



Anti-Müllerian Hormone in the Prediction of Assisted Reproduction Results at the Poissy Hospital (France)

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Abstract

Background: Assessment of ovarian reserve is an essential parameter in the evaluation of female infertility. It has been established that serum anti-müllerian hormone (AMH) is the best marker of ovarian reserve and predicts the quality of ovarian response to stimulation. However, there is some controversy about the prediction of pregnancy rates. Can the serum AMH assay predict the chances of successful *in vitro* fertilization (IVF)? **Objective:** To analyze the association between AMH levels, the number of mature oocytes obtained by oocyte puncture after stimulation and the outcome of *in vitro* fertilization at the first attempt. **Methods:** An analytical cross-sectional study with retrospective data collection on 123 patients who had consulted for a desire to conceive and undergone ovarian reserve evaluation followed by IVF in the assisted reproduction department of the Poissy-Saint-Germain-en-Laye Intercommunal Hospital Centre at its Poissy hospital site between January 2020 and March 2022. **Results:** Of the 123 patients, 43.8% had normal AMH levels. At the end of stimulation, almost half of patients (52.84%) had a sub-optimal ovarian response and almost a quarter had a poor response (27.64%). We found a statistically significant positive correlation [$r = 0.42$ $b = 0.70$ CI 95% (0.43 - 0.97) $p = 0.000$] between AMH level and the number of mature oocytes collected. Of all patients with low AMH, 83.3% had a clinical pregnancy, while 61.1% of patients with normal AMH had no pregnancy. These differences were statistically significant ($p = 0.001$). AMH level was

significantly associated with predicting ovarian response to stimulation and had a decreasing association with IVF outcome. Indeed, compared with patients with low AMH levels, those with normal AMH levels were 7 times more likely to have a good response to stimulation [OR = 7.12 CI 95% (1.98 - 30.79) p = 0.003] and less likely to achieve pregnancy after embryo transfer. **Conclusion:** AMH assay is an important marker of ovarian reserve and predicts ovarian response to stimulation. It correlates with the number of mature oocytes that can be collected at puncture but does not predict the chances of IVF pregnancy.

Subject Areas

Gynecology & Obstetrics

Keywords

In Vitro Fertilization, Anti-Mullerian Hormone, Mature Oocytes, Ovarian Stimulation

1. Introduction

Anti-müllerian hormone (AMH) is a glycoprotein of gonadal origin belonging to the Transforming Growth Factor β family (TGF- β), secreted in women by granulosa cells [1] [2], and is an important prognostic marker in the management of infertile couples. Infertility is a major public health problem: almost 80 million people are affected [3] [4].

Over the last few decades, the proportion of couples consulting for infertility has increased from 3.5 to 16.7% in developed countries, whereas in developing countries from 6.9 to 9.3% [3] [4].

In France, the infertility prevalence varies from 15 to 25%, *i.e.*, one couple in five consulting a doctor because of parental project [5].

The couple's infertility assessment is an essential step for identifying the aetiology, guiding the treatment and estimating the prognosis. When the treatment for natural conception fails or the natural conception is not possible, assisted reproductive technologies (ART) are an appropriate option.

Determining the ovarian reserve (OR), defined as the follicular stock at a given time, is an essential step in the assessment of female infertility, predicting the ovarian response to stimulation. It involves the hormonal assessment: serum follicle stimulating hormone (FSH), estradiol and AMH assay and the antral follicles count (AFC) between the day 2 and day 4 of the cycle [1] [2].

Numerous studies, comparing all these markers, concluded that serum AMH was the best due to its correlation with the number of antral follicles [6] [7], the stability during the cycle, low variability and reproducibility from one cycle to another [7] [8] [9] [10].

Concerning the prediction of pregnancy rate, the results were more contro-

versial; Klinkert *et al.* [11] in 2005 demonstrated that there is a significant correlation between OR and clinical pregnancy rate in assisted reproduction. Hagen CP *et al.* in 2012 demonstrated that low AMH was not predictive of reduced natural fertility [12]. Some studies have found a correlation between AMH and oocyte quality [13] [14] [15] or with embryo quality [16], while others have found no correlation with either oocyte and embryo quality or in the prediction of pregnancy rate [17] [18] [19].

According to the above mentioned statement, the question of the role of AMH in predicting the chances of success of intraconjugal *in vitro* fertilization (IVF) was raised. Would it be reasonable to propose systematic recourse to oocyte donation based on serum AMH levels?

In the current study, we aimed to analyze the correlation between AMH and the chances of pregnancy in couples undergoing intraconjugal IVF treatment at an intercommunal hospital in Ile de France.

2. Materials and Methods

2.1. Location and Type of Study

This was across-sectional study with retrospective data collection carried out from January 2020 to March 2022 in the reproductive medicine department of the Poissy-Saint-Germain-en-Laye Intercommunal Hospital Centre.

2.2. Selection Criteria

All patients who sought care for infertility with assessment of ovarian reserve and underwent conventional IVF or intracytoplasmic sperm injection (ICSI).

2.3 Exclusion Criteria

Patients with endometrial abnormalities, those who had undergone oocyte donation and those whose partner had severe spermogram abnormalities after preparation of the sperm were excluded.

2.4. Stimulation Protocol

Our selection criteria did not consider any particular stimulation protocol, given that no protocol has been shown to be more effective than another, and that the choice of stimulation regimen is best adapted to the patient's characteristics, such as age, ovarian reserve, and results of the previous treatments (Bologna and Poseidon). In addition, for reasons of professional secrecy, the centre's authorisation to carry out this study requires us to keep the stimulation protocols confidential.

2.5. Statistical Analysis

Data were collected from computerised medical records using Médifirst© software, recorded in a Microsoft Access 2013 database and analysed using STATA SE 17.0 software.

Comparison of means was done by using student's t-test and those of propor-

tions by using Pearson's chi-square. Odds ratios were used to assess the strength of association between variables. The test was considered significant for p-value < 0.05.

3. Results

3.1. Sociodemographic, Clinical, and Therapeutic Profile

The study concerned 123 patients, the majority of whom were nulliparous (60.1%). Most couples had never conceived together (69.9 %). The age of the patients ranged from 27 to 44 years, with an average of 35 ± 4.4 years, and 44% of the patients were overweight or obese. Most patients (80%) and their partners (60.98%) had never used tobacco, alcohol or any other form of drug.

Primary infertility was 59.35% male, 52.84% mixed and 42.28% female. Unexplained infertility was the first indication for IVF in 27.64%, followed by tubal infertility (26.8%) and endometriosis (22.76%). Isolated sperm abnormalities accounted for 8.13% of indications. The spermogram was normal in 87.8% of partners and 6.5% had oligozoospermia. Most transfers (56.1%) did not achieve a pregnancy.

Table 1 shows that the mean AMH level was 2.9 ± 2.72 ng/ml with 43.8 % of patients having a normal AMH level (1 - 2.9 ng/ml). At the end of stimulation, the average number of mature oocytes retrieved was 6.94 ± 4.5 and almost half the patients (52.84%) had had a sub-optimal response to ovarian stimulation (4 to 9 oocytes punctured).

3.2. Relationship between AMH and Other Characteristics

According to **Table 2**, there was a positive and significant correlation ($p = 0.000$) between AMH on the one hand and AFC and the number of M2 oocytes retrieved

Table 1. Paraclinical characteristics of patients.

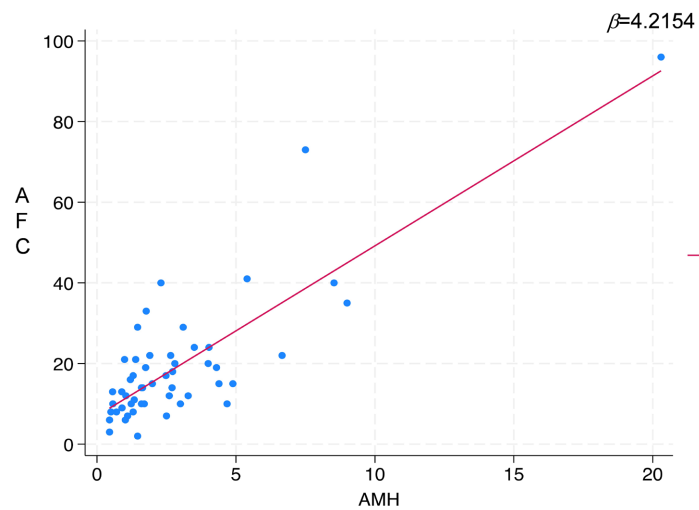
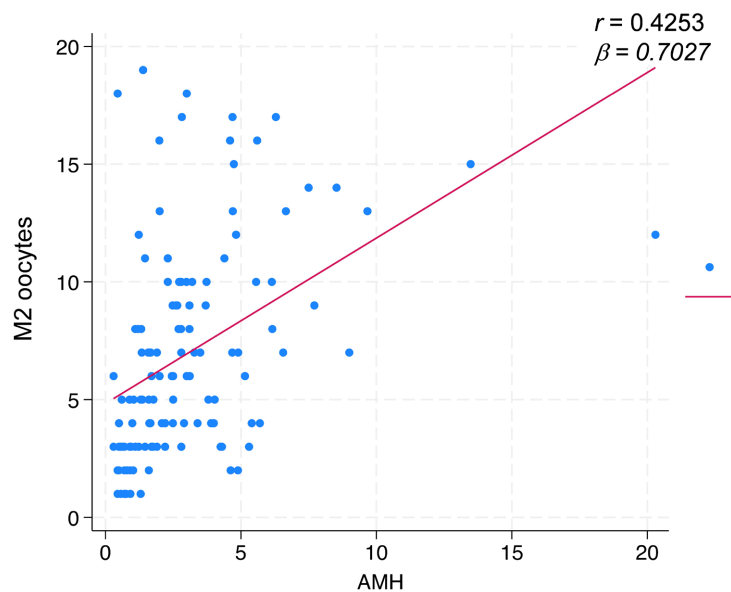
Characteristics	Total (n = 123)	Mean \pm SD	Min	Max
AMH (ng/ml)				
Low (0.3 - 0.6)	9.76	2.99 ± 2.72	0.3	20.3
Normal-low (0.7 - 0.9)	9.76			
Normal (1 - 2.9)	43.86			
High (3 - 6)	26.83			
Very high (>6)	8.94			
Oocytes retrieved		6.94 ± 4.50	1	19
Poor response (<4 oocytes)	27.64			
Sub-optimal response (4 to 9 oocytes)	52.84			
Optimal response (10 to 15 oocytes)	19.52			
Excessive response (>15 oocytes)	0			

Legend: n = number; SD = standard deviation; Min = minimum; Max = maximum; % = percentage.

Table 2. Relationship between AMH and other characteristics.

Variables	<i>r</i>	β	CI95%	CI95%
Age	-0.0738	0.4564	-0.1565 - 0.6529	0.417
Duration	0.0305	0.6025	-0.2946 - 0.4151	0.737
BMI	-0.0354	-0.0207	-0.1258 - 0.8439	0.697
AFC	0.8232	4.2154	3.3894 - 5.0413	0.000
M2oocytes	0.4253	0.7027	0.4335 - 0.9719	0.000

Legend: *r* = correlation coefficient; β = linear regression coefficient; CI 95% = 95% confidence interval; *p* = significance level of the statistical test.

**Figure 1.** Relationship between AFC and AMH.**Figure 2.** Relationship between M2 oocytes and AMH.

on the other hand, and a non-significant correlation with the duration of infer-

tility ($p = 0.737$). AMH was negatively and non-significantly correlated with age and Body mass index (BMI). In fact, an increase in AMH of one unit was associated with an increase in BMI. An increase in AMH of one unit was associated with an AFC increase of four antral follicles [$b = 4.21$ CI 95% (3.38 - 5.04) $p = 0.000$] with a strong correlation ($r = 0.82$) (Figure 1) and also associated with an increase in the number of oocytes retrieved of 70% [$b = 0.70$ CI 95% (0.43 - 0.97) $P = 0.000$] with a moderate correlation strength ($r = 0.42$) (Figure 2).

3.3. Relationship between Age and IVF Indication

The tubal indication predominated in patients aged 35 to 39 (42.4%), with premature ovarian failure (POF) and endometriosis in those less than 35 (60% vs 57.1%). Also, the majority (80%) of patients whose partner had a sperm abnormality were less than 40 (Figure 3).

3.4. Relationship between Age and Ovarian Response

According to Figure 4, the optimal response concerned mainly patients less than 35 years old (69.6%). Patients aged 35 and more had a poor response in 61.8% of cases.

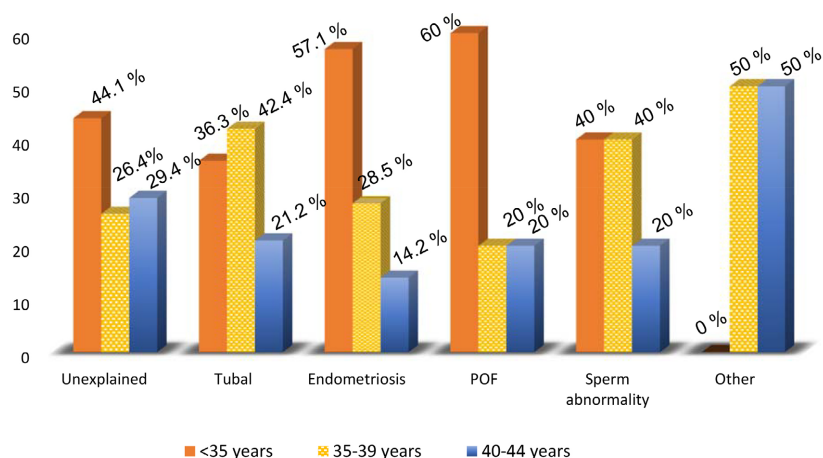


Figure 3. Relationship between age and IVF indication.

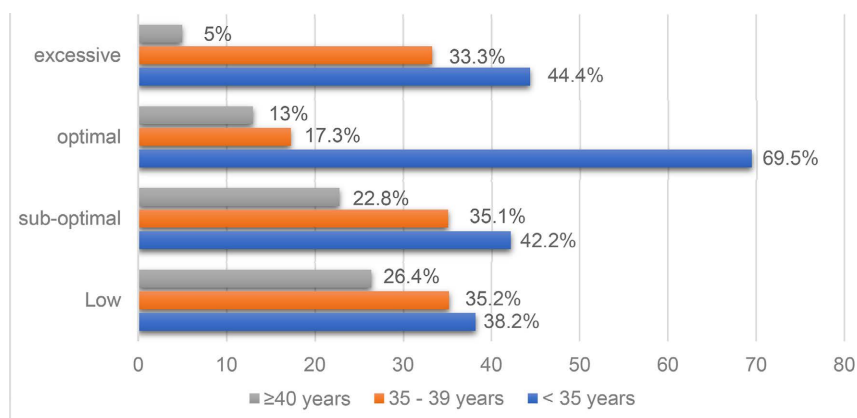


Figure 4. Relationship between age and ovarian response.

3.5. Relationship between AMH and First Test Result

Of all the patients with a normal AMH level, 38.8% had a clinical pregnancy with an embryo showing cardiac activity at the first test, 5.5% had a biochemical pregnancy and 55.5% did not achieve a pregnancy.

Of the patients with a low AMH level, 83.3% had a clinical pregnancy with an embryo showing cardiac activity at first check; only 16.6% did not achieve pregnancy.

These differences were not statistically significant ($p = 0.118$), subject to the smallness of patients concerned.

3.6. Relationship between AMH and Second Test Result

In the evolution of process, biochemical pregnancies with a doubtful prognosis had not progressed, those with a favorable prognosis had a good evolution, and some patients (18%) that got pregnant were lost to follow up. Among the patients with a low AMH level, 83.3% had a clinical pregnancy, in contrary, almost a third (33.3%) of patients with normal-low AMH. Most patients with normal AMH (61.1%) and those with high AMH (54.4%) did not achieve a pregnancy.

These differences were statistically significant ($p = 0.001$) (Figure 5).

3.7. Factors Predicting Ovarian Response and IVF Outcome

No clinical variable was significantly associated with ovarian response or IVF outcome in univariate analysis. The AMH level was significantly associated with the prediction of ovarian response to stimulation and with the results of IVF. Indeed, patients with a normal AMH level were 7 times more likely to have a good response compared with those with a low level [OR = 7.12 CI 95% (1.98 - 30.79) $p = 0.003$] and were less likely to obtain a pregnancy after embryo transfer

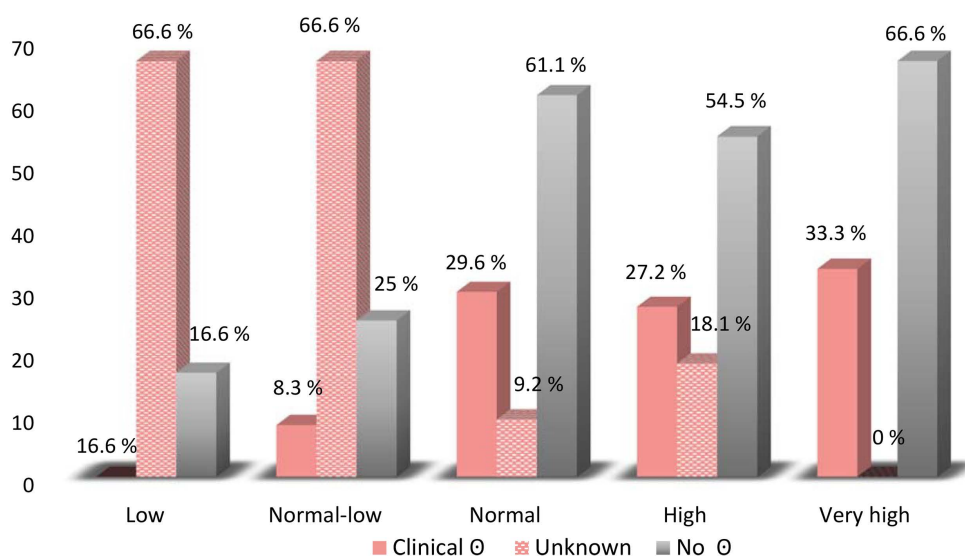


Figure 5. Relationship between AMH and IVF outcome at second check ($p = 0.001$). Legend: \odot = pregnancy.

Table 3. Relationship between ovarian response and paraclinical characteristics.

Variables	OR	CI 95%	p
AMH	1		
Low (0.3 - 0.6)	0.4	0.01 - 0.69	
Normal-low (0.7 - 0.9)	7.12		0.353
Normal(1 - 2.9)	11.2	1.98 - 30.79	0.003
High (3 - 6)	1	2.42 - 51.81	0.002
Very high (>6)	1		
Indication			
Tubal	1		
Unexplained	1.21	0.41 - 3.49	0.728
Sperm abnormality	1.01	0.21 - 4.74	0.985
POF	0.04	0.00 - 0.43	0.007
Endometriosis	5.65	1.12 - 28.51	0.036
Hyperprolactinemia	0.43	0.02 - 7.66	0.569

Legend: OR = odd ratio; CI = confidence interval; p = significance level of a statistical test.

[OR = 0.12 CI95% (0.02 - 0.63) p = 0.012]. This finding, shown in **Table 3**, was statistically significant.

Compared with patients with a tubal indication, those with POF had a significant 96% risk of having a poor response [OR = 0.04 CI 95% (0.00 - 0.43) p = 0.007]. Those with endometriosis were statistically significantly 6 times more likely to have a good response [OR = 5.65 CI 95% (1.12 - 28.51) p = 0.036] (**Table 3**).

3.8. Relationship between IVF Outcome and Paraclinical Variables

According to **Table 4**, patients with low AMH had a significantly progressively greater chance of a good IVF result than those with normal-low, normal, high, or very high AMH.

Table 4. Relationship between IVF outcome and paraclinical variables.

Variables	OR	CI 95%	p
AMH	1		
Low (0.3 - 0.6)	0.1		
Normal-low (0.7 - 0.9)	0.12	0.01 - 0.69	0.020
Normal(1 - 2.9)	0.16	0.02 - 0.63	0.012
High (3 - 6)	0.1	0.03 - 0.88	0.035
Very high (> 6)	1	0.01 - 0.69	0.020

Legend: OR = odd ratio; CI = confidence interval; p = significance level of a statistical test.

4. Discussion

The aim of the current study was to investigate the correlation between AMH and the number of M2 oocytes and the results of the first IVF attempt, the discussion is focused on the characteristics of the patients, their relationship with AMH and the results and the predictive ability of AMH IVF result.

Patients' age ranged from 27 to 44 years with an average of 35 ± 4.4 years (54%). These results are consistent with some studies in our setting Mboloko *et al.* [20], and Bikuelo. The same with perinatal surveys carried out in France in 2022, which reported an increasing in the average age of the first pregnancy in recent decades throughout the world. This is due to changes in the society mindset for women: access to higher education and to get to the more comfortable positions in the society [21] [22] [23]. The consequence is that these patients are in the period of the onset of ovarian ageing according to Broekmans *et al.* findings [24], with accelerated depletion of the follicular capital and consequent reduction in the quantity and quality of oocytes [24] [25] [26]. These factors are often responsible for a reduced prognosis in terms of oocyte quantity and IVF outcome. In the current study, age and BMI were negatively correlated to AMH (Table 2); that is consistent with the abovementioned statement [24]. However, there is a positive and statistically significant correlation with AFC and M2 oocytes.

Jeppesen's study shows that AMH is secreted by primary, pre-antral and small antral follicles [27] [28]. The AFC, as the name suggests, represents sonographically visible follicles that are counted, in other words, follicles recruited from the cohort of those secreting AMH [9]. It is currently established that AFC and AMH are the most relevant markers of ovarian reserve [6] [9] [17]. This explains the positive correlation between these two markers in the present study ($r = 0.823$, $p = 0.000$).

Furthermore, the different levels of AMH and M2 oocytes were compared with the low level of AMH, the normal level was associated with 7 times more chance [OR = 7.12 CI 95% (1.98 - 30.79) $p = 0.003$] of having a good response and the high level with 11 times more chance of having a good response [OR = 11.2 CI 95% (2.42 - 51.81) $p = 0.002$]. In the contrary, the very high level of AMH showed no trend. It should be pointed out that in addition to its role as a marker of ovarian reserve, AMH is a protective factor for ovarian reserve. In fact, it counteracts prematurely excessive initial and cyclical recruitment of follicles [29] [30]. This may explain why very high AMH levels are not associated with a good response. Endometriosis was significantly associated with a 6-fold greater chance of a good ovarian response, unlike POF. Endometriosis is known to be associated with an early quantitative and quality alteration of ovarian reserve [31]. That finding can be explained by the youth of the endometriosis patient (57%, see Figure 3) who had no history of ovarian surgery [32]. As for patients with POF, the association with poor response was consistent with their condition [33] [34].

Patient characteristics showed no association in predicting IVF outcome. Only the AMH marker had a decreasing association with IVF results: the higher the patient's AMH level, the lower the chance of obtaining a good IVF result, except for very high levels, which had the same chances as low normal levels. Large-scale, better-structured studies could explain this finding.

5. Conclusions

This study confirmed that AMH is a predictive marker of response to ovarian stimulation. However, it is not predictive of the chances of pregnancy in IVF.

Therefore, unless constrained by the patient's age, the AMH level should not justify a direct decision to donate oocytes; prior stimulation should be attempted.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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