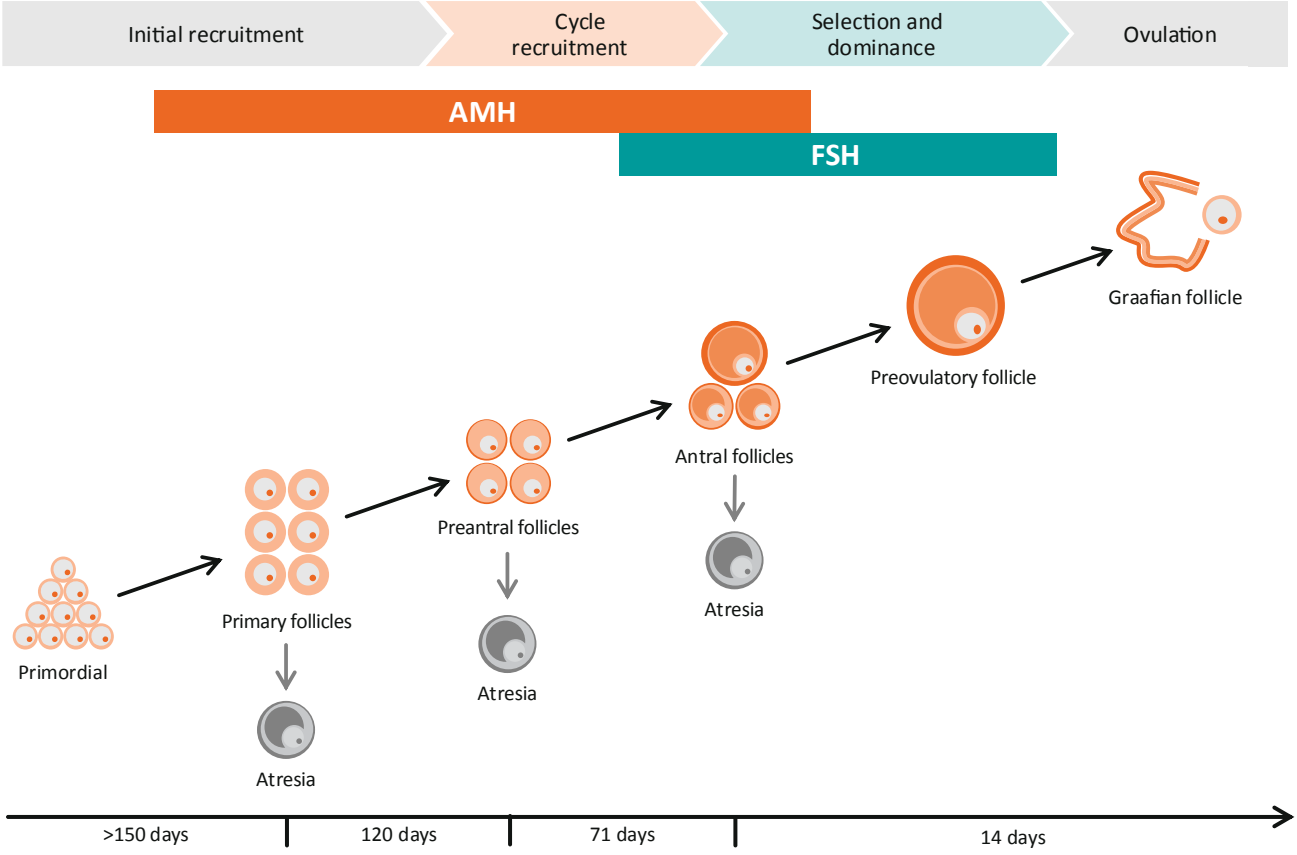


Figure 3. Follicular maturation.

Primordial egg-containing follicles mature via a process called folliculogenesis that drives growth and differentiation (Figure 3). Most will not survive and are removed by atresia (a physiologic process resulting in follicular degeneration and egg loss).

Growing follicles develop into preantral follicles (the antrum is a fluid-filled sac containing the egg). 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") (Figure 3).

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 mature egg. As levels of FSH decline and LH surges, the mature egg is released (ovulation) and becomes available for fertilization and subsequent pregnancy (Figure 3).

COS and superovulation in IVF

In IVF, levels of FSH can remain elevated (via treatment). 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 4).

Thus, a high COS response that produces several mature egg-containing follicles is a crucial component for IVF success. With COS, at least five to greater than 15 mature follicles are desirable. Conversely, four or fewer mature follicles are typically considered a poor response associated with higher failure rates for a successful fertilization.[11,12]

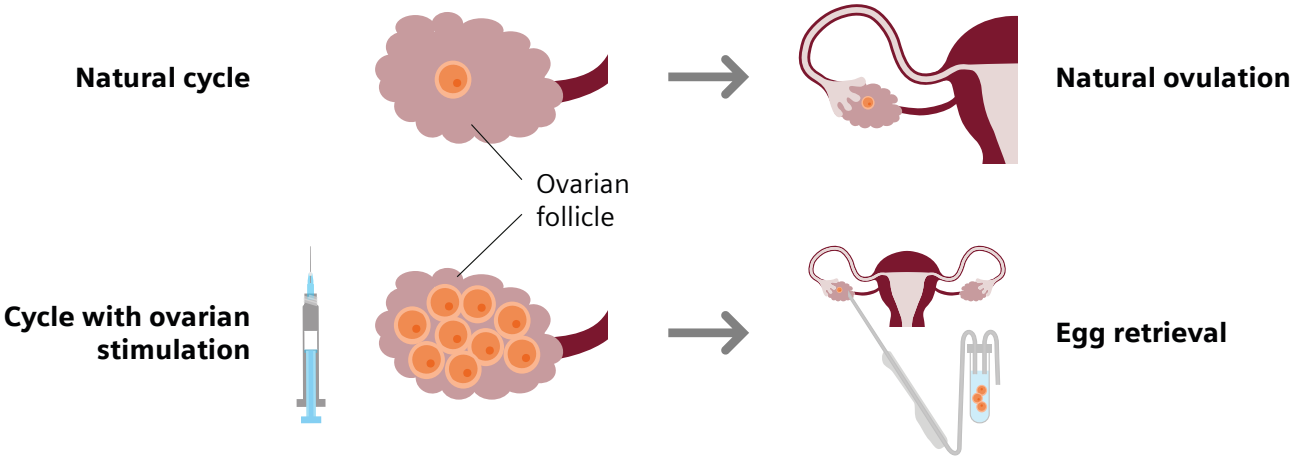


Figure 4. Egg retrieval following COS.

Assessment of ovarian reserve (OR) when considering IVF

OR can impact a decision to pursue IVF

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 lower likelihood of successful pregnancy.[10-13] 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) 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.[15,19,20]

Methods to determine OR

- **Antral Follicular Count (AFC) by ultrasonography:**[15,19,20] Transvaginal ultrasound is used to visualize and count antral follicles in the range of 2–10 mm in diameter. A higher AFC (example: >15) reflects a good potential, while low counts are predictive of reduced fertility.[20]
  - However, the procedure is invasive, requires a trained operator, and is subject to inter-operator variability.[19,20]
  - 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 might mask some antral follicles), and can vary with the equipment used.[15,19,20]
- **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 early antral follicles.
  - However, they perform more poorly compared to either AFC or AMH as predictors of OR, especially if measured independently (versus collectively).[15,20]
  - Timing of testing is important, and utility can be challenged by variability between assays and the influence of reproductive aging.[15,21]
  - 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.[22-24]
- **Anti-Müllerian hormone (AMH)**
  - AMH has now largely supplanted older hormonal measurements for the estimation of OR.[14-16,21,22,24,26] AMH is a better predictor of the number of oocytes retrieved compared to age and day three FSH levels.[15,20,25]
  - AMH is produced principally by primary and growing antral follicles, and quantitative levels can be determined in the blood (serum, plasma) by immunoassay.[26-28] 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).[14,15,24] This means testing is not limited to timing of the menstrual cycle (i.e., such as day three of menstruation).[15,28-30]
  - Since AMH levels reflect the pool of growing follicles (Figure 5 A), values are predictive of the OR. AMH is highly associated with AFC and so provides an effective (and typically more convenient) alternative.[14-16,24] Use of either method (AMH or AFC) is recommended in European Guidelines.[30] AMH levels can differ significantly between women, reflecting the variable number of growing follicles with subjects of similar age.[31] (Figure 5 B).
  - As a simple blood test that can be drawn at any point during the cycle, AMH has operational advantages over AFC and offers greater convenience.[15] However, AMH assays currently lack international standardization so quantitative values can vary between assays.[28]
  - While AMH levels are predicative of mature oocyte yield, like other methods it does not measure egg quality or the probability of a successful pregnancy.[15,32]

"For predicting high and poor response to ovarian stimulation, use of either antral follicle count (AFC) or anti-Müllerian hormone (AMH) is recommended over other ovarian reserve tests."

Level of evidence: **Strong.**

Source: Guideline of the European Society of Human Reproduction and Embryology.[30]

AMH: clinical applications in IVF

The measurement of AMH, in conjunction with other clinical and laboratory findings, is commonly used as an aid in the assessment of OR and the prediction of a poor response to COS.

- **Assessment of OR:** AMH levels can indicate if a woman's ovaries may be aging too quickly or if good reserve remains in women attempting pregnancy later in life. As AMH is expressed by growing follicles, it indicates a measure of the true reserve, informing a decision to attempt IVF or pursue alternatives.[15,33-35] As assays are not standardized, values are assay-specific.
- **Determination of response to COS:** AMH levels can indicate how well the ovaries are responding to fertility stimulation.[29,30,32] Women with a sub-optimal number of mature follicles (and thus fewer eggs) will generally have lower AMH concentrations, supporting a decision to cease or continue the process, alter the protocol, or consider other options. As assays are not standardized, values that support estimation of the COS response are assay specific.

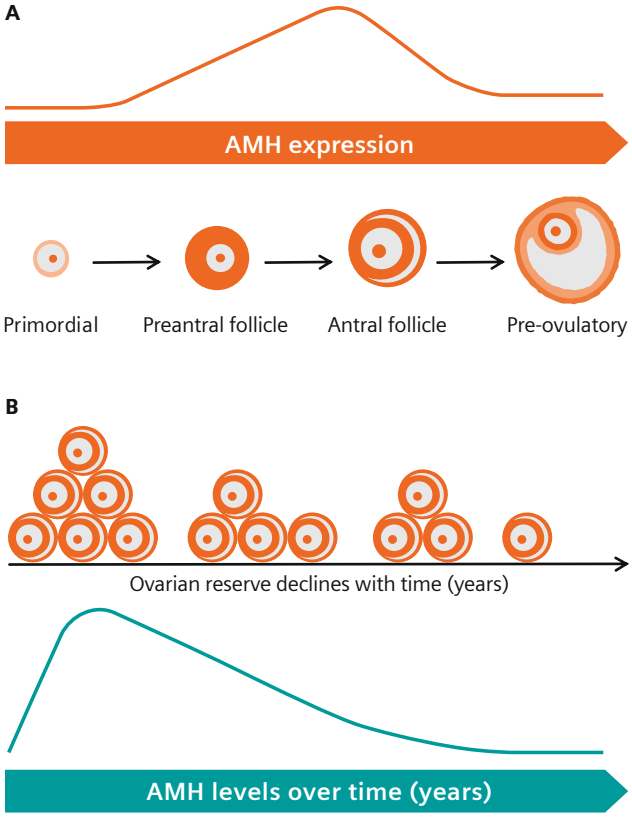


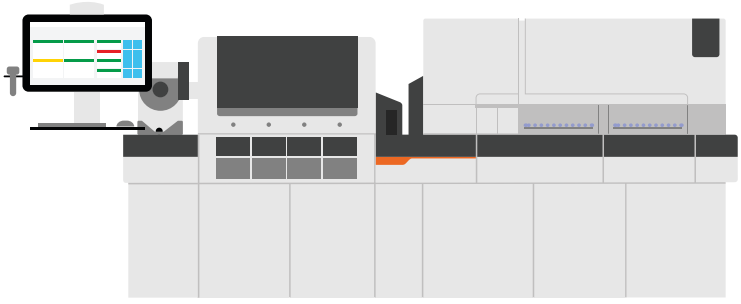
Figure 5. Adapted from reference 26 (Loes et al.)

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).[35-38] 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 with the overall clinical context as a part of the decision to pursue IVF.

Automated 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

AMH is synthesized as a larger precursor protein (140 kDa), that undergoes post-translational processing to generate identical 70 kDa disulfide-linked subunits.[29] Further processing results in the active (hormonal) fragment and an inactive fragment, with both biologically active and inactive AMH isoforms present in the blood.[27,28] Two mouse monoclonal anti-AMH antibodies are used in the assay, which is designed to detect total AMH.[29] Either serum or plasma can be used.[29]

Clinical cut-points when using the AMH Assay from Siemens Healthineers

- **Prediction of OR:**[29] A result of >1.77 ng/ml is associated with a higher than normal OR.
  - 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 1** and support use of the 1.77 ng/mL as a decision value for predicting OR.
- **Prediction of a poor COS response:**[29] A value of ≤0.93 ng/mL can be utilized to predict a poor response (defined as ≤4 oocytes retrieved following a COS protocol).
  - This was determined by comparing the AMH Assay from Siemens Healthineers to a commercially available assay, using the published cut-off value of 0.93 ng/mL for predicting a poor response.
  - When that value of 0.93 ng/mL was applied to the AMH Assay from Siemens Healthineers, high agreement was observed, including a 99.2% positive percent agreement (PPA). **Table 2**

Table 1.[29]

Limit of Detection (LoD)	Measuring interval	AMH result	Sensitivity/Specificity	PPV/NPV
0.013 ng/mL (0.093 pmol/L)	0.013–24.0 ng/mL (0.093–171 pmol/L)	**Prediction of OR: >1.77 ng/mL or ≤1.77 ng/mL**	90.5%/52.0%	68.1%/82.8%

Source: Atellica AMH IFU

Table 2. A cutoff of ≤0.93 ng/mL predicts a poor ovarian response.[29]

Parameter	Number of measurements	Estimate	95% Confidence interval
Positive predictive agreement (PPA)	471	99.2% (467/471)	(97.84, 99.67)
Negative predictive agreement (NPA)	62	95.2% (59/62)	(86.71, 98.34)

Source: Atellica AMH IFU

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

Available AMH assays are not currently standardized using a common reference material.[28] 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 reports examined the AMH from Siemens Healthineers (run either on the Centaur or Atellica analyzers which utilize the same set of reagents) to the assays available from Beckman and Roche.[27,28]

AMH on the ADVIA Centaur compared to Roche and Beckman AMH assays:[27]*

In a published study, 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, correlation differences for 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.
- Significant bias was observed with the Roche assay when compared to results from either Beckman or Siemens Healthineers AMH assays.

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 NPV's 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 4)**.

Table 3.[27]*

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.[27] 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%

The authors noted that use of the 1.77 ng/mL for the SH assay value 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.

Study was supported by Siemens Healthineers

Atellica IM AMH compared to Beckman and Roche[28]*

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 a strong correlation across the range of AMH concentrations, differences were observed **(Table 5)**.

The authors noted that, while Siemens Healthineers Atellica and Beckman DxI AMH assays exhibited minimal bias when compared, results from the Roche cobas Elecsys® e801 AMH assay were misaligned with those of Beckman and Siemens Healthineers.

Table 5.[28] 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

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 bias in the reported value is vital.

Conclusion

Use of AMH supports important decision-making for women considering or undergoing IVF. Now, in addition to aiding the assessment of ovarian reserve, the AMH Assay from Siemens Healthineers may be used to aid the prediction of poor ovarian response (defined as ≤4 oocytes retrieved) to COS. This added utility supports in-vitro fertilization across the COS process, supporting informed decisions that help mitigate unsuccessful outcomes. 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.

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

Study was supported by Siemens Healthineers

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

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