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ARTICLE

The performance of the Elecsys® anti-Müllerian hormone assay in predicting extremes of ovarian response to corifollitropin alfa

BIOGRAPHY

Professor Polyzos is the Clinical and Scientific Director of the Department of Reproductive Medicine of Dexeus University Hospital and a Professor of Reproductive Endocrinology in the University of Aarhus. He is the author of more than 150 publications, with special focus on reproductive endocrinology, ovarian reserve and poor ovarian response.

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KEY MESSAGE

The Elecsys® anti-Müllerian hormone (AMH) assay demonstrated an excellent clinical performance in identifying both low and high ovarian response. To the best of our knowledge, this is the first study to provide evidence on the ability of the automated Elecsys® AMH assay to predict ovarian response in a corifollitropin alfa antagonist protocol.

ABSTRACT

Research question: What is the performance of anti-Müllerian hormone (AMH) as measured by the Elecsys® AMH assay in predicting ovarian response in women treated with 150 µg corifollitropin alfa (CFA)?

Design: Multicentre, prospective study conducted between December 2015 and April 2018. Women were aged 18–43 years, had regular menstrual bleeding, a body mass index of 17–35 kg/m^2 and weighed 60 kg or over. Exclusion criteria: previous oophorectomy, history of ovarian hyperstimulation syndrome, a previous IVF and intracytoplasmic sperm injection cycle producing over 30 follicles measuring 11 mm or wider, basal antral follicle count (AFC) over 20 or polycystic ovarian syndrome. All women were treated with 150 µg CFA followed by recombinant FSH (150–300 IU/day) in a fixed gonadotrophin releasing hormone antagonist protocol.

Results: Of the 219 patients enrolled, 22.8% had low ovarian response (three or fewer oocytes), 66.2% had normal response and 11% had high ovarian response (15 or more oocytes). The AMH and AFC presented an area under the curve of 0.883 (95% CI 0.830 to 0.936) and 0.879 (95% CI 0.826 to 0.930), respectively, for low ovarian response; and an AUC of 0.865 (95% CI 0.793 to 0.935) and 0.822 (95% CI 0.734 to 0.909) for high ovarian response. An AMH cut-off of 1.0 ng/ml provided a sensitivity of 92.0% and a specificity of 66.9% in the prediction of low ovarian response; a cut-off of 2.25 ng/ml predicted high ovarian response with a sensitivity of 54.2% and a specificity of 91.8%.

Conclusions: The automated Elecsys® AMH assay predicts ovarian response in a CFA antagonist protocol. The best predictors of ovarian response in CFA-treated patients were AMH and AFC.

KEYWORDS

Anti-Müllerian hormone
Corifollitropin alfa
Electrochemiluminescence immunoassay
Ovarian reserve
Ovarian response

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INTRODUCTION

Ovarian stimulation is a key part of the IVF and intracytoplasmic sperm injection (ICSI) procedure. The anticipated yield of oocytes varies according to patient age and ovarian reserve. Therefore, individualization of ovarian stimulation is of paramount importance, which makes ovarian reserve marker analysis essential for treatment and dose selection (*Popovic-Todorovic et al., 2003*; *Olivennes et al., 2011*; *La Marca and Sunkara, 2014*). Therefore, a wide range of markers have been proposed as predictors of ovarian response. Antral follicle count (AFC) and anti-Müllerian hormone (AMH) are now recognized as the most accurate (*Broer et al., 2011*; *2014*; *Martínez et al., 2013*; *Polyzos et al., 2013*; *Lerman et al., 2017*; *Scheinhardt et al., 2018)*. The advantages of AMH include its low intra- and inter-cycle variability (*Van Disseldorp et al., 2010*; *Polyzos et al., 2013*; *Kissell et al., 2014*; *Gracia et al., 2018*), as well as the fact that it is less prone to observer biases compared with AFC (*Iliodromiti et al., 2015*). Different AMH assays have been developed over the past few years (*Li et al., 2016*; *Iliodromiti et al., 2017*). Most studies have been used manual plate-based ELISAs and, although these reports provide valuable information, the reproducibility of the results among different laboratories has been controversial (*Zuvela et al., 2013*). Conversely, excellent intra- and inter-assay correlation has been reported among different automated assays (*Li et al., 2016*). Comparisons between manual and automated assays, however, should be analysed with caution, taking into account the 20–30% higher values reported with manual assays (*Gassner and Jung, 2014*).

The Elecsys® AMH assay is an electrochemiluminescence immunoassay for quantitative determination of serum AMH and was the first automated AMH assay to be approved by the US Food and Drug Administration. Its analytical performance, in terms of precision and increased sensitivity, has been reported in several trials (*Anderson et al., 2015*; *Anckaert et al., 2016*; *2019*; *Jacobs et al., 2019*). Few studies have assessed the new automated Elecsys® AMH assay in predicting ovarian response to stimulation, and all were focused on patients receiving daily gonadotrophin injections with different daily doses. Corifollitropin alfa (CFA) is a fusion product of human FSH and the C-terminal peptide of the β-subunit of HCG, produced by recombinant DNA technology (*Fauser et al., 2009*). It has the same activity as FSH and recombinant FSH, with an increased serum half-life, which allows it to induce and sustain multi-follicular growth for 7 days after a single subcutaneous injection (*Fauser et al., 2009*). Its proven superiority in the number of oocytes retrieved, ongoing pregnancy rates and live birth rates compared with daily recombinant FSH (*Corifollitropin Alfa Dose-finding Study Group, 2008*; *Devroey et al., 2009*; *Boostanfar et al., 2015*; *Pouwer et al., 2015*; *Griesinger et al., 2016*), have generalized its use among reproductive medicine physicians.

The scope of our study was to evaluate the predictive ability of AMH as measured using the Elecsys® AMH assay in women treated with CFA.

MATERIALS AND METHODS

A multicentre, prospective study of patients who underwent an IVF/ICSI cycle was conducted between December 2015 and April 2018. Patients were recruited in five centres (Dexeus University Hospital; Centre for Reproductive Medicine of the Universitair Ziekenhuis (UZ) Brussel, Belgium; IVI Madrid, Spain; Universitäres Kinderwunschzentrum Lübeck und Manhagen, Lübeck, Germany; Università degli Studi di Modena e Reggio Emilia, Italy).

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and with approval of the Institutional Review Board of the institutions involved in the study (Dates of final approval: Dexeus University Hospital, Barcelona, 23 January 2017; Centre for Reproductive Medicine of the Universitair Ziekenhuis (UZ) Brussel, 23 December 2015; IVI Madrid, 7 July 2016; Universitäres Kinderwunschzentrum Lübeck und Manhagen, 23 September 2016; Università degli Studi di Modena e Reggio Emilia, 8 November 2016). After detailed written and oral information regarding the study, all patients signed an informed consent sheet.

Patient selection criteria

The study included patients aged between 18 and 43 years old, with regular menstrual bleeding, a body mass index (BMI) of 17–35 kg/m^2, weighing over 60 kg who planned to undergo ovarian stimulation with 150 μg CFA followed by recombinant FSH either for IVF or ICSI or in order to undergo fertility preservation for social or medical reasons. Patients were excluded if they had undergone a previous oophorectomy, had a history of ovarian hyperstimulation syndrome (OHSS), had undergone a previous ovarian stimulation cycle that resulted in more than 30 follicles measuring 11 mm or wider as determined by ultrasound examination, a basal AFC over 20, polycystic ovarian syndrome according to the Rotterdam criteria (*Teede et al., 2018*) or clinically relevant endocrine disorders.

Stimulation protocol

All women planned to be treated with 150 μg CFA followed by recombinant FSH in a fixed gonadotrophin releasing hormone (GnRH) antagonist protocol were enrolled. They received no pre-treatment with oral contraceptive pills. On day 2 or 3 of the menstrual cycle, a single subcutaneous injection of 150 μg CFA was administered (stimulation day 1). Starting on stimulation day 6, patients received a daily subcutaneous injection of 0.25 mg ganirelix up to and including the day of HCG administration to prevent premature LH surges. From stimulation day 8 onwards, treatment continued with a daily subcutaneous dose of recombinant FSH (150–300 IU/day) up to the day of HCG administration based on the patient's ovarian reserve. Recombinant FSH dose was selected based on age, AFC, AMH and BMI as described in previous studies (*Yovich et al., 2018*). No stepping up or down was allowed. In the case of monofollicular development and in the case of non-tubal factor infertility and adequate sperm quality, either rescue intrauterine insemination was considered or the cycle was cancelled. In case of no follicular development the treatment cycle was cancelled. Criteria for cycle cancellation or rescue intrauterine insemination were implemented on stimulation day 10. In both circumstances, the cycle was considered cancelled and the number of oocytes imputed was set to zero. These patients were included in the low response group. The allocated interventions are presented in FIGURE 1.

Ovulation trigger and oocyte retrieval

As soon as three follicles measuring 17 mm or wider were observed by

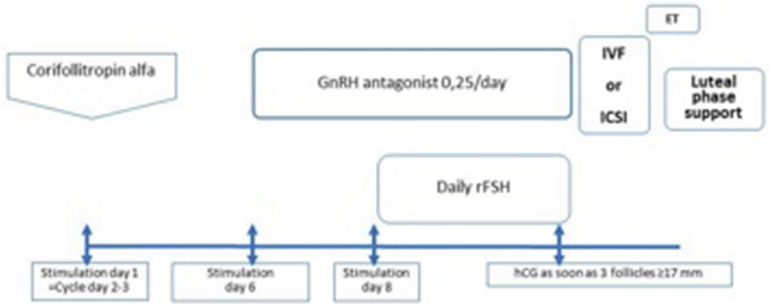

FIGURE 1 Treatment schedule. GnRH, gonadotrophin releasing hormone; ICSI, intracytoplasmatic sperm injection; rFSH, recombinant FSH.

ultrasound, recombinant 250 µg subcutaneous HCG was administered the same day or the day after to induce final oocyte maturation.

In case of excessive ovarian response (17 or more follicles measuring more than 11 mm on the day of final oocyte maturation) triggering with a GnRH agonist (triptorelin 0.2 mg) was used for safety reasons, followed by either freezing all embryos, or fresh embryo transfer with modified luteal phase support. About 34–36 h thereafter, oocyte retrieval followed by IVF/ICSI was carried out.

Blood sampling and sample analysis

At the routine blood sampling visit on the day of initiation of CFA, part of the blood sample collected for routine analysis was used to allow the measurement of AMH, oestradiol, FSH and LH.

Blood drawn in plain serum tubes for routine blood analysis during treatment underwent centrifugation within 1 h, and serum was separated and immediately stored at −80°C until analysis. All samples were analysed together at the end of the study in the central laboratory of UZ Brussel.

Elecsys® AMH assay (Roche Diagnostics GmbH, Germany) was used to analyse AMH, with a coefficient of variation for intermediate precision less than 3.0% and a detection range of 0.01 to 23 ng/ml (0.07–164 pmol/l).

Outcomes

The primary outcome was to evaluate the incidence of low and high ovarian response by serum AMH level. Secondary outcomes were to evaluate the ability of AFC, age and FSH to predict low and high ovarian response and to compare the ability of AMH, AFC, age and FSH as predictors of ovarian response. The cut-off for defining low ovarian response was three or fewer oocytes retrieved, in accordance with the Bologna criteria for ovarian response (*Ferraretti et al., 2011*). Excessive ovarian response was defined as more than 15 oocytes retrieved, in accordance with earlier studies demonstrating an association between more than 15 oocytes retrieved and OHSS (*Steward et al., 2014*).

Statistical analysis

Sample size calculation was carried out on the assumption that up to five predictive factors will be selected in each logistic regression model. Therefore, assuming that for each predictive factor at least 10 events are required, a total of around 50 events are needed (*Moons et al., 2009*). A total sample size of 200 participants was planned, with an additional 20 participants (10%) to compensate for discontinued participants.

Continuous variables were expressed as mean and SD, and categorical variables as frequencies and percentages. Univariate logistic regression analysis was conducted to identify the best predictors for high (more than 15 oocytes) and low (fewer than three oocytes) ovarian responses. Categorical variables were compared using chi-squared test or Fisher's exact test. Continuous variables were compared using the Student's t-test or Wilcoxon Mann–Whitney Test. All tests were bilateral with a significance level set to 0.05. Receiver operating characteristic (ROC) curves were generated and the area under the ROC curves (AUC) was determined to assess the discriminative power of independent ovarian reserve markers (AMH, AFC, age and FSH) to predict low and high ovarian response. Subsequently, associated factors for prediction of high and low ovarian reserve were entered into logistic regression models. The performance of each model was evaluated using ROC curves and AUCs. The pROC package (Robin *et al*., 2011) in R Software (R Core Team, 2018) was used for statistical analyses.

RESULTS

A total of 219 patients were enrolled in the study, of whom 22.8% (n = 50) had a low ovarian response, 66.2% (n = 145) had a normal response and 11% (n = 24) had a high ovarian response. Patients' baseline characteristics and ovarian stimulation cycle characteristics according to the level of ovarian response are presented in TABLE 1.

Predictors of low ovarian response

The clinical performance of the different ovarian reserve markers in the prediction of low ovarian response was assessed by ROC curve analysis (FIGURE 2A). The best predictors of low ovarian response were AMH and AFC, with an AUC of 0.883 (95% CI 0.830 to 0.936) and an AUC of 0.879 (95% CI 0.826 to 0.930). A cut-off of 1.0 ng/ml provided a sensitivity of 92.0% and a specificity of 66.9% in the prediction of three or fewer oocytes retrieved, with a positive likelihood ratio (LR+) of 2.78 and a negative likelihood ratio (LR–) of 0.12. The addition of AFC, age and FSH to AMH in the prediction model slightly increased the AUC to 0.926 (95% CI 0.871 to 0.981), although the difference was not statistically significant compared with AMH or AFC alone (FIGURE 3).

Predictors of high ovarian response

The ROC curve analysis also confirmed that AMH and AFC were the best predictors of high ovarian response, with an AUC of 0.865 (95% CI 0.793 to 0.935) and 0.822 (95% CI 0.734 to 0.909), respectively (FIGURE 2B). A cut-off of 2.25 ng/ml predicted more than 15 oocytes retrieved, with a sensitivity of 54.2% and a specificity of 91.8%, with a LR+ of 6.60 and a LR– of 0.50. Adding AFC, age and FSH to AMH slightly increased the AUC of the predictive model to 0.918 (95% CI 0.856 to 0.981), although the difference was not statistically significant compared with AMH or AFC alone (FIGURE 4). Of note, the oocyte retrieval process yielded more than 20 oocytes in only nine patients (4.1%).

TABLE 1 PATIENT AND CYCLE CHARACTERISTICS

		Normal ovarian response (n=145)	*High ovarian response (n=24)*	*Low ovarian response (n=50)*	*P-value (HOR versus others)*	*P-value (LOR versus others)*
Age, years		36.08 ± 4.24	33.58 ± 4.62	37.54 ± 3.19	0.003	0.011
BMI, kg/m^2		24.18 ± 4.49	25.27 ± 3.02	24.25 ± 3.99	0.120	0.360
Race, % (*n*)	White	80.0 (116)	91.7 (22)	84.0 (42)		
	Asian	4.8 (7)	0	2.0 (1)	0.507	0.599
	Black or African American	2.8 (4)	4.2 (1)	6.0 (3)		
	Other	12.4 (18)	4.2 (1)	8.0 (4)		
Smokers		21.4 (31)	29.2 (7)	20.0 (10)	0.363	0.709
Cause of infertility	Male	39.3 (57)	54.2 (13)	26.0 (13)		
	Idiopathic	33.1 (48)	33.3 (8)	28.0 (14)		
	Endometriosis	6.2 (9)	8.3 (2)	14.0 (7)	0.554	0.001
	Tubal	6.9 (10)	4.2 (1)	10.0 (5)		
	Ovulatory disorders	2.8 (4)	0	18.0 (9)		
	Single mother	2.1 (3)	0	2.0 (1)		
	Information not available	9.7 (14)	0	2.0 (1)		
Type of infertility	Primary	66.2 (96)	45.8 (11)	48.0 (24)	0.139	0.052
	Secondary	33.8 (49)	54.2 (13)	52.0 (26)		
Duration of infertility		34.61 ± 33.29	24.13 ± 15.18	51.00 ± 45.28	0.064	0.003
AMH, ng/ml		1.3 ± 0.72	2.47 ± 1.08	0.50 ± 0.38	<0.001	<0.001
AFC, *n*		9.47 ± 3.91	14.29 ± 8.35	4.76 ± 2.44	<0.001	<0.001
FSH, mIU/ml		8.52 ± 2.64	7.16 ± 1.97	10.14 ±3.72	0.026	0.040
LH, mIU/ml		6.43 ± 2.35	6.36 ± 2.32	6.72 ± 2.66	0.790	0.748
Oestradiol, pg/ml		42.04 ± 20.88	40.52 ± 17.87	48.33 ± 68.18	0.986	0.253
Progesterone, ng/ml		0.74 ± 3.31	0.30 ± 0.17	0.39 ± 0.21	0.249	0.586
Duration of stimulation, days		9.57 ± 2.16	9.88 ± 1.42	9.58 ± 2.95	0.502	0.729
Total gonadotrophin dose, IU		646.63 ± 413.5	617.05 ± 342.90	865.85 ± 664.78	0.778	0.103
Oocytes retrieved, *n*		8.21 ± 3.11	20.71 ± 4.14	1.46 ± 1.15	<0.001	<0.001
Mature oocytes retrieved, *n*		6.34 ± 3.28	14.13 ± 6.37	1.39 ± 0.86	<0.001	<0.001
Fertilized oocytes, *n*		4.08 ± 3.01	9.63 ± 5.63	0.71 ± 0.87	<0.001	<0.001

Quantitative variables are presented as mean ± SD and qualitative variables as or % (*n*).

AFC, antral follicle count; AMH, anti-Müllerian hormone; HOR, high ovarian response; LOR, low ovarian response.

DISCUSSION

In this prospective, multicentre study, the Elecsys® AMH assay demonstrated excellent clinical performance in identifying low and high ovarian response in women treated with CFA in an antagonist protocol. For a cut-off of 1.0 ng/ml, low ovarian response was predicted with a sensitivity of 92.0% and a specificity of 66.9%. Similarly, excellent predictive ability was demonstrated for high ovarian response. A cut-off of 2.25 ng/ml presented a sensitivity of 54.2% and a specificity of 91.8% in the prediction of high ovarian response. ROC curve analysis comparing different ovarian reserve markers also confirmed that AMH and AFC performed best as predictors of ovarian response for both low and high responders.

Anti-Müllerian hormone has proven its clinical utility in the prediction of ovarian response among different ovarian stimulation protocols (*Anckaert et al., 2012*; *2019*; *Baker et al., 2018*). In patients stimulated with CFA, previous studies have also reported AMH as the best predictor of ovarian response (*Polyzos et al., 2013*; *Oehninger et al., 2015*; *Lerman et al., 2017*). These studies, however, were carried out with manual plate-based ELISA assays, with inherent variability within and between laboratories (*Zuvela et al., 2013*). To the best of our knowledge, this is the first study to analyse the ability of the automated Elecsys® AMH assay to predict ovarian response in a CFA antagonist protocol. The AUC for the prediction of low ovarian response in this study was 0.883, which is in line with previous studies reporting AUCs between 0.836 and 0.929 with other assays (*Oehninger et al., 2015*; *Lerman et al., 2017;Baker et al., 2018*; *Scheinhardt et al., 2018;Jacobs et al., 2019*). Similarly, the AUC for the prediction of high ovarian response was 0.865, which is in line with previous

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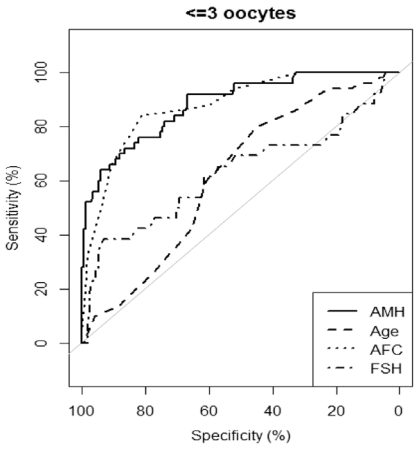
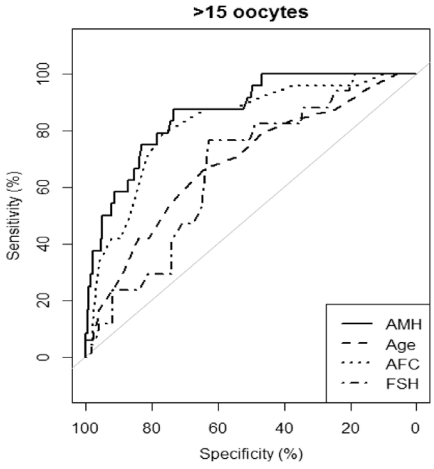


	Cut-off	Sensitivity	Specificity	LR+	LR-	AUC	95% CI
AMH (ng/mL)	1.0	92.0%	66.9%	2.78	0.12	0.883	0.830-0.936
AFC	6.0	84.0%	81.1%	4.44	0.20	0.879	0.826-0.930
Age (years)	35.0	80.0%	45.0%	1.45	0.44	0.619	0.537-0.700
FSH (IU/L)	12.1	38.5%	93.0%	5.5	0.66	0.629	0.489-0.768

b)



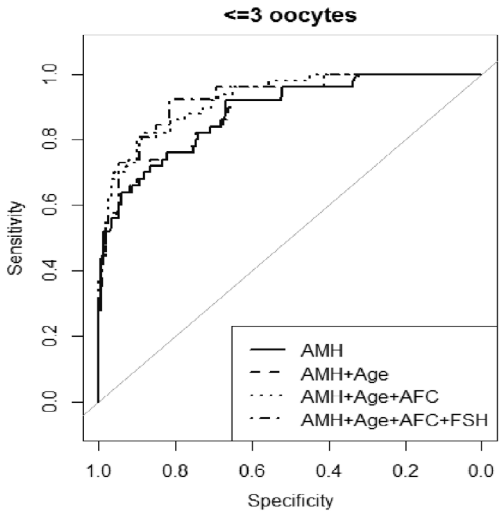
	Cut-off	Sensitivity	Specificity	LR+	LR-	AUC	95% CI
AMH (ng/mL)	2.25	54.2%	91.8%	6.60	0.50	0.865	0.793-0.935
AFC	10.0	79.2%	76.4%	3.36	0.27	0.822	0.734-0.909
Age (years)	35.0	66.7%	64.1%	1.86	0.52	0.686	0.570-0.802
FSH (IU/L)	7.2	62.6%	76.5%	2.66	0.49	0.667	0.541-0.791

FIGURE 2 Receiver operating characteristics curve analysis for ovarian reserve markers as predictors of low (a) and high (b) ovarian response. AFC, antral follicle count; AMH, anti-Müllerian hormone; AUC, area under the curve; LR, likelihood ratio.

reports varying between 0.821 and 0.890 (*Polyzos, et al., 2013*; *Oehninger et al., 2015*; *Lerman et al., 2017*; *Scheinhardt et al., 2018*; *Anckaert et al., 2019*).

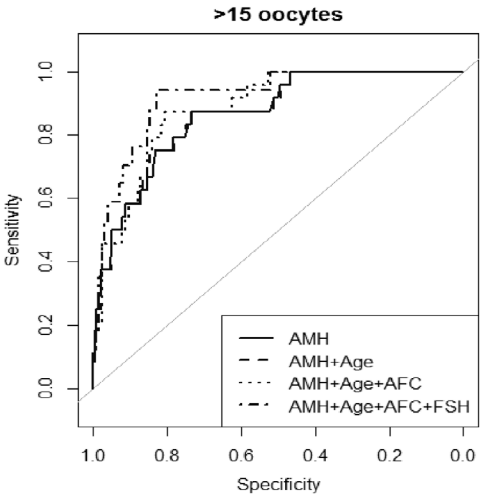
Regarding AMH optimal cut-off levels for the prediction of the extremes of ovarian response, the cut-off of 1.0 ng/ml, identified as the value with maximal sensitivity and specificity for predicting low response, seems to be comparable with previous studies and close to the proposed cut-offs defined by the Bologna criteria (*Ferraretti et al., 2011*). *Baker et al. (2018)* reported a cut-off of 0.93 ng/ml in the prediction of four or fewer oocytes retrieved, whereas *Lerman et al. (2017)* and *Oehninger et al. (2015)* described a cut-off of 0.91 ng/ml and 1.03 ng/ml, respectively, as the most accurate to predict six or fewer oocytes retrieved.

For high responders, the cut-off of 2.25 ng/m was selected to optimize specificity. Taking into account that a GnRH antagonist protocol and GnRH agonist trigger were used, and that only women with a basal AFC of 20 or lower were included in the study, in accordance with the summary of product characteristics of CFA, the risk of OHSS is remarkably low. Therefore, a cut-off point with a higher specificity at the expense of a lower sensitivity was selected for high responders. The cut-off of 2.25 ng/ml is similar to the cut-offs of 2.24–3.52 ng/ml previously suggested using the manual assays (*Polyzos et al., 2013*; *Oehninger et al., 2015*; *Lerman et al., 2017*).



	AUC	95% CI
AMH	0.883	0.830-0.936
AMH+Age	0.883	0.830-0.937
AMH+Age+AFC	0.925	0.886-0.965
AMH+Age+AFC+FSH	0.926	0.871-0.981

FIGURE 3 Receiver operating characteristics curve analysis for predictors of low ovarian response. AFC, antral follicle count; AMH, anti-Müllerian hormone; AUC, area under the curve.



	AUC	95% CI
AMH	0.865	0.793-0.935
AMH+Age	0.865	0.794-0.937
AMH+Age+AFC	0.883	0.824-0.944
AMH+Age+AFC+FSH	0.918	0.856-0.981

FIGURE 4 Receiver operating characteristics curve analysis for predictors of high ovarian response. AFC, antral follicle count; AMH, anti-Müllerian hormone; AUC, area under the curve.

As far as AFC is concerned, this biomarker also showed a good clinical performance in predicting low and high responders (AUC 0.879 and 0.822, respectively). Although AFC has some disadvantages, including inter-observer variability (*Iliodromiti et al., 2015*) and controversies about its variation during the menstrual cycle (*Rombauts et al., 2011*; *Mavrelos et al., 2016*; *Coelho Neto et al., 2018*), the present study demonstrated that the predictive ability of AFC for low and high ovarian responses is comparable in women treated with CFA. Finally, in line with previous studies, age and FSH performed poorly in the prediction of both low and high ovarian responses (*Lerman et al., 2017*).

A limitation of this study is that, although sample size was accurately calculated as per CFA administration instruction and in accordance with the summary of product characteristics, patients' inclusion had to be restricted based on previous AFC values. Another limitation is that the dose of recombinant FSH administered from stimulation day 8 onwards was not fixed, varying between 150–300 IU/day. Although this dose was calculated based on patients' age and ovarian reserve (*Devroey et al., 2009*; *Boostanfar et al., 2015*), no specific protocol criteria have been followed. This could, however, be seen as a strength because it allows the results to be generalized to the IVF population. Moreover this dose adjustment after stimulation day 8 is highly unlikely to have influenced the number of oocytes retrieved. In fact, considering that follicular recruitment occurs during the first days of ovarian stimulation, the number of growing follicles is not likely to be affected by these dose modifications. Furthermore, recent studies on the individualization of ovarian stimulation protocols have not demonstrated such a significant effect (*Andersen et al., 2017*; *Oudshoorn et al., 2017*; *van Tilborg et al., 2017*; *Lensen et al., 2018*).

Finally, AMH was measured systematically on day 2–3 of the cycle, and this may limit the extrapolation of the results to samples obtained on other days of the cycle. Previous studies, however, have demonstrated limited inter- and intracycle variation in AMH levels (*Kissell et al., 2014*; *Lambert-Messerlian et al., 2016*; *Gracia et al., 2018*).

A major strength of our study is its prospective, multicentric design, optimizing the extrapolation of our results to the general IVF population. Furthermore, to our knowledge, this was the first study to evaluate the ability of the Elecsys® AMH assay to predict ovarian response in women treated with CFA. Therefore, our results provide clinical guidance for treatment and can be used in the prediction of low ovarian response in patients treated with CFA.

In the era of the OHSS-free clinic, closely related to the segmentation concept (*Devroey et al., 2011*), it might seem that ovarian reserve markers are losing ground. Clinical practice, however, is still guided by validated recombinant FSH dose algorithms, such as the PIVET algorithm, adjusting for patient parameters such as age, AFC, BMI, AMH, day-2 FSH and history of smoking (*Yovich et al., 2016*). In particular, this algorithm has also been validated for use with CFA, showing the critical role of age and AFC and the modulator role of AMH in defining recombinant FSH treatment dose (*Yovich et al., 2018*). These findings highlight the importance of keeping an accurate evaluation of ovarian reserve markers in our daily practice.

In conclusion, considering the limitations discussed above, our results demonstrate that AMH and AFC are the best predictors of ovarian response in patients treated with CFA, identifying with high sensitivity and specificity patients who are likely to be poor responders. With AMH, a cut-off of 1.00 ng/ml identifies with high sensitivity and specificity patients who are likely to be poor responders. As for high responders, a cut-off of 2.25 ng/ml selects with high specificity those are at risk of high response. It is also of great interest that when the threshold of 20 AFC is set for the selection of patients treated with CFA, only 11% of patients will respond with more than 15 oocytes and 4.1% with more than 20 oocytes, thereby minimizing the risk for OHSS.

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