Predicting ovarian reserve and reproductive outcome using antimüllerian hormone (AMH) and antral follicle count (AFC) in patients with previous assisted reproduction technique (ART) failure

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Summary

Purpose of investigation: The main objective of our prospective, observational, analytical research work was to determine whether Anti-Müllerian hormone (AMH) and antral follicle count (AFC) could be effectively used as predictors of ovarian reserve and, possibly, of reproductive outcome with ART. *Methods:* We studied 143 IVF/ET cycles in patients with a previous history of ART failure, all of them supposed to be of poor prognosis, who had agreed to another ART attempt after knowing their AMH, AFC, and base hormone (FSH, LH, 17 β-estradiol) levels. *Results:* AMH and AFC showed a positive correlation with the number of oocytes retrieved ($p = 0.0016$) and ($p < 0.0001$), respectively and with percentage of MII oocytes, ($p = 0.00756$) and ($p < 0.001$). The combined use of these markers showed an area under the curve of 82.2% for oocytes retrieved. Our results shows a very high cancelation (22% of started cycles) and very low pregnancy rates (6.7% and 9.8%) in low and normoresponders, respectively. *Conclusions:* AMH levels and AFC are reliable indicators of ovarian reserve. Patients with ovarian reserve levels that predict a very low probability of success should be informed that the poor prognosis associated with these values may not justify the expense of IVF/ET.

Key words: Anti-Müllerian hormone (AMH); Antral follicle count (AFC); 3D ultrasound; AVC; VOCAL; Inverse mode; Low responders.

Introduction

Advanced age is currently the main cause of female sterility in Spain. This is a troubling problem since Spain is the country with the lowest birthrate in the world [1]. We now face older patients desirous of procreation who have a low probability of success due to low ovarian reserve along with poor quality of remaining oocytes. Starting at 37.5 years of age, it is estimated that 70 to 80% of oocytes are bearers of chromosomal or genetic defects [2-4], since the best oocytes are recruited in the early reproductive years [5].

Menopause appears when there are about one thousand follicles left. However, it is estimated that the decline in fecundity precedes menopause by about 13.5 years [5]. Data from the onset of reproductive biology indicate that about 10% of the female population experience an accelerated reduction in the oocyte pool before age 32 [6]. By age 37 this accelerated reduction increases to 25%. The probability of spontaneous gestation after 40 years of age is less than 4% [6].

Ovarian reserve is more of a biological than a chronological function and because of this, the onset of accelerated decline can occur at an early age [6]. Numerous hormones (FSH, LH, estradiol, inhibin B) and dynamic tests (clomiphene challenge test, gonadotrophin challenge test, GnRh agonist stimulation test, etc.), upon which there were high expectations that they would serve as markers of ovarian reserve, have turned out to be tests with little or no predictive value [7].

At present anti-Müllerian hormone (AMH) is considered to be an excellent marker of ovarian reserve, response to gonadotropin stimulation, in vitro fertilization (IVF) reproductive success, and even of approaching menopause [8-21]. Determinations of AMH levels along with antral follicle count (AFC) with last-generation ultrasound (US) modes seem to open new avenues to avoid, or at the very least to reduce, the number of patients with no possibility or very low probability of reproductive success who are subjected to artificial reproduction therapy (ART) [22].

The cost of the medications used in ART, the associated psychological discomfort, the risk of complications, and the avoidance of predictable failures justify the need to obtain prognostic information prior to the initiation of therapy [23]. At present, when safety and cost/benefit are very important considerations in ART, excessive and deficient responses to ovarian stimulation should be avoided.

Material and Method

The study consisted of 143 IVF/ICSI patients, all of whom had a history of ART failure (i.e., ovulation induction, IVF/ICSI), normal menses, both ovaries present, no previous history of major diseases, endocrinopathies, chemotherapy and/or radiotherapy; and whom in spite of past failures, and with knowledge about their AMH levels, agreed to undergo

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another cycle of IVF/ICSI. Couples with severe masculine factor (< 5 million sperm/ml) were eliminated.

Once the stimulation cycle results were obtained, the following subgroup analyses were carried out, taking into account the number of oocytes recovered:

Group 1. Cycles cancelled or ≤ 5 oocytes recovered

A) Cancelled cycles: n = 34 (23.7%)

B) Low response not cancelled: n = 44 (30.7%)

Group 2. Normal responders > 5 and < 15 oocytes recovered: n = 65 (45.45%).

We determined basal hormonal levels (FSH, LH, AMH, and 17 β-E_2), on day 3 of the previous cycle (enzyme-linked immuno-sorbent assay (ELISA) - Human Gessellschaft für Biochemica und Diagnostica MbH. Wiesbaden, Germany) with an analytical sensibility of 0.4 mIU/ml for FSH, 0.5 mIU/ml for LH, 3 pg/ml for 17 β-E_2). For AMH we used the AMH/MIS immunoassay (Laboratory Instrument & Beckman-Coulter, Vienna, Austria). Estimated analytic sensibility (ELISA) of 0.1 ng/ml (0.7 pmol/l).

AFC was carried out with transvaginal 2D/3D US (Voluson E8, GE, equipped with a RAB 4-8D transvaginal probe) at the onset of the stimulation cycle [24].

Ovarian induction was carried out using 225-300 IU of rFSH (Puregon, MSD, Madrid, Spain) during six days, and continued depending on follicle size and 17β-E_2 levels. On day 5 (or follicles of 15 mm), a GnRH antagonist 0.25 mg (Orgalutran, MSD Madrid, Spain) was given daily until the administration of rHCG (Ovitrelle, Serono, Madrid).

The following data were taken into account.

– Age

– History of ART

– Total oocytes recovered, percentage of mature oocytes obtained and fertilized

– Total number of GI, GII, and other quality embryos obtained. Number and quality of embryos transferred. Number of gestations achieved and clinical evolution (number of abortions, number of ectopic pregnancies, and number of gestations in evolution).

Seventy-eight (67.5%) were low responders, and the remaining 65 (32.5%) were normal responders. All data were included in a Filemaker program.

US modes used for AFC

AFC was carried out using 2D and 3D vaginal US in surface, inverse, VOCAL, and AVC modes [22, 25, 26] (Figure 1).

Statistical studies

The InfoStat (2008) statistical package (InfoStat Group, FCA, National University of Cordoba, Argentina) was employed using ANOVA (analysis of variance) as the parametric test. To verify its significance we used the Kruskal-Wallis non-parametric test to a < 0.05 level of significance.

To accept the normalcy of data we used the Kolmogorov-Smirnov test (also known as K-S test), a non-parametric test used to determine the adjustment compatibility between the standardized residues of two distributions of probability.

Results

AFC vaginal 2D US versus 3D US using AVC. From the onset of the study, clear counting differences were appreciated since observations with 2D US are only in two planes. The images observed with 3D (AVC mode) (Figure 1) were superior, and quantification of AF was faster. Use of these modes saved time, and, as others have reported [21, 22, 24], they eliminate intra- and inter-observer differences.

Table 1. — *Median and p values for age and determined hormones*.

	Low		Normal		*p* value
	n	mean ± sd	n	mean ± sd	
Age (years)	74	36.6 ± 3.5	64	33.9 ± 4.6	0.0002
FSH	73	9.1 ± 8.9	65	7.4 ± 3.5	ns
AMH	75	9.9 ± 9.5	64	15.7 ± 12.0	0.0016
LH	59	6.6 ± 7.6	40	6.8 ± 4.1	ns
E2	42	61.3 ± 56.5	39	51.2 ± 32.5	ns
AFC	71	5.7 ± 4.4	64	11.2 ± 7.3	< 0.0001

AVC modes quantified and measured diameters and volumes automatically (Figure 1). Due to the advantages of 3D AFC, no statistical comparison analysis with 2D were performed. Our results are based on 3D US AFC.

General results of ovarian aging markers: Table 1 shows the median and p values for age and determined hormones.

Age versus ovarian response showed statistically significant differences (Table 1).

AMH in low and normal responders indicates a statistically significant correlation (Table 1) of clinical interest. An AMH value of 9.28 pmol/l predicted a low response with a sensitivity and specificity of 69% and 65% respectively (Figure 2).

Recovered oocytes versus basal FSH, LH, and 17 ß-estradiol were not statistically significant (Table 1).

Differences in AFC between normal and low responders: The difference was statistically significant (Table 1 and Figure 2).

AMH versus AFC: Although the lineal regression did not indicate a correlation ($p = 0.0614$), we observed a trend between both variables, suggesting that both parameters are indicators of ovarian reserve.

AMH versus mature oocytes: Analysis of variance between mean levels of AMH and the proportion of mature oocytes in low vs normo-responders showed a statistically significant positive correlation ($p = 0.0756$).

AFC versus mature oocytes showed a statistically significant positive correlation ($p < 0.0001$).

AMH, AFC and number of fertilized oocytes: There was a significant relationship ($p = 0.00756$) and ($p < 0.001$), respectively.

AMH, AFC and G1 and G2 embryonic quality: The *p* value for AMH was a straight line of 0.0852; the model was incapable of detecting a relationship. Nevertheless *p* for AFC was significant ($p < 0.001$).

AMH and reproductive outcome: No significant differences were detected ($p = 0.1242$) between women who did or did not conceive. Our results showed that the combination of AMH and AFC was the best parameter with an area under the curve of 82.2% (Figure 3).

Reproductive outcome: Our definitive reproductive outcome showed a high cancelation rate (22%) of initiated cycles, and very low pregnancy rates for low (6.7%) and

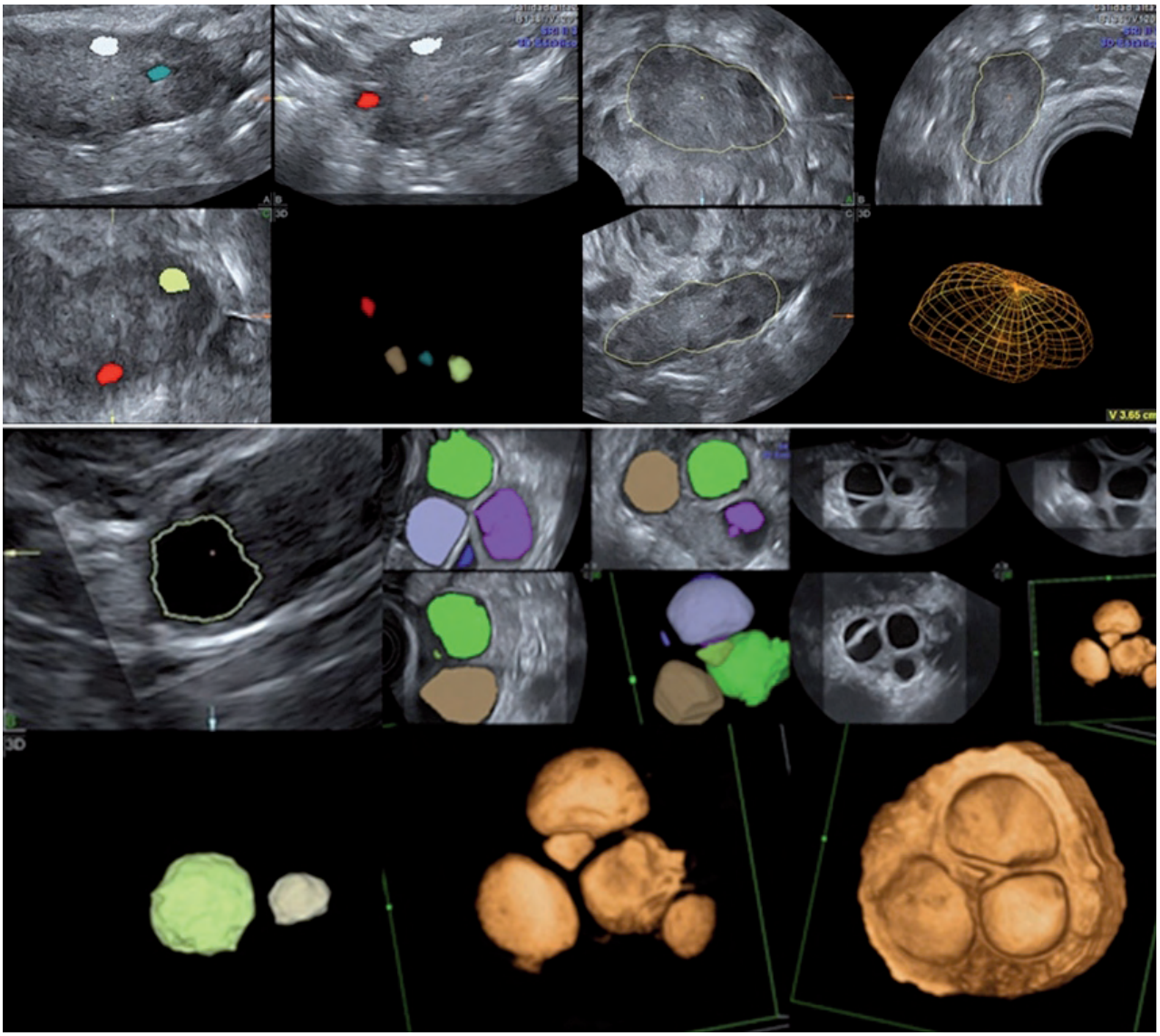

Figure 1. — Low responder AVC (above), inverse mode (below left) and 3D surface mode with VOCAL of the follicles (below right).

normo-responders (9.8%). There were 24 (16.7%) gestations out of 143 initiated cycles, and only nine (6.2%) were ongoing pregnancies.

Discussion

Several parameters for evaluation of ovarian reserve have been proposed during the past decade. Age is included as a basic criterion in all protocols, but several authors [8-22] have reported that AMH levels seem to be more predictive of ovarian reserve than age alone.

A recent report [26] concludes that many of the hormonal and laboratory tests (i.e., FSH, LH, inhibin B, basal estradiol, ovarian reserve (EFORT), and clomiphene tests), are of limited value, and indicates that the most predictive tests and the ones with best clinical application are AFC, AMH, and stimulation tests with agonists (GAST) [8, 25]. Our results are in agreement with these conclusions.

The main goal is to identify younger women with an already reduced ovarian reserve, as well as older women who still have adequate ovarian reserve. If this goal can be achieved, we can then optimize treatment by identifying patients who have already experienced ovarian ageing and offer them appropriate ART, such as oocyte donation.

The basal determination of FSH and 17 β-estradiol have generated interest due to the clinical repercussions. Our results showed no significant differences between low and normal responders, and poor prediction power.

Our results also showed that AMH was predictive of oocyte maturity which is of clinical interest. The more AMH values the more mature the oocytes recovered. Knowledge about the remaining oocyte pool is impossible, but AFC is closely related to it. Numerous US param-

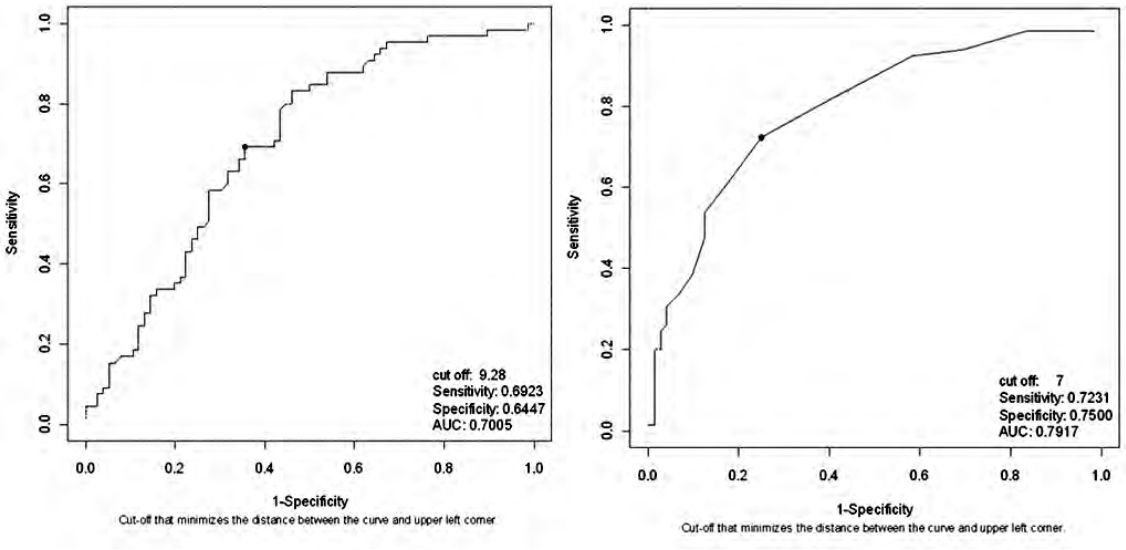

Figure 2. — ROC curve for AMH (left), and ROC Curve for AFC (right).

eters have been used, which have different sensitivities [7, 27-30].

Less than 3 cm^3 of *ovarian volume* has been associated with low response and a high cancellation index [31-33]. Ovarian volume has proven to be a good predictor of low response when excessively small, but there are better US parameters [7, 20].

AFC with vaginal 2D US provides excellent results, however vaginal 3D US with inverse and AVC provide even better results [7, 22, 27, 29, 34-40] allowing the observation of follicles from 2 to 3 mm [29, 34-40]. We observed a relationship between AFC and oocytes recovered, which provides useful prognostic information for low responders [5, 29, 34, 36, 37]. A number of AFC from 1-6 is a poor prognostic index [5, 29, 33, 41-43].

Results of AFC with 3D US modes have proven to be highly reproducible [7, 22, 24, 29, 44, 45]. Observation of the number of AF with 3D orthogonal planes is more precise than with 2D US. Comparing low with normal responders, we observed that in low responders, AFC was diminished and the existing follicles were somewhat larger (between 5 and 7 mm instead of between 2 and 3 mm), which is a manifestation of reduced recruitment and of precocious growth due to the effect of circulating levels of FSH in the higher ranges of normality [27].

Using *AVC and inverse modes* the number and volume of any structure can be calculated with great precision as previously mentioned [7, 22, 44]. *Tomographic US image and inverse mode* allow storage in cine loop and observation of follicles in all spatial angles [46-48]. In our opinion, these modes along with AVC are the most promising [7, 22, 24, 25, 43, 47-49].

A comparison between vaginal 2D and these 3D modes for AFC revealed that 3D modes were superior, saved time, and reduced inter- and intra-observer differences [22, 25, 49].

Angio-power Doppler has been used for vascular evaluation of follicular development, ovulatory follicle, corpus luteum, hyperstimulation, PCOS and to identify anovulatory patients, however, it cannot predict low reserve [7, 26].

Anti-Müllerian hormone (AMH). The relationship between AMH and AFC was the best ageing parameter. Lower levels of AMH are associated with lower number of AFC observed with all 3D US modes. This association indicates that both parameters reflect existing ovarian reserve. Differences in AMH levels and in AFC between low responders and normal responders were statistically significant.

AMH and AFC were better predictors of ovarian reserve than age. The observed stability of AMH throughout the cycle, regardless of age, allows its use as a marker of ovarian reserve with much more confidence than other hormones [12, 13].

The associations of AMH and AFC with FSH, LH, and 17 β-estradiol lack statistical significance and should be eliminated from ovarian reserve protocols. Regarding FSH, if it is true that with values above 10 IU there were only two false-positives, it is also true that the number of women with normal values who did not conceive is high. Levels of 17 β-estradiol, which have received so much attention in the medical literature, have no relationship with AMH levels or with AFC.

A comparison of mean values of AMH and AFC in groups with AFC of ≤ 5 vs > 5 and oocyte maturity reveals that both parameters are predictive of ovarian reserve.

Results of adjusted lineal regression comparing AMH levels and AFC with the quality of G1 and G2 embryos showed an almost horizontal line. The model was incapable of detecting any association.

The relationship between AMH, AFC, and reproductive outcome showed no predictive value.

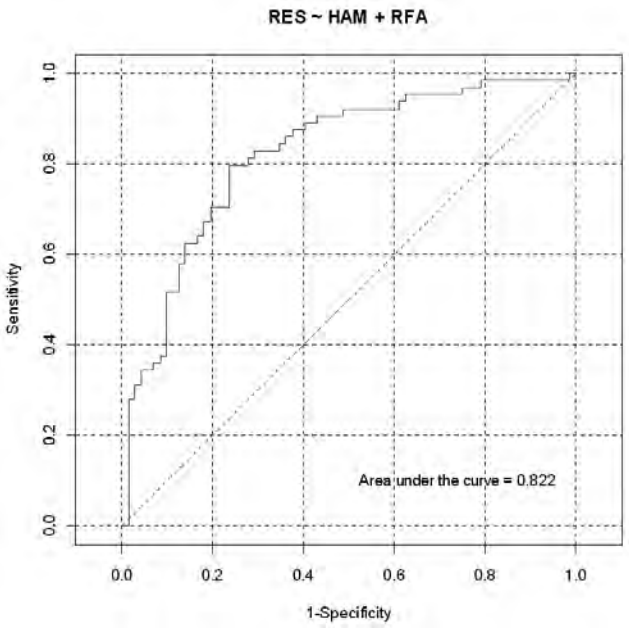

Figure 3. — Area under the curve for AMH and AFC.

Conclusion

We propose AMH levels and AFC as the only reliable parameters to determine ovarian reserve. Very low levels of AMH (1.15 ng/ml = 8.9 pmol/l) are predictive of minimal possibilities of success, and patients should be so informed and recommended alternative techniques such as oocyte donation.

The fact that we have had a few successful pregnancies in women with very low AMH and AFC values, does not allow us, as we had wished, to deny IVF services to women with low values if, as will most certainly happen, they request this service having been informed of, and knowing about, the very low probability of success. Cost/benefit considerations hardly justify the economic expense when there are such dismal probabilities of success. Results of AMH level determinations and their association with the probability of success with ART would offer excellent and reliable support when faced with a decision not to recommend IVF/ET, or to recommend consideration of other alternatives with a better prognosis, such as oocyte donation or adoption.

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