International Journal of Gynecology and Obstetrics 133 (2016) 64–68

Contents lists available at ScienceDirect

International Journal of Gynecology and Obstetrics

journal homepage: www.elsevier.com/locate/ijgo

CLINICAL ARTICLE

Anti-Müllerian hormone concentrations and antral follicle counts for the prediction of pregnancy outcomes after intrauterine insemination

Francesca Moro [a,*], Anna Tropea [a], Elisa Scarinci [a], Emanuele Leoncini [b], Stefania Boccia [b], Alex Federico [a], Ornella Alesiani [a], Antonio Lanzone [a,1], Rosanna Apa [a,1]

[a] Institute of Obstetrics and Gynecology, Università Cattolica del Sacro Cuore, Rome, Italy
[b] Institute of Public Health, Section of Hygiene, Università Cattolica del Sacro Cuore, Rome, Italy

ARTICLE INFO

Article history:
Received 14 April 2015
Received in revised form 3 August 2015
Accepted 8 December 2015

Keywords:
Anti-Müllerian hormone
Antral follicle count
Intrauterine insemination
Ovulation induction
Prediction

ABSTRACT

Objective: To evaluate anti-Müllerian hormone (AMH) concentrations and antral follicle counts (AFCs) in the prediction of pregnancy outcomes after controlled ovarian stimulation among women undergoing intrauterine insemination. *Methods:* A retrospective study included women with unexplained infertility aged 41 years or younger who attended a fertility clinic in Italy between December 2009 and May 2014. Ovarian stimulation was achieved with recombinant follicle-stimulating hormone or highly purified human menopausal gonadotropin. Receiver operating characteristic curves were generated to predict ongoing pregnancy. The primary outcome was the association between AMH/AFC and ongoing pregnancy, and was assessed by logistic regression. *Results:* Overall, 276 women were included, of whom 43 (15.6%) achieved ongoing pregnancy. Multivariate analysis showed that women with a serum day-3 concentration of AMH higher than 2.3 ng/mL were more likely to have ongoing pregnancy than were those with a concentration lower than 2.3 ng/mL (odds ratio 5.84, 95% confidence interval 2.38–14.31; $P < 0.001$). No associations were recorded for AFCs. *Conclusion:* AMH should be used to predict the pregnancy outcome of intrauterine insemination.

1. Introduction

Intrauterine insemination (IUI) with controlled ovarian stimulation (COS) is frequently used to treat couples with unexplained infertility [1,2]. Although COS combined with IUI (COS–IUI) has been found to result in a significantly higher pregnancy rate per couple as compared with IUI during the natural cycle [3], COS has also been associated with an increased risk of ovarian hyperstimulation syndrome [4,5]. Cancellation of a cycle owing to hyper-response has been reported to occur in 15% of COS–IUI treatments [6]. By contrast, some relatively young women with a reduced ovarian reserve, and some women whose fertility is naturally impaired by age might have a low response to COS [7]. An extreme response or, conversely, a poor response to COS can occur unexpectedly; therefore, there is a real need to find predictive factors that can be used in daily clinical practice to predict the stimulation and pregnancy outcomes for an individual.

Among the available biomarkers of ovarian follicular status, much interest has been paid to anti-Müllerian hormone (AMH) and antral follicle count (AFC) as accurate reproducible predictors of in vitro fertilization (IVF) outcomes [8]. AMH inhibits the initial recruitment of primordial follicles and has a role in the intrafollicular and interfollicular coordination of follicle development [9]. Different studies have demonstrated that AMH is a strong predictor of ovarian response in IVF cycles [8,10]. AFC (i.e. number of antral follicles present in the ovaries) is strongly related to serum AMH concentrations. The most practical method for assessment of AFC in clinical practice is to count all follicles with a diameter of 2–9 mm [11,12]; however, follicles with a diameter of 6 mm or less express the greatest levels of AMH [13]. Therefore, the latter subset of antral follicles might be better predictors of ovarian reserve and ovarian response [14].

Few studies have evaluated the prognostic value of AMH and AFC in IUI cycles. Moreover, there is no information regarding the performance of the AFC of smaller follicles in predicting the ovarian response and pregnancy outcome of IUI cycles. The aim of the present study was therefore to evaluate the role of AMH and different sizes of antral follicles (2–4 mm, 2–6 mm, and 2–9 mm) in predicting both ovarian response to COS and pregnancy outcome among women undergoing their first IUI cycle.

2. Materials and methods

The present retrospective study included women with unexplained infertility attending the Day Hospital of Physiopathology of Human Reproduction, Policlinico Gemelli, Rome, Italy, for COS–IUI between December 1, 2009, and May 31, 2014. The eligibility criteria were:

* Corresponding author at: Università Cattolica del Sacro Cuore, Largo A. Gemelli 8, 00168 Rome, Italy. Tel.: +39 06 30156770; fax: +39 06 30155897.
E-mail address: morofrancy@gmail.com (F. Moro).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.ijgo.2015.08.021
0020-7292/

good health; age 41 years or younger; duration of infertility of more than 1 year; regular ovulatory menstrual cycles; bilateral tubal patency, as defined by hysterosalpingo-contrast sonography or dye hydrotubation; and normal day-3 hormonal pattern, including follicle-stimulating hormone (FSH; 2.5–11 mIU/mL) and 17β-estradiol (E2; 30–100 pg/mL). The exclusion criteria were: polycystic ovarian syndrome, or any systemic disease or endocrine or metabolic abnormalities; pelvic inflammatory disease; endometriosis; neoplasms; and breast pathology incompatible with gonadotropin stimulation. Women were also excluded if infertility was due to a male factor. Women were included for one cycle and only if it was their first cycle of treatment. The study was a retrospective analysis and neither ethical approval nor written informed consent was required.

The treatment protocol has been previously described [15]. Ovarian stimulation was achieved with recombinant FSH (follitropin alpha; Gonal-F, Merck Serono, Geneva, Switzerland), starting at 75 IU/day for patients aged 35 years or younger, or with highly purified menotropin (HP-hMG ; Meropur, Ferring Pharmaceuticals, Copenhagen, Denmark), starting at 150 IU/day for patients older than 35 years. The gonadotrophin preparations were subcutaneously administered. The drug dose was adjusted individually depending on the follicular response of the patient.

Ovarian stimulation was started on the third day of the menstrual cycle after hormonal assays for AMH, FSH, and E2, and a basal ultrasonography examination. FSH and E2 were measured by electrochemiluminescence immunoassays and AMH (normal range 0.42–6.50 ng/mL) by an enzyme-linked immunosorbent assay. For the AFC, the number of follicles measuring 2–4 mm, 2–6 mm, and 2–9 mm was evaluated by transvaginal ultrasonography (Voluson *i*, GE Medical Systems, Milwaukee, WI, USA). The count in both ovaries was used for calculations.

Follicular development was monitored by ultrasonography and serum E2 levels. On the day that the leading follicle reached a diameter of more than 17 mm, one intramuscular injection of 10 000 IU of human chorionic gonadotropin (hCG; Gonasi, Ibsa Farmaceutici Italia Srl, Lodi, Italy) was administered. Three responses were defined [16]: hyporesponse occurred when there was only one follicle bigger than 17 mm, and no other follicles with a diameter of 14 mm or greater; normal response when there were two or three follicles with a diameter of 14 mm or greater, a maximum of two follicles bigger than 17 mm, and no need for dose reduction during stimulation; and hyper-response when there were more than three follicles with a diameter of 14 mm or greater and a maximum of two follicles bigger than 17 mm on the day of hCG administration, or dose reduction during stimulation.

Approximately 34–36 hours after hCG injection, a single IUI was carried out. Ovulation was confirmed by progesterone assay and ultrasonography. Sperm was prepared as previously described [15]. At 6 weeks after IUI, transvaginal ultrasonography was used to confirm clinical pregnancy. Ongoing pregnancy was defined as a pregnancy that continued after 12 weeks.

The primary outcome was the association between AMH or numbers of antral follicles of different sizes (2–4 mm, 2–6 mm, and 2–9 mm) and ongoing pregnancy. The secondary outcome was the ability of AMH or antral follicles to predict both hyporesponse and hyper-response in two different cohorts of patients: women aged 35 years or younger who were stimulated with recombinant FSH, and women older than 35 years who were stimulated with HP-hMG.

Statistical analyses were done with Stata version 13 (StataCorp, College Station, TX, USA). Data were summarized as median (interquartile range). The Box–Tidwell test was used to test for nonlinearity of continuous variables. Data were compared by the Mann–Whitney *U* test and the χ^2 test for proportions. The Cuzick test was used to examine trend across ordered groups, and the Kruskal–Wallis test was used to compare three groups. All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant.

Receiver operating characteristic (ROC) curves were generated for serum AMH concentration, AFC, and serum FSH concentration to compare the ability of parameters to predict poor or excessive ovarian response, and ongoing pregnancy. A test for equality of the ROC curves was used. AMH concentration was dichotomized as guided by the ROC curve because the linearity assumption was violated. The sensitivity, specificity, positive and negative predictive values, and exact binomial 95% confidence interval (CI) for selected cutoff levels of the parameters were also calculated. The relationship between ongoing pregnancy and serum AMH concentration, serum FSH concentration, or AFC was evaluated by using logistic regression analysis to determine the adjusted odds ratio (OR) and 95% CI.

3. Results

During the study period, 276 women undergoing their first COS cycle and IUI treatment were enrolled in the study and therefore 276 cycles were analyzed. Clinical pregnancy was achieved in 50 (18.1%) and ongoing pregnancy in 43 (15.6%).

The demographic and baseline characteristics of the 276 study women are shown in Table 1, grouped by attainment of ongoing pregnancy. The starting dose, duration of stimulation, and number of follicles bigger than 17 mm that developed during COS are also shown by pregnancy group. Women who achieved an ongoing pregnancy had significantly higher AMH and lower FSH serum levels as compared with those who did not ($P < 0.001$ for both). The pregnancy group had also a higher count of follicles measuring 2–9 mm ($P < 0.001$), 2–4 mm ($P = 0.013$), and 2–6 mm ($P < 0.001$).

Although AMH showed a trend toward better prediction of ongoing pregnancy than FSH level or AFC in the ROC curves (Fig. 1), the differences among these parameters were not significant.

Multivariate analysis indicated that the only independent predictor of ongoing pregnancy was AMH (Table 2). Specifically, women who had AMH levels higher than 2.3 ng/mL were more likely to have an ongoing pregnancy than were those with AMH concentrations lower than or equal to 2.3 ng/mL ($P < 0.001$). On the basis of the ROC curve for serum AMH concentration, a cutoff of 2.3 ng/mL correctly identified 218 (79.0%) of the 276 observations. At this threshold, the assay sensitivity was 63% (95% CI 47%–77%), the specificity was 82% (95% CI 76%–87%), the positive predictive value was 39% (95% CI 28%–52%), and the negative predictive value was 92% (95% CI 88%–95%).

Among the 131 patients aged 35 years or younger, 33 (25.2%) were classified as hyporesponders (for one woman, the cycle was interrupted owing to no response), 79 (60.3%) as normal, and 19 (14.5%) as hyper-

Table 1
Demographic and clinical characteristics by pregnancy outcome.[a]

Characteristic	Ongoing pregnancy		P value[b]
	Yes (n = 43)	No (n = 233)	
Age, y	33 (31–39)	35 (33–37)	0.113
Body mass index[c]	22 (20–23)	22 (21–23)	0.089
Duration of infertility, y	2 (2–3)	2 (2–3)	0.335
Anti-Müllerian hormone on day 3, ng/mL	2.5 (1.2–3.4)	1.2 (0.7–2.2)	<0.001
Follicle-stimulating hormone on day 3, IU/L	5.7 (4.7–6.8)	6.7 (5.7–7.9)	<0.001
Antral follicle count			
2–9 mm	15 (14–16)	13 (10–15)	<0.001
2–4 mm	7 (5–8)	5 (3–8)	0.013
2–6 mm	12 (11–13)	10 (6–13)	<0.001
Estradiol, pg/mL	41 (32–55)	41 (28–53)	0.195
Starting dose, IU			0.061[d]
75	27 (62.3)	104 (44.6)	
150	16 (37.2)	129 (55.3)	
Duration of stimulation, d	7 (6–8)	7 (6–8)	0.998
No. of follicles >17 mm	1 (1–2)	1 (1–2)	0.531

[a] Values are given as median (interquartile range) or number percentage, unless indicated otherwise.
[b] By Mann–Whitney *U* test unless indicated otherwise.
[c] Calculated as weight in kilograms divided by the square of height in meters.
[d] By χ^2 test.