

## ARTICLE



# The performance of the Elecsys<sup>®</sup> anti-Müllerian hormone assay in predicting extremes of ovarian response to corifollitropin alfa



## BIOGRAPHY

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## KEY MESSAGE

The Elecsys<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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<sup>2</sup> 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<sup>®</sup> AMH assay predicts ovarian response in a CFA antagonist protocol. The best predictors of ovarian response in CFA-treated patients were AMH and AFC.

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## KEYWORDS

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

## 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  $\beta$ -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

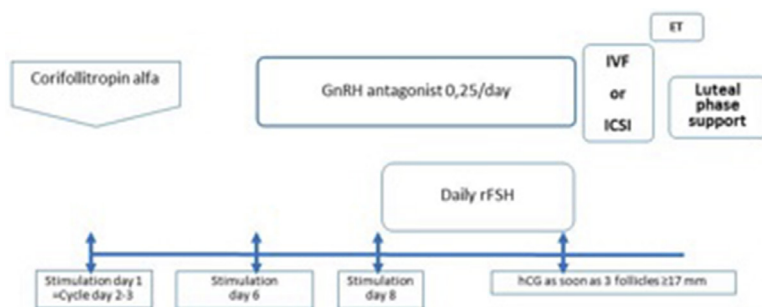
index (BMI) of 17–35 kg/m<sup>2</sup>, weighing over 60 kg who planned to undergo ovarian stimulation with 150 µg CFA followed by recombinant FSH either for IVF or ICSI or in order to undergo fertility preservation for social or medical reasons. Patients were excluded if they had undergone a previous 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, intracytoplasmic 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  $-80^{\circ}\text{C}$  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**

		<b>Normal ovarian response (n=145)</b>	<b>High ovarian response (n=24)</b>	<b>Low ovarian response (n=50)</b>	<b>P-value (HOR versus others)</b>	<b>P-value (LOR versus others)</b>
Age, years		36.08 ± 4.24	33.58 ± 4.62	37.54 ± 3.19	0.003	0.011
BMI, kg/m <sup>2</sup>		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)	0.507	0.599
	Asian	4.8 (7)	0	2.0 (1)		
	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)	0.554	0.001
	Idiopathic	33.1 (48)	33.3 (8)	28.0 (14)		
	Endometriosis	6.2 (9)	8.3 (2)	14.0 (7)		
	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

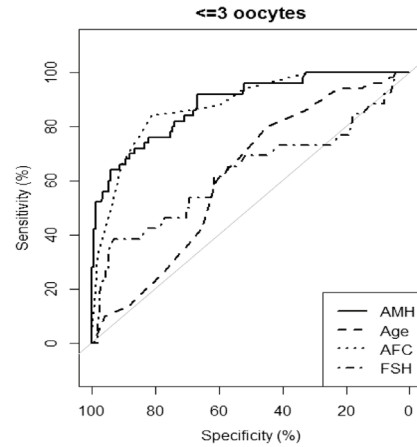
In this prospective, multicentre study, the Elecsys® AMH assay demonstrated excellent clinical performance in identifying low and high ovarian response in women treated with CFA in an antagonist protocol. For a cut-off of 1.0 ng/ml, low ovarian response was predicted with a sensitivity of 92.0% and a 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

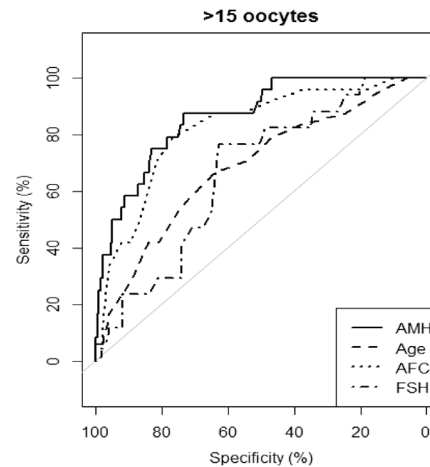
inherent variability within and between laboratories (Zuvela *et al.*, 2013). To the best of our knowledge, this is the first study to analyse the ability of the automated Elecsys® AMH assay to predict ovarian response in a CFA antagonist protocol. The AUC for the prediction of low ovarian response in 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

a)



	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).

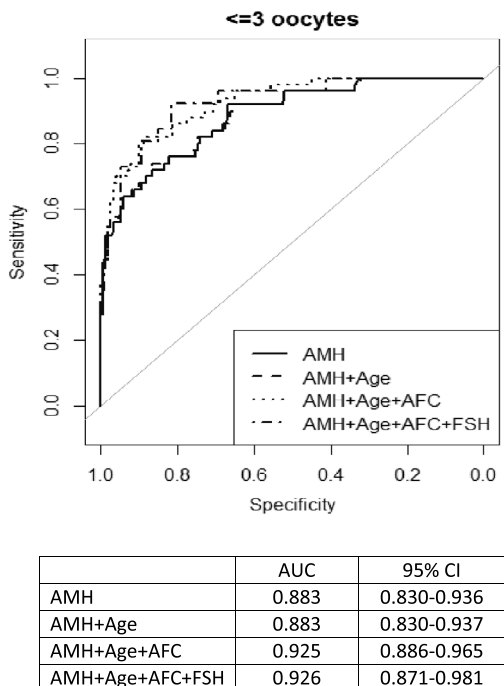
Regarding AMH optimal cut-off levels for the prediction of the extremes of ovarian response, the cut-off of 1.0 ng/ml, identified as the value with maximal sensitivity and specificity for predicting low response, seems to be comparable with previous studies and close to the proposed cut-offs defined 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.

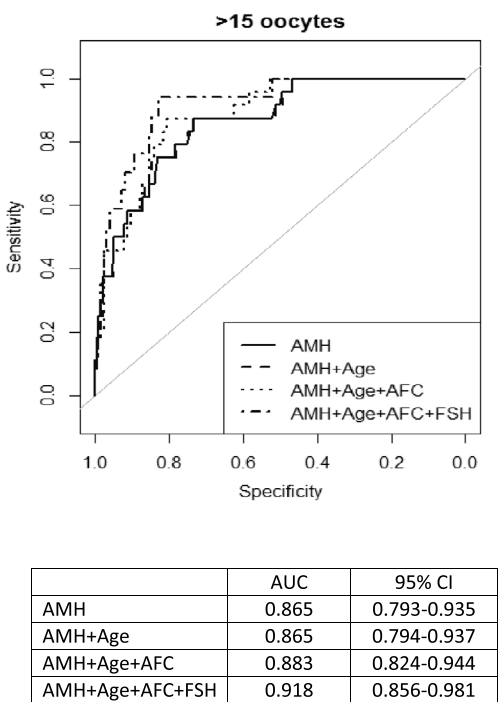
For high responders, the cut-off of 2.25 ng/ml was selected to optimize specificity. Taking into account that a GnRH antagonist protocol and GnRH

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).





**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.



**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; Mavrelou 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 who 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.

## ACKNOWLEDGEMENT

ARN conducted the literature search, contributed to data interpretation and wrote the manuscript; IR performed data collection and statistical analysis; BC, GG, GV, LM, DP, HT and MA contributed to the critical review of the manuscript; NPP designed the study, contributed to the writing and editing of the manuscript and provided critical review of the manuscript; all authors read and approved the final manuscript

## REFERENCES

- Anckaert, E, Denk, B., He, Y., Torrance, H.L., Broekmans, F., Hund, M. **Evaluation of the Elecsys® anti-Müllerian hormone assay for the prediction of hyper-response to controlled ovarian stimulation with a gonadotrophin-releasing hormone antagonist protocol.** European Journal of Obstetrics & Gynecology and Reproductive Biology, 2019; 236: 133–138 <https://doi.org/10.1016/j.ejogrb.2019.02.022>
- Anckaert, E, Öktem, M., Thies, A., Cohen-Bacrie, M., Daan, N.M.P., Schiettecatte, J., Milczynski, C. **Multicenter analytical performance evaluation of a fully automated anti-Müllerian hormone assay and reference interval determination.** Clinical Biochemistry 2016; 49: 260–267
- Anckaert, Ellen, Smits, J., Schiettecatte, J., Klein, B.M., Arce, J.C. **The value of anti-Müllerian hormone measurement in the long GnRH agonist protocol: Association with ovarian response and gonadotrophin-dose adjustments.** Human Reproduction 2012; 27: 1829–1839
- Anderson, R.A., Anckaert, E., Bosch, E., Dewailly, D., Dunlop, C.E., Fehr, D., Hund, M. **Prospective study into the value of the automated Elecsys antimüllerian hormone assay for the assessment of the ovarian growing follicle pool.** Fertility and Sterility 2015; 103: 1074–1080.e4
- Baker, V.L., Gracia, C., Glassner, M.J., Schnell, V.L., Doody, K., Coddington, C.C., Broyles, D.L. **Multicenter evaluation of the Access AMH antimüllerian hormone assay for the prediction of antral follicle count and poor ovarian response to controlled ovarian stimulation.** Fertility and Sterility 2018; 110: 506–513.e3
- Boostanfar, R., Shapiro, B., Levy, M., Rosenwaks, Z., Witjes, H., Stegmann, B.J., Yeko, T. **Large, comparative, randomized double-blind trial confirming noninferiority of pregnancy rates for corifollitropin alfa compared with recombinant follicle-stimulating hormone in a gonadotropin-releasing hormone antagonist controlled ovarian stimulation protocol.** Fertility and Sterility 2015; 104: 94–103.e1
- Broer, S., Broekmans, F., Laven, J., Fauser, B. **Anti-Müllerian hormone: Ovarian reserve testing and its potential clinical implications.** Human Reproduction Update 2014; 20: 688–701
- Broer, S., Dölleman, M., Opmeer, B., Fauser, B., Mol, B., Broekmans, F. **AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: A meta-analysis.** Human Reproduction Update 2011; 17: 46–54
- Coelho Neto, M.A., Ludwin, A., Borrell, A., Benacerraf, B., Dewailly, D., da Silva Costa, F., Martins, W.P. **Counting ovarian antral follicles by ultrasound: a practical guide.** Ultrasound in Obstetrics and Gynecology 2018; 51: 10–20
- Corifollitropin Alfa Dose-finding Study Group **A randomized dose-response trial of a single injection of corifollitropin alfa to sustain multifollicular growth during controlled ovarian stimulation.** Human Reproduction 2008; 23: 2484–2492
- Devroey, P., Boostanfar, R., Koper, N.P., Mannaerts, B.M.J.L., Ijzerman-Boon, P.C., Fauser, B.C.J.M. **A double-blind, non-inferiority RCT comparing corifollitropin alfa and recombinant FSH during the first seven days of ovarian stimulation using a GnRH antagonist protocol.** Human Reproduction 2009; 24: 3063–3072
- Devroey, Paul, Polyzos, N.P., Blockeel, C. **An OHSS-Free Clinic by segmentation of IVF treatment.** Human Reproduction 2011; 26: 2593–2597
- Fauser, B.C.J.M., Mannaerts, M.J.L., Devroey, P., Leader, A., Boime, I., Baird, D.T. **Advances in recombinant DNA technology: Corifollitropin alfa, a hybrid molecule with sustained follicle-stimulating activity and reduced injection frequency.** Human Reproduction Update 2009; 15: 309–321
- Ferraretti, A.P., La Marca, A., Fauser, B.C.J.M., Tarlatzis, B., Nargund, G., Gianaroli, L. **ESHRE consensus on the definition of “poor response” to ovarian stimulation for in vitro fertilization: the Bologna criteria.** Human Reproduction 2011; 26: 1616–1624
- Gassner, D., Jung, R. **First fully automated immunoassay for anti-Müllerian hormone.** Clinical Chemistry and Laboratory Medicine 2014; 52: 1143–1152
- Gracia, C., Shin, S., Prewitt, M., Chamberlin, J., Lofaro, L., Jones, K., Broyles, D. **Multicenter clinical evaluation of the Access AMH assay to determine AMH levels in reproductive age women during normal menstrual cycles.** J Assist Reprod Genet 2018; 35: 777–783
- Griesinger, G., Boostanfar, R., Gordon, K., Gates, D., McCrary Sisk, C., Stegmann, B.J. **Corifollitropin alfa versus recombinant follicle-stimulating hormone: an individual patient data meta-analysis.** Reproductive BioMedicine Online 2016; 33: 56–60
- Iliodromiti, S., Anderson, R.A., Nelson, S.M. **Technical and performance characteristics of anti-Müllerian hormone and antral follicle count as biomarkers of ovarian response.** Human Reproduction Update 2015; 21: 698–710
- Iliodromiti, S., Salje, B., Dewailly, D., Fairburn, C., Fanchin, R., Fleming, R., Nelson, S.M. **Non-equivalence of anti-Müllerian hormone automated assays - Clinical implications for use as a companion diagnostic for individualised gonadotrophin dosing.** Human Reproduction 2017; 32: 1710–1715
- Jacobs, M. H., Reuter, L. M., Baker, V. L., Craig, L. B., Sakkas, D., Surrey, E., ... Timm, B. (2019). **A multicentre evaluation of the Elecsys® anti-Müllerian hormone immunoassay for prediction of antral follicle count.** Reproductive BioMedicine Online, (pii), S1472-6483(19)30006-9. <https://doi.org/10.1016/j.rbmo.2018.12.041>
- Kissell, K.A., Danaher, M.R., Schisterman, E.F., Wactawski-Wende, J., Ahrens, K.A., Schliep, K., Mumford, S.L. **Biological variability in serum anti-Müllerian hormone throughout the menstrual cycle in ovulatory and sporadic anovulatory cycles in eumenorrhic women.** Human Reproduction 2014; 29: 1764–1772
- La Marca, A., Sunkara, S.K. **Individualization of controlled ovarian stimulation in IVF using ovarian reserve markers: From theory to practice.** Human Reproduction Update 2014; 20: 124–140
- Lambert-Messerlian, G., Plante, B., Eklund, E.E., Raker, C., Moore, R.G. **Levels of antimüllerian hormone in serum during the normal menstrual cycle.** Fertility and Sterility 2016; 105: 208–213.e1
- Lensen, SF, Wilkinson, J, Leijdekkers, JA, La Marca, A, Mol, BWJ, Marjoribanks, J, Torrance,

- H, B. F. **Individualised gonadotropin dose selection using markers of ovarian reserve for women undergoing in vitro fertilisation plus intracytoplasmic sperm injection (IVF/ICSI).** *Cochrane Database of Systematic Reviews* 2018; 2
- Lerman, T., Depenbusch, M., Schultze-Mosgau, A., von Otte, S., Scheinhardt, M., Koenig, I., Griesinger, G. **Ovarian response to 150 µg corifollitropin alfa in a GnRH-antagonist multiple-dose protocol: a prospective cohort study.** *Reproductive BioMedicine Online* 2017; 34: 534–540
- Li, H.W.R., Wong, B.P.C., Ip, W.K., Yeung, W.S.B., Ho, P.C., Ng, E.H.Y. **Commercial immunoassays for anti-Müllerian hormone measurement.** *Human Reproduction* 2016; 31: 2796–2802
- Martínez, F., Clua, E., Carreras, O., Tur, R., Rodríguez, I., Barri, P.N. **Is AMH useful to reduce low ovarian response to GnRH antagonist protocol in oocyte donors?** *Gynecological Endocrinology* 2013; 29: 754–757
- Mavrelou, D., Al Chami, A., Talaulikar, V., Burt, E., Webber, L., Ploubidis, G., Yasmin, E. **Variation in antral follicle counts at different times in the menstrual cycle: does it matter?** *Reproductive BioMedicine Online* 2016; 33: 174–179
- Moons, K., Royston, P., Vergouwe, Y., Grobbee, D., Altman, D. **Prognosis and prognostic research: what, why, and how?** *BMJ* 2009; 338: b375
- Nyboe Andersen, A., Nelson, S.M., Fauser, B.C.J.M., García-Velasco, J.A., Klein, B.M., Arce, J.C., Arce, J.C. **Individualized versus conventional ovarian stimulation for in vitro fertilization: a multicenter, randomized, controlled, assessor-blinded, phase 3 noninferiority trial.** *Fertility and Sterility* 2017; 107: 387–396.e4
- Oehninger, S., Nelson, S.M., Verweij, P., Stegmann, B.J. **Predictive factors for ovarian response in a corifollitropin alfa/GnRH antagonist protocol for controlled ovarian stimulation in IVF/ICSI cycles.** *Reproductive Biology and Endocrinology* 2015; 13: 1–7
- Olivennes, F., Howies, C.M., Borini, A., Germond, M., Trew, G., Wikland, M., Alam, V. **Individualizing FSH dose for assisted reproduction using a novel algorithm: The CONSORT study.** *Reproductive BioMedicine Online* 2011; 22: S73–S82
- Oudshoorn, S.C., van Tilborg, T.C., Eijkemans, M.J.C., Oosterhuis, G.J.E., Friederich, J., van Hooff, M.H.A., Torrance, H.L. **Individualized versus standard FSH dosing in women starting IVF/ICSI: an RCT. Part 2: The predicted hyper responder.** *Human Reproduction* 2017; 32: 2506–2514
- Polyzos, N.P., Nelson, S.M., Stoop, D., Nwoye, M., Humaidan, P., Anckaert, E., Tournaye, H. **Does the time interval between antimüllerian hormone serum sampling and initiation of ovarian stimulation affect its predictive ability in in vitro fertilization – intracytoplasmic sperm injection cycles with a gonadotropin-releasing hormone antagonist? A retrospective single-center study.** *Fertility and Sterility* 2013; 100: 438–444
- Polyzos, N.P., Tournaye, H., Guzman, L., Camus, M., Nelson, S.M. **Predictors of ovarian response in women treated with corifollitropin alfa for in vitro fertilization/intracytoplasmic sperm injection.** *Fertility and Sterility* 2013; 100: 430–437
- Popovic-Todorovic, B., Loft, A., Ejdrup Bredkjær, H., Bangsbøll, S., Nielsen, I.K., Nyboe Andersen, A. **A prospective randomized clinical trial comparing an individual dose of recombinant FSH based on predictive factors versus a “standard” dose of 150 IU/day in “standard” patients undergoing IVF/ICSI treatment.** *Human Reproduction* 2003; 18: 2275–2282
- Pouwer, A., Farquhar, C., Kremer, J. **Long-acting FSH versus daily FSH for women undergoing assisted reproduction.** *Database of Systematic Reviews* 2015; 7
- Rombauts, L., Onwude, J.L., Chew, H.W., Vollenhoven, B.J. **The predictive value of antral follicle count remains unchanged across the menstrual cycle.** *Fertility and Sterility* 2011; 96: 1514–1518
- Scheinhardt, M.O., Lerman, T., König, I.R., Griesinger, G. **Performance of prognostic modeling of high and low ovarian response to ovarian stimulation for IVF.** *Human Reproduction* 2018; 33: 1499–1505
- Steward, R.G., Lan, L., Shah, A.A., Yeh, J.S., Price, T.M., Goldfarb, J.M., Muasher, S.J. **Oocyte number as a predictor for ovarian hyperstimulation syndrome and live birth: An analysis of 256,381 in vitro fertilization cycles.** *Fertility and Sterility* 2014; 101: 967–973
- Teede, H., Misso, M., Costello, M., Dokras, A., Laven, J., Moran, L., Network, I.P. **Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome.** *Human Reproduction* 2018; 33: 1602–1618
- Van Disseldorp, J., Lambalk, C.B., Kwee, J., Looman, C.W.N., Eijkemans, M.J.C., Fauser, B.C., Broekmans, F.J. **Comparison of inter- and intra-cycle variability of anti-Müllerian hormone and antral follicle counts.** *Human Reproduction* 2010; 25: 221–227
- van Tilborg, T.C., Torrance, H.L., Oudshoorn, S.C., Eijkemans, M.J.C., Koks, C.A.M., Verhoeve, H.R., Torrance, H.L. **Individualized versus standard FSH dosing in women starting IVF/ICSI: an RCT. Part 1: The predicted poor responder.** *Human Reproduction* 2017; 32: 2496–2505
- Yovich, J.L., Alsbjerg, B., Conceicao, J.L., Hinchliffe, P.M., Keane, K.N. **PIVET rFSH dosing algorithms for individualized controlled ovarian stimulation enables optimized pregnancy productivity rates and avoidance of ovarian hyperstimulation syndrome.** *Drug Design, Development and Therapy* 2016; 10: 2561–2573
- Yovich, J.L., Keane, K.N., Borude, G., Dhaliwal, S.S., Hinchliffe, P.M. **Finding a place for corifollitropin within the PIVET FSH dosing algorithms.** *Reproductive BioMedicine Online* 2018; 36: 47–58
- Zuvela, E., Walls, M., Matson, P. **Within-laboratory and between-laboratory variability in the measurement of anti-müllerian hormone determined within an external quality assurance scheme.** *Reproductive Biology* 2013; 13: 255–257

Received 26 October 2019; received in revised form 21 March 2020; accepted 30 March 2020.