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Original Research Article

Anti-Mullerian hormone (AMH) as predictor of ovarian reserve

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ABSTRACT

Background: Anti-Müllerian hormone (AMH) is produced by the granulosa cells of preantral and small antral follicles and its levels can be assessed in serum. Since the number of ovarian follicles declines with increasing age, AMH levels might be used as a marker for ovarian ageing. Therefore, we studied the relationship between AMH levels and ovarian response during ovarian stimulation for In vitro fertilization.
Methods: A total of 100 patients who have undergone their ICSI treatment cycle using a GnRH antagonist protocol were retrospectively included. Co-relation between AMH and antral follicular count (AFC) was assessed.
Results: In present study, 36% patients had normal AMH, 18% patients were in low normal range, 5% patients had low values and 2% patients had very low values. 41% of patients had values in high range suggestive of PCOS. Amongst this, 21% had values between 4 to 8 ng/ml where we got good AFC count and good result in terms of pregnancy. 80% were good responders while 20% were poor responders. When we evaluated the relationship of retrieved oocyte counts with the parameters included, we found that only basal AMH levels and the number of antral follicles were statistically correlated.
Conclusions: High AMH levels correlated with low cancellation rates, retrieval of more eggs, higher live birth rates and a high chance for freezing of embryos. Low AMH levels (alone) do not predict low success rates in women under 35 years of age.

Keywords: Anti-mullerian hormone, Antral follicular count, Oocytes

INTRODUCTION

Ovarian reserve is defined as the functional potential of the ovary, and reflects the number and quality of the oocytes in the ovary at any given time.[1]

With the understanding that chronological age alone is an inadequate predictor of the ovarian reserve, multiple tests have been developed to assess ovarian function (i.e., "ovarian reserve" tests). Some of these tests include basal FSH, basal inhibin B, the clomiphene citrate (CC) challenge test, basal E_2, the GnRH challenge test, the ovarian antral follicle count (AFC) as assessed by transvaginal ultrasound examination, and serum levels of anti-Müllerian hormone (AMH). Although such tests are frequently labeled ovarian reserve tests, they are more accurately ovarian response tests.[2]

Anti-Müllerian hormone (AMH) is produced by the granulosa cells of preantral and small antral follicles and its levels can be assessed in serum. Since the number of ovarian follicles declines with increasing age, AMH levels might be used as a marker for ovarian ageing.[3]

Human female serum contains measurable amounts of AMH during the reproductive life span.[3] Since AMH is

solely produced in the growing ovarian follicles, serum levels may be used as a marker for ovarian reserve, representing the quantity and quality of the ovarian follicle pool.[3] The ovarian reserve, constituted by the size of the ovarian follicle pool and the quality of the oocytes therein, declines with increasing age, resulting in the decrease of a woman's reproductive function.[3]

Anti-Müllerian hormone seems to be the best endocrine marker for assessing the age-related decline of the ovarian pool in healthy women; thus, it has a potential ability to predict future reproductive lifespan. The most established role for AMH measurements is before in vitro fertilization is initiated, because AMH can be predictive of the ovarian response, namely poor and hyper-responses.[4]

Plasma AMH assessments are superior to FSH in identifying women with reduced ovarian reserve.[4] Anti-müllerian hormone assessment should be considered as a useful adjunct to FSH/oestradiol levels and antral follicle count when estimating ovarian reserve.[5]

It is widely accepted that the reduction of AMH levels in serum is the first indication of a decline in the follicular reserve of the ovaries.[6] AMH concentration remains stable throughout the menstrual cycle.[6] In conditions with high LH and normal or low FSH levels, as in PCOS, AMH concentrations are positively correlated with LH concentrations, while they are not negatively correlated with FSH.[6]

In a normal ovary, AMH works to slow and prevent the premature development of the follicles before they are mature – keeping the ovary from developing eggs prematurely.[7]

Present study was undertaken to determine the predictive value of antimüllerian hormone (AMH) as a marker for ovarian reserve.

METHODS

It was a retrospective analysis carried out at in vitro fertilization (IVF) clinic. A total of 100 patients who have undergone their ICSI treatment cycle using a GnRH antagonist protocol were retrospectively included.

All patients recruited for ICSI, irrespective of indications, were included in the study. On day 2 of a spontaneous cycle, patients underwent a transvaginal ultrasound examination to assess the number of antral follicles, measuring 2–5 mm and endometrial thickness. Patients with thin endometrium were recruited. On the same day a venous blood sample was obtained for the measurement of AMH, FSH, estradiol (E2) and inhibin B

Ovulation induction was initiated with recombinant FSH, 150–300 IU/day, depending on AMH, age and AFC. After more than three follicles larger than 18 mm were observed, 250 microgram of recombinant hCG was administered subcutaneously and 35 hours later oocyte retrieval was done under general anesthesia.

The study group was divided into two subgroups according to the number of oocytes retrieved. Patients with an oocyte count of five or more were considered good responders, and patients with less than five oocytes as poor responders. ICSI (intracytoplasmic sperm injection) was done in all patients.

The transfer of the embryos was performed 48 hours after the procedure. Four cell grade 1 embryos were transferred. A maximum of three embryos were transferred. On the 12th to 14th days of the transfer, a serum β-hCG test was performed to confirm pregnancy. To support the luteal phase, micronized progesterone was given.

The main outcome measures of the study were the number of oocytes retrieved i.e. ovarian response to stimulation. As described previously, poor response was defined as fewer than 4 oocytes obtained.

RESULTS

Maximum patients i.e. 42% patients were between 31-35 years. 29% patients were between 26-30 years, 16% patients were between 36-40 years, 12% patients were between 21-25 years while only 1% of patients were above 40 years of age.

Table 1: Age group.

Age group	No. of patients	Percent
21-25 years	12	12
26-30 years	29	29
31-35 years	42	42
36-40 years	16	16
>40 years	1	1

Table 2: Type of infertility.

Type of infertility	No. of patients	Percent
Primary	74	74
Secondary	26	26

In present study, 74% of patients had primary infertility while 26% of patients had secondary infertility.

Table 3: Indication for ICSI.

Indication for ICSI	No. of patients	%
Tubal factor	22	22
Male factor	20	20
PCOS	17	17
Endometriosis	5	5
Unexplained infertility	18	18
More than 1 factor	18	18

In present study, 22% of patients had tubal factor, 20% of patients had male factor, 17% of patients had polycystic ovarian syndrome (PCOS) while 5% patients had endometriosis. 18% of patients had unexplained infertility.

Table 4: Number of ICSI cycle.

No. of ICSI cycle	No. of patients	%
First	93	93
Second	3	3
Third	2	2
Fourth	2	2

In present study, 93% of patients had their first cycle of ICSI, 3% of patients had their second cycle of ICSI, 2% of patients had third cycle of ICSI and 2% of patients had their fourth cycle of ICSI.

Table 5: AMH levels.

	AMH levels	No. of patients	%
High	Over 4.0ng/ml	41	41
Normal	1.5 - 4.0ng/ml	36	36
Low normal range	1.0 - 1.5ng/ml	18	18
Low	0.5 - 1.0ng/ml	5	5
Very low	Less than 0.5ng/ml	2	2

In present study, 36% patients had normal AMH, 18% patients were in low normal range, 5% patients had low values and 2% patients had very low values. 41% of patients had values in high range suggestive of PCOS. Amongst this, 21% had values between 4 to 8 ng/ml where we got good AFC count and good result in terms of pregnancy.

Table 6: Ovarian response.

Ovarian response	No. of patients	%
Good responders	80	80
Poor responders	20	20

The study group was divided into two subgroups according to the number of oocytes retrieved. Patients with an oocyte count of five or more were considered good responders, and patients with less than five as poor responders. In present study, 80% were good responders while 20% were poor responders.

Table 7: Pregnancy outcome.

Pregnancy outcome	No. of patients	%
Total no. of pregnancies	54	54
Triplets	3	3
Twins	7	7
Abortion	10	10
Biochemical pregnancies	8	8

In present study, fifty four patients conceived. Three patients had triplets, seven of them continued as twin pregnancies, and forty four patients as singleton pregnancy. Ten pregnancies ended up in abortion and eight were biochemical pregnancies.

When we evaluated the relationship of retrieved oocyte counts with the parameters included, we found that only basal AMH levels and the number of antral follicles correlated well.

DISCUSSION

Maximum patients i.e. 42% patients were between 31-35 years. 29% patients were between 26-30 years, 16% patients were between 36-40 years,12% patients were between 21-25 years while only 1% of patients were above 40 years of age. Patients with low and very low values of AMH were above 35 years of age.

K. Hansen et al found that even after correcting for chronological age, two of the tests, the ovarian AFC and serum levels of AMH, were still significantly correlated with the ovarian primordial follicle number. In addition, approximately 74% of the variation in ovarian primordial follicle count could be explained with only two of the parameters, chronological age and the ovarian AFC.2 Kelton P et al found that plasma AMH levels remained relatively static (20–25 pmol/L) from 18 to 29 years of age. By 30 years of age, plasma AMH levels start to drop rapidly, reaching only10 pmol/L by 37 years.[5]

In present study, 74% of patients had primary infertility while 26% of patients had secondary infertility. 93% of patients had their first cycle of ICSI, 3% of patients had their second cycle of ICSI, 2% of patients had third cycle of ICSI and 2% of patients had their fourth cycle of ICSI.

In present study, 36% patients had normal AMH, 18% patients were in low normal range, 5% patients had low values and 2% patients had very low values. 41% of patients had values in high range suggestive of PCOS. Amongst this, 21% had values between 4 to 8 ng/ml where we got good AFC count and good result in terms of pregnancy.

The study group was divided into two subgroups according to the number of oocytes retrieved. Patients with an oocyte count of five or more were considered good responders, and patients with less than five as poor responders. In present study, 80% were good responders while 20% were poor responders. When we evaluated the relationship of retrieved oocyte counts with the parameters included, we found that only basal AMH levels and the number of antral follicles were statistically correlated.

Kelton P et al found that using a cut off value of 8.1 pmol/L, plasma AMH assessment could predict poor ovarian reserve on a subsequent IVF cycle with a

sensitivity of 80% and a specificity of 85%.[5] Kelton P et al found that plasma AMH assessments are superior to FSH in identifying women with reduced ovarian reserve. Anti-müllerian hormone assessment should be considered as a useful adjunct to FSH/oestradiol levels and antral follicle count when estimating ovarian reserve.[5]

Fiçicioğlu C et al found that patients with fewer than five retrieved oocytes had lower day 3 AMH levels, fewer antral follicles, and lower hCG day E2 levels. Thus, basal antral follicle count and basal AMH levels are good tools for use in counseling patients.[8] C Fiçicioğlu et al found that levels of AMH would predict the number of oocytes with a positive predictive rate of 96%, although it had little value for predicting pregnancy.8 C Fiçicioğlu et al revealed that the most sensitive and specific indicator of ovarian reserve is the level of AMH, it does not indicate pregnancy success as well when 0.25 pg/mL is taken as a cut-off value.[8] Fiçicioğlu C et al demonstrated an association between early follicular phase serum AMH and number of retrieved oocytes despite clinically similar day.[8] Fiçicioğlu C et al measured serum FSH and E2 levels in patients of all age groups. Baseline FSH, LH, and E2 levels are good predictors of ovarian reserve.[8] C Fiçicioğlu et al found that basal antral follicle count is correlated but weakly with the number of retrieved oocytes during assisted reproduction cycles.[8] Fiçicioğlu C et al found that AMH was the best indicator of ovarian reserve with a high sensitivity and specificity.[8]

Better AMH than FSH specificity has been previously demonstrated and is also supported by Fiçicioğlu C et al. AMH <1.05 ng/mL, however, does not define DOR. It only defines DOR with significantly decreased live-birth chances. It also does not warrant withholding of treatment because even DOR patients with very low to undetectable AMH still achieve rather surprising live-birth rates.[8]

Van Rooij et al found that serum AMH levels were highly correlated with the number of antral follicles ($r = 0.77$; $P < 0.01$) and the number of oocytes retrieved ($r = 0.57$, $P < 0.01$).[9] Van Rooij et al combined antral follicle count with AMH and inhibin B to provide for better prediction.[9,12] Dillon K et al found that participants with a pre-treatment AMH level >2 ng/mL recovered at a rate of 11.9% per month after chemotherapy, whereas participants with pre-treatment AMH levels ≤2 ng/mL recovered at a rate of 2.6% per month after therapy.[10]

Akira Ivase et al found that the median AMH level was 2.98 ng/mL and 3.92 ng/mL before operation and was significantly reduced to a median level of 2.24 ng/mL and 3.29 ng/mL at 1 month after operation in the endometrioma group (n = 29) and the non-endometrioma group (n = 21), respectively.[11] Kelton T found that Serum AMH is a sensitive marker of age-related decline in ovarian reserve status. A serum AMH result >36 pmol L−1, or above the 75th percentile for age, is highly suggestive of a diagnosis of PCOS. A serum AMH result below the 10th percentile for age suggests accelerated loss of ovarian reserve, while an AMH result exceeding 20 pmol L−1 suggests an increased risk of OHSS during IVF treatment.[12] Brodin T et al found that all ORTs correlated significantly with each other, with the strongest correlation between AFC and AMH ($r = 0.71$, $p < 0.0001$). Univariately, AMH and age equivalently predicted live birth (c-statistic 0.61), and together they provided a significantly better model (c-statistic 0.64). For prediction of poor and excessive response the best model included AMH, AFC and age (c-statistic 0.89).[13]

Lauren Z et al found that among 97 women who underwent AMH testing, 32 (33.0%) had elevated AMH levels. Hyperandrogenism was reported by 8 (25.0%) women with elevated AMH and none with AMH concentrations lower than 4.7 ng/mL ($P < 0.001$). Irregular menstrual cycles before hormonal contraceptive use were reported by 16 (24.6%) of 65 women with AMH concentrations lower than 4.7 ng/mL and 11 (34.4%) with elevated AMH ($P = 0.34$). Of the 20 women with elevated AMH who returned for further evaluation, 16 (80.0%) had polycystic ovaries and 13 (65.0%) were diagnosed with PCOS (Rotterdam criteria).[14]

CONCLUSION

When basal AMH levels and the number of antral follicles were correlated, high AMH levels correlate with low cancellation rates, retrieval of more eggs, higher live birth rates and a high chance for freezing of embryos. Low AMH levels (alone) do not predict low success rates in women less than 35 years of age. Couples should not be excluded from attempting assisted reproductive cycles due to low AMH values alone because live birth success rates were reasonable in these cases.

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