HIGHLIGHTS

- Serum AMH levels can predict ovarian response following a GnRH antagonist cycle
- A strong positive correlation was observed between AMH level and oocyte yield
- AMH cut-offs for excluding a low or high response were 6.4 and 14.2 pmol/l
- 47% of the variation in ovarian response could be attributed to AMH alone

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ARTICLE

Prediction of ovarian response using the automated Elecsys anti-Müllerian hormone assay in gonadotrophin-releasing hormone antagonist cycles

BIOGRAPHY

Ernesto Bosch was born in Philadelphia in 1968, graduated in 1992 and specialized in obstetrics and gynaecology in 1997. He obtained his PhD in 1999 and a Global Executive MBA in 2020. He is currently Clinic Director at Instituto Valenciano de Infertilidad in Valencia, where he has worked since 2000.

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KEY MESSAGE

Before ovarian stimulation, ovarian response following a GnRH antagonist cycle can be predicted with optimal accuracy using a single determination of serum AMH. The AMH cut-offs for excluding a low, suboptimal or high response using the Elecsys AMH immunoassay were 6.4, 13.4 and 14.2 pmol/l, respectively. Accurate prediction of ovarian response and oocyte yield will optimize patient counselling.

ABSTRACT

Research question: What is the capability of serum anti-Müllerian hormone (AMH) measured using the automated Elecsys® AMH immunoassay to determine ovarian response after fertility treatment?

Design: Single-centre, retrospective, observational, cohort study including women undergoing ovarian stimulation. Serum AMH concentrations were determined using the Elecsys AMH immunoassay based on one blood sample drawn 6 months or less before treatment (GnRH). Stimulation was conducted in accordance with a gonadotrophin-releasing hormone antagonist protocol. Patients were divided into four ovarian response categories based on their oocyte yield: low (0–3), suboptimal (4–9), optimal (10–15) and high (>15). Areas under the curve were calculated for each ovarian response group.

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KEYWORDS

Anti-Müllerian hormone
Gonadotropin-releasing hormone
Immunoassay
In vitro fertilization
Ovarian reserve
Ovarian response

Results: Overall, 1248 patients were enrolled. The AMH concentration had a strong positive correlation with oocyte yield (Spearman's rho = 0.74, $P < 0.001$). Areas under the curve (95% CI) for AMH predicting ovarian response were 0.85 (0.83 to 0.88) for low and 0.89 (0.87 to 0.91) for high response. Optimal serum AMH cut-offs for predicting a low and high response using the Elecsys AMH immunoassay were 6.4 pmol/l (0.89 ng/ml) and 14.2 pmol/l (1.99 ng/ml), respectively. Multivariable regression analysis showed that 47% ($R^2 = 0.470$) of variation in ovarian response could be attributed to AMH alone, increasing to 50.9% ($R^2 = 0.509$) with the addition of age, body weight, and total dose of gonadotrophin.

Conclusion: Ovarian response and oocyte yield after stimulation in a GnRH antagonist cycle can be predicted with high accuracy using a single determination of serum AMH before ovarian stimulation.

INTRODUCTION

Oocyte quantity declines with age, eventually leading to reproductive senescence (*Practice Committee of the American Society for Reproductive Medicine, 2020*). Ovarian reserve, defined as the number of oocytes remaining in the ovary, is typically evaluated before starting an ovarian stimulation cycle for IVF (*Practice Committee of the American Society for Reproductive Medicine, 2020*). Individualization of gonadotrophin doses based on ovarian reserve markers may help to optimize the ovarian response (defined herein as the number of oocytes retrieved) in women undergoing IVF, and thereby reduces the likelihood of treatment cancellation owing to poor response or risk of hyper-response and ovarian hyperstimulation syndrome, a potentially severe or lethal complication (*Lensen et al., 2018*).

Apart from ultrasound estimates of antral follicle count (AFC) (*Practice Committee of the American Society for Reproductive Medicine, 2020*), several biochemical tests have been developed as markers of ovarian reserve, including basal FSH (*Muasher et al., 1988*), oestradiol (*Licciardi et al., 1995*; *Smotrich et al., 1995*), inhibin B (*Seifer et al., 1997*) and anti-Müllerian hormone (AMH) (*Broer et al., 2014*). Recent guidelines from the Practice Committee of the American Society for Reproductive Medicine and the European Society of Human Reproduction and Embryology advise that AFC and AMH are the most sensitive and reliable measures of ovarian reserve (Ovarian Stimulation *TEGGO et al., 2020*; *Practice Committee of the American Society for Reproductive Medicine, 2020*). Although these two measures demonstrate a similar predictive performance for ovarian reserve (*Broer et al., 2013*), in patients undergoing IVF serum AMH is significantly more accurate than AFC to predict low (three or fewer oocytes) and high (15 or more oocytes) ovarian response (*Arce et al., 2013*).

The Elecsys® AMH assay (Roche Diagnostics International Ltd., Rotkreuz, Switzerland) is a fully automated immunoassay for the in-vitro quantitative determination of AMH (Roche Diagnostics GmbH, Basel, Switzerland). A prospective multicentre study has previously shown that serum AMH concentrations determined using the Elecsys AMH immunoassay have a strong positive correlation with AFC (*Anderson et al., 2015*), without affecting inter-observer and cyclical variation. A more recent prospective multicentre study conducted at US fertility clinics found that an optimal serum AMH cut-off of 1.77 ng/ml (12.6 pmol/l) identified women with an AFC above 15 with high specificity of 69.01% and sensitivity of 89.63% when using the Elecsys AMH assay (*Jacobs et al., 2019*).

Previous studies propose classification of ovarian response based on the likelihood of achieving a live birth after a fresh embryo transfer and the cumulative live birth rate (*Drakopoulos et al., 2016*; *Polyzos et al., 2018*). The following four ovarian response categories were suggested: low (zero to three oocytes), suboptimal (four to nine oocytes), optimal (10–15 oocytes), and high (>15 oocytes) (*Drakopoulos et al., 2016*; *Polyzos et al., 2018*). This study aimed to evaluate the capability of a single serum AMH determination to predict these four ovarian response categories, using the automated Elecsys AMH immunoassay, in an unselected, sub-fertile population of women undergoing ovarian stimulation with gonadotrophins and gonadotrophin-releasing hormone (GnRH) antagonists.

MATERIALS AND METHODS

Study design

This was a single-centre (Instituto Valenciano de Infertilidad, Valencia, Spain), retrospective, observational, cohort study in women undergoing fertility treatment. All patients presented for treatment between January 2015 and January 2017.

Ethical approval

This study was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all individuals included in this study before starting IVF treatments, of which AMH measurement is part of routine clinical practice. Ethical approval (1803-VLC-022-EB) was provided by the Clinical Research Ethics Committee of Instituto Valenciano de Infertilidad (Valencia, Spain) on 30 January 2018.

Study population

Eligible women were aged over 18 years, presenting for ovarian stimulation cycles for the purpose of IVF, fertility preservation or oocyte donation. The study population was unselected and so no exclusion criteria were applied.

Study procedures

For all women, a GnRH antagonist protocol was used for ovarian stimulation: starting on day 2–3 of the menstrual cycle, a customized dose of gonadotrophin was administered according to the clinician's judgment. Administered gonadotrophins included recombinant FSH (Gonal-F) (Merck, Darmstadt, Germany), Puregon (MSD, Kenilworth, NJ, USA) or Bemfola (Gedeon Richter, Budapest, Hungary), highly purified human menopausal gonadotrophin (Menopur) (Ferring Pharmaceuticals, Copenhagen, Denmark), or a combination of both. Ovarian response was monitored using transvaginal ultrasound and measurement of oestradiol (using the Elecsys E2 Gen II assay [Roche Diagnostics International Ltd., Rotkreuz, Switzerland]). Gonadotrophin releasing hormone antagonists were introduced once the lead follicle reached 12–13 mm in diameter.

TABLE 1 BASELINE PATIENT CHARACTERISTICS (*N* = 1248)

Characteristic	Mean ± SD	Range (minimum–maximum)
Age, years	36.4 ± 5.0	18–48
BMI, kg/m^2	23.1 ± 3.7	16.0–49.7
AMH, pmol/l	15.3 ± 15.2	1–53
AFC, *n*	12.8 ± 8.5	1–57
Oocytes retrieved (*n*)	11.0 ± 5.0	0–51

AFC, antral follicle count; AMH, anti-Müllerian hormone; BMI, body mass index.

Measurement of anti-Müllerian hormone

Serum AMH concentrations were determined in a single blood sample drawn 6 months or less before starting treatment, at any time and on any day of the menstrual cycle. Determination of AMH took place immediately after taking the blood sample. All measurements were carried out in-house using the fully automated Elecsys AMH assay according to manufacturer's instructions on the e 411 module of the cobas 6000 analyzer (all Roche Diagnostics International Ltd., Rotkreuz, Switzerland). The Elecsys AMH assay is an electrochemiluminescence immunoassay that uses the sandwich principle, with a total duration of 18 min and a sample volume of 50 µl (*Anderson et al., 2015*; Roche Diagnostics GmbH, Basel, Switzerland). The limit of quantitation (functional sensitivity) for this assay is 0.03 ng/ml (0.22 pg/ml), and the coefficients of variation for repeatability and reproducibility are 1.0–1.8% coefficients of variation and 2.7–4.4% coefficients of variation, respectively (Roche Diagnostics GmbH, Basel, Switzerland).

In-house quality-control checks were carried out daily using control reagents provided by Roche Diagnostics International Ltd. (Rotkreuz, Switzerland). The assays were also calibrated whenever a new reagent pack was used or an outcome outside the normal measuring range was observed. Furthermore, external quality-control assessment of every assay was carried out monthly at the Spanish Society of Clinical Biochemistry and Molecular Pathology (Barcelona, Spain).

Statistical analysis

Patients were divided into four categories based on the number of oocytes retrieved (ovarian response): low (zero to three oocytes), suboptimal (four to nine oocytes), optimal (10–15 oocytes), and high (>15 oocytes). The correlation between serum AMH concentration and number of oocytes was determined using Spearman's rho. To define the predictive capability of serum AMH for the low and high ovarian response categories, a receiver operating characteristic (ROC) curve analysis was conducted and the area under the curve (AUC) calculated for each category compared with the remaining population. The Youden derived optimal cut-point was derived using the CUTPT command (*Clayton, 2013*). To define the range of AMH values associated with the suboptimal and optimal ovarian response categories, generalized linear models were applied between each of the following comparisons: low response versus suboptimal response; suboptimal response versus optimal response; and optimal response versus high response. The estimated coordinates of the optimal cut-off point were then derived using ROC analysis. A multinomial logistic regression analysis was conducted to further analyse the relationship between AMH and ovarian response. Potential confounding variables were controlled for by adjusting for age, body weight and total dose of gonadotrophins.

RESULTS

Study population

In total, 1248 patients aged between 18 and 48 years were enrolled (TABLE 1). Patients had a mean age ± SD of 36.4 ± 5.0 years old and a mean number of oocytes ± SD of 11.0 ± 5.0 oocytes (TABLE 1). Briefly, 1448 ovarian stimulation cycles for IVF (*n* = 1119), fertility preservation (*n* = 252) or oocyte donation (*n* = 77) were completed. Of these, 270 cycles (18.6%) led to a low response, 539 (37.2%) suboptimal response, 341 (23.5%) optimal response and 298 (20.6%) high response. Overall mean gonadotrophin dose was 2801 ± 1089 international units per day; mean gonadotrophin dose did not seem to vary greatly by ovarian response category (Supplementary Table 1).

Measurement of anti-Müllerian hormone

A strong positive correlation was observed between serum AMH concentration and the number of oocytes retrieved (Spearman's rho = 0.74; $P < 0.0001$). The mean (95% CI) serum AMH concentrations in patients with low, suboptimal, optimal and high responses were 5.0 pmol/l (4.4 to 5.7) or 0.70 ng/ml (0.62 to 0.79), 9.6 pmol/l (8.9 to 10.2) or 1.34 ng/ml (1.25 to 1.43), 17.5 pmol/l (16.3 to 18.7) or 2.45 ng/ml (2.28 to 2.62), and 32.5 pmol/l (30.3 to 34.8) or 4.55 ng/ml (4.24 to 4.87), respectively.

The percentiles for serum AMH values in the whole population, from the 2.5th percentile to the 97.5th percentile, are presented in TABLE 2. Number of oocytes retrieved for each serum AMH decile is presented in FIGURE 1. The number of oocytes retrieved in each serum AMH group, defined by percentile, is presented in detail in Supplementary Table 2.

Development of anti-Müllerian hormone cut-offs for low, suboptimal, optimal and high ovarian response

On the basis of ROC curve analysis, use of serum AMH concentrations to predict ovarian response showed AUC (95% CI) of 0.85 (0.83 to 0.88) for low and 0.89 (0.87 to 0.91) for high response ($P < 0.001$ for both categories) (FIGURE 2A–FIGURE 2B). The optimal serum AMH cut-off for predicting a low ovarian response was 6.4 pmol/l (0.89 ng/ml), with 74.4% sensitivity and 79.8% specificity (TABLE 3).

TABLE 2 MEAN ANTI-MÜLLERIAN HORMONE VALUES BY PERCENTILE IN THE WHOLE POPULATION

Percentile	2.5	5	10	25	50	75	90	95	97.5
AMH, pmol/l (ng/ml)	0.7 (0.10)	1.5 (0.21)	2.6 (0.36)	5.6 (0.78)	10.5 (1.47)	20 (2.8)	34.2 (4.8)	44.6 (6.2)	58.9 (8.2)

AMH, anti-Müllerian hormone.

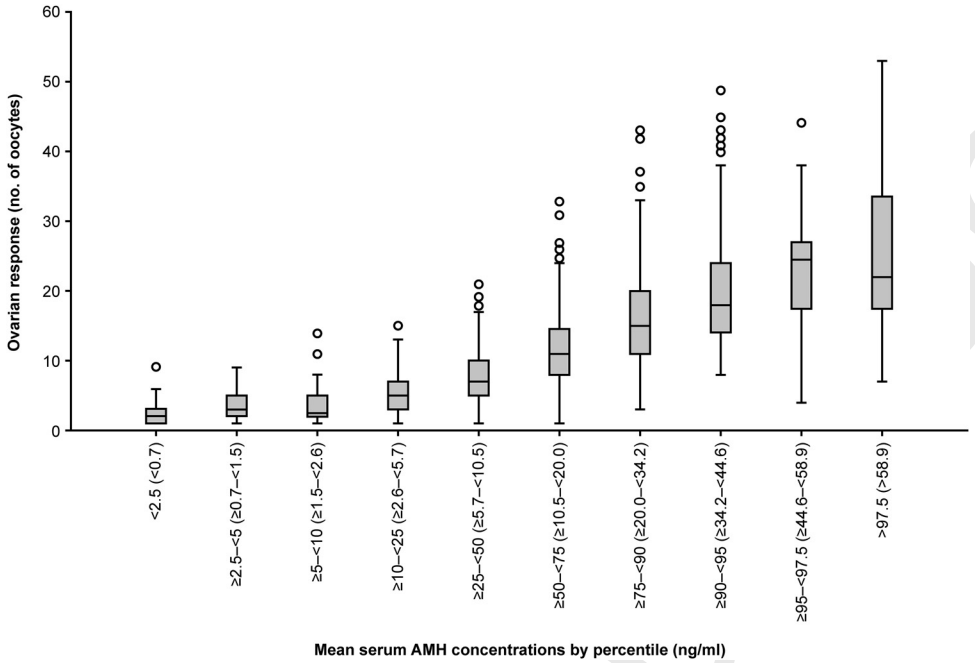

FIGURE 1 The distribution of ovarian response (number of oocytes retrieved) by serum anti-Mullerian hormone (AMH) percentile in whole population (n = 1248). The circular data points indicate outliers.

For predicting a high ovarian response, the optimal cut-off was 14.2 pmol/l (1.99 ng/ml), with 88.3% sensitivity and 74.8% specificity (TABLE 3). For a suboptimal ovarian response, the predicted AMH range was 4.9 pmol/ (0.69 ng/ml; sensitivity: 59.8%; specificity: 65.2%) to 11.3 pmol/l (1.58 ng/ml; sensitivity: 67.2%; specificity: 73.7%) ((FIGURE 2C). For an optimal ovarian response, the predicted AMH range was 11.3 pmol/l (1.58 ng/ml) to 20.9 pmol (2.9 ng/ml; sensitivity and specificity for this upper value of 68.5% and 72.7%, respectively) (FIGURE 2C and TABLE 3).

The multinomial logistic regression analysis showed that 47% (R^2 = 0.470) of the variation in ovarian response could be attributed to serum AMH alone. The addition of age, body weight and total dose of gonadotrophins had limited effect on the model, increasing this value to 50.9% (R^2 = 0.509).

DISCUSSION

The present study demonstrates that a single determination of serum AMH, performed at any time of day, on any day of the menstrual cycle, 6 months or less before an ovarian stimulation cycle, can predict the magnitude of ovarian response with high precision. The accuracy of sensitivity and specificity is highest when predicting a low (zero to three oocytes obtained) or high (>15 oocytes obtained) ovarian response. Serum AMH determination, however, also has utility in predicting suboptimal (four to nine oocytes) and optimal (10–15 oocytes) responses. A strong correlation was observed between serum AMH concentrations and the number of oocytes obtained, despite the variation in, and adjustments, to gonadotrophin dosage. By defining optimal cut-off values for predicting a low and high ovarian response using the Elecsys AMH immunoassay, a single serum AMH measurement could aid accurate prediction of the number of oocytes to be obtained in a GnRH antagonist cycle.

Serum AMH determination is known to be a good predictor of low ovarian response (*Broekmans et al., 2006*). An individual patient data meta-analysis, including 5705 women undergoing IVF, found that AMH had an AUC of 0.78 for predicting poor ovarian response (defined as fewer than three to four dominant follicles measuring over 12 mm in diameter) (*Broer et al., 2013*). Furthermore, a retrospective data analysis including 523 women undergoing their first IVF cycle following a progestin-primed ovarian stimulation protocol found that AMH had an AUC of 0.86 for the prediction of poor ovarian response (fewer than four oocytes), corresponding to an optimal cut-off of 1.26 ng/ml on the Access AMH assay (Beckman Coulter, Inc., Brea, CA, USA) (*Huang et al., 2019*). A recent prospective cohort study including data from 472 participants validated a lower AMH cut-off of 0.93 ng/ml for poor ovarian response (fewer than four oocytes) on the Access AMH assay, with an AUC of 0.85 (*Baker et al., 2018*; *2021*). Both cut-offs previously validated for the Access AMH assay are lower than that derived herein for predicting a low ovarian response (three or fewer oocytes) on the Elecsys AMH assay (6.4 pmol/l [0.89 ng/ml]); however, predictive performance between cut-offs for poor ovarian response on the Access AMH and Elecsys AMH assays was similar (AUC 0.85–0.86 versus AUC 0.85). The automated Elecsys AMH immunoassay has previously shown strong concordance with the Access AMH assay in calibration; however, both assays provide substantially lower AMH measurements compared with pre-existing assays (*Nelson et al., 2015*). Therefore, AMH cut-offs for poor ovarian response may vary depending on the assay used, and assay-specific AMH cut-offs for poor ovarian response should be reported in subsequent studies (*Baker et al., 2021*).

Anti-Müllerian hormone has previously been shown to have good predictive capability for excessive ovarian response. An individual patient data meta-analysis that included 4786 women demonstrated the high predictive value

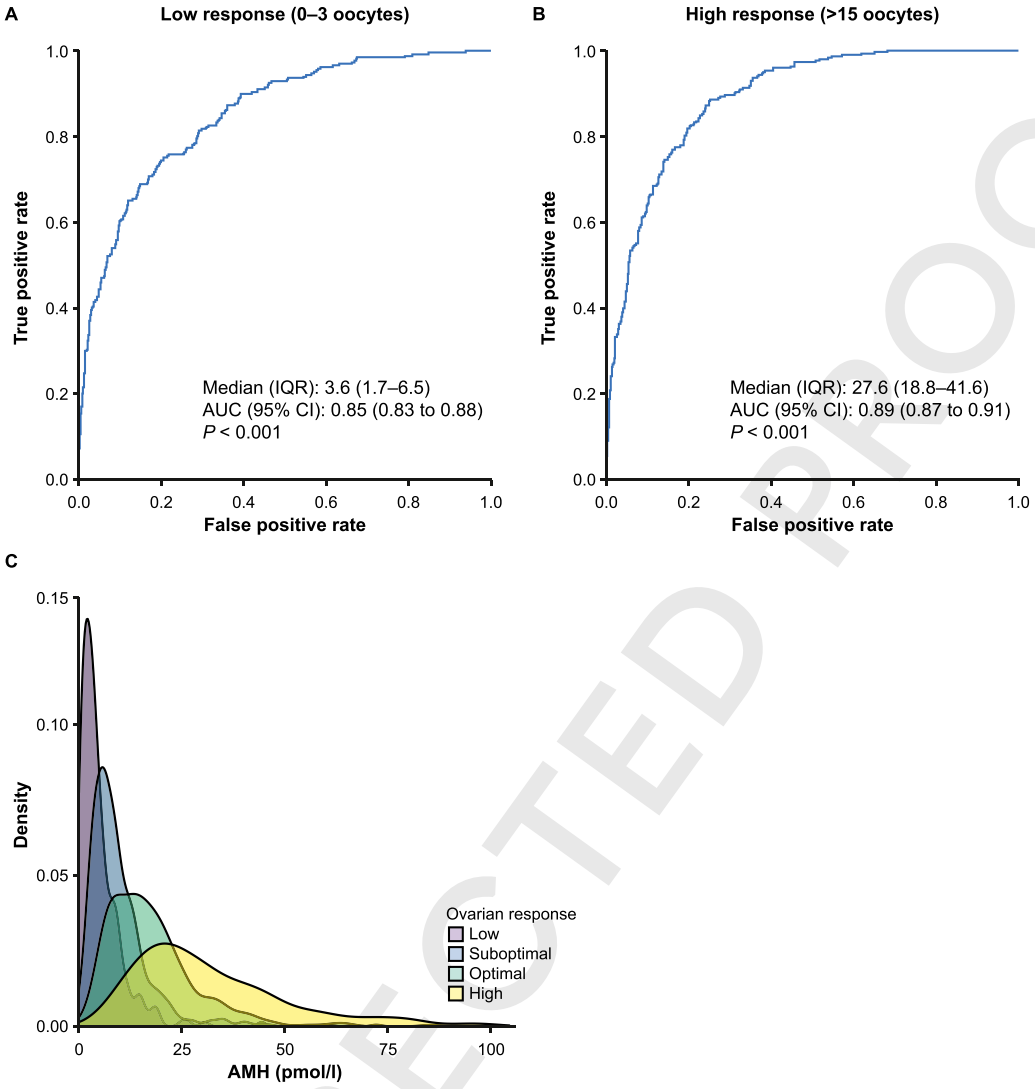

FIGURE 2 Receiver operating characteristic curves for predicting ovarian response based on serum anti-Müllerian hormone (AMH) concentrations in (A) low and (B) high response groups, and (C) a multinomial model for suboptimal and optimal ovarian response categories; density refers to the proportion of values observed.
AUC, area under the curve; IQR, interquartile range.

of AMH for excessive ovarian response (AUC 0.82) (Broer *et al*., 2013). More recently, *Anckaert et al. (2019)* validated an AMH cut-off of 15.0 pmol/l (2.1 ng/ml) for prediction of hyper-response to ovarian stimulation (more than 15 oocytes retrieved) using the Elecsys AMH assay, with good predictive performance (sensitivity: 81.3%; negative predictive value: 96.6%). The AMH cut-off derived herein for exclusion of a high ovarian response was marginally lower (14.2 pmol/l [1.99 ng/ml]) than that of *Anckaert et al. (2019)* but achieved higher sensitivity (88.3% versus 81.3%). To the best of our knowledge, however, no previous studies have analysed the capability of serum AMH to discriminate within more detailed sub-categories of ovarian response, i.e. low, suboptimal, optimal and high.

Key strengths of this study include the large, unselected population of women enrolled, robust statistical analysis and measurement of samples using the

TABLE 3 SERUM ANTI-MÜLLERIAN HORMONE CUT-OFFS FOR PREDICTING A LOW, SUBOPTIMAL, OPTIMAL OR HIGH OVARIAN RESPONSE USING THE ELECSYS ANTI-MÜLLERIAN HORMONE IMMUNOASSAY

Ovarian response	AMH cut-off, pmol/l (ng/ml)	Sensitivity (%)	Specificity (%)
Low[a]	6.4 (0.89)	74.4	79.8
High[a]	14.2 (1.99)	88.3	74.8
Suboptimal	4.9–11.3 (0.69–1.58)	59.8 67.2	65.2 73.7
Optimal	11.3–20.9 (1.58–2.9)	67.2 68.5	73.7 72.7

[a] Values derived from the receiver operating characteristic area under the curve analysis using the CUTPT command.
Sensitivity and specificity values for suboptimal and optimal ovarian response refer to the upper and lower anti-Müllerian hormone cut-off values, respectively.
AMH, anti-Müllerian hormone.

fully automated and internationally accessible Elecsys AMH immunoassay. We acknowledge that the present study also has some limitations. Primarily, the dose of gonadotrophin (FSH and human menopausal gonadotrophin) given to patients was not solely defined using serum AMH concentrations, but administered at the physicians' discretion, whereby factors such as age, body weight and the patient's response to previous ovarian stimulation cycles were also considered. This is reflective, however, of current practice in most fertility treatment centres. Nonetheless, our multinomial logistic regression analysis showed that 47% of the variation in ovarian response could be attributed to serum AMH concentrations alone; the addition of age, body weight or total dose of gonadotrophins had minimal effect on this model. In addition, we did not treat or analyse women differently depending on the reason for ovarian stimulation, e.g. IVF, fertility preservation or oocyte donation. A minor limitation is that the data were gathered over 5 years ago from the time of writing, and practices may have changed in the intervening years. Furthermore, previous studies have reported between-method variability in commercially available AMH immunoassays (*Su et al., 2014*; *Punchoo and Bhoora, 2021*); therefore, the present results may not be directly transferable to assays from other manufacturers. The Elecsys AMH immunoassay, however, is still available in many markets, and data are lacking on this topic; therefore, we believe the data are still valuable. Additionally, AMH was determined at any time and on any day of the menstrual cycle, which may have affected its predictive value (*Kissell et al., 2014*; *Biniasch et al., 2021*). A recent study, however, showed a strong correlation (r = 0.92) between AMH concentrations measured on a random day of the cycle at screening (up to 3 months before ovarian stimulation) and AMH concentrations measured at the start of stimulation on cycle day 2–3 (*Nelson et al., 2019*). Moreover, it was shown that the predicted number of oocytes obtained between a random AMH determination and a determination at the start of stimulation was equal in 75.3% of women, with a difference in ovarian response of ±1 oocyte in 95% of women (*Nelson et al., 2019*). Furthermore, 97.3% of women with an AMH concentration lower than 15 pmol/l (2.1 ng/ml) attained the same number of oocytes (*Nelson et al., 2019*). These findings, and the findings of our study, support that AMH can be determined using a serum sample drawn on any day of the menstrual cycle, without intra-cycle variation impairing its predictive performance for ovarian response. Future research should consider the effects of adding other variables into this model, such as the day of the cycle in which serum AMH is determined or comorbidities within the patient population.

In conclusion, the present study shows that ovarian response after stimulation in a GnRH antagonist cycle can be predicted with high accuracy using a single determination of serum AMH, measured on any day of the menstrual cycle. These findings will support medical decision-making for physicians counselling patients on assisted reproductive technology.

UNCITED REFERENCE

Roche Diagnostics GmbH, 2021

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.rbmo.2022.10.012.

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