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Severe endometriosis: low value of AMH did not affect oocyte quality and pregnancy outcome in IVF patients

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Abstract. – OBJECTIVE: The aim of this pilot study was to determine whether the low anti-müllerian hormone (AMH) serum level, due to severe endometriosis, was associated with diminished oocyte yield, poor oocyte/embryo quality and reduced *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) clinical outcomes in young patients (<37 years old).

PATIENTS AND METHODS: A total of 50 IVF cycles of patients younger than 37 with severe endometriosis were retrospectively analyzed in a single center between November 2016 and July 2018. The clinical outcome was then compared to a control group of 84 patients with no story of endometriosis and normal AMH value. AMH value was evaluated within three months before the stimulation. In these two groups, number and maturation of retrieved oocytes, embryo quality, and pregnancy outcomes were evaluated and compared using Student's *t*-test and Fisher's test.

RESULTS: The number of oocytes retrieved per cycle and the percentage of mature oocytes (MII) were significantly lower ($p < 0.001$) in IVF patients with severe endometriosis and AMH value ≤ 1.1 ng/ml (Group A; 3.8±2.6 retrieved oocytes, 70% MII) compared to patients without endometriosis and AMH levels > 1.1 ng/ml (Group B; 6.9±4.6 retrieved oocytes, 83% MII). On the other hand, embryo morphology, implantation rate (31% *vs.* 33%; $p = 0.833$) and pregnancy rate (50% *vs.* 49%; $p = 1$) were comparable in the two groups.

CONCLUSIONS: This study shows that younger patients with an impairment of the ovarian reserve due to severe endometriosis, displayed a diminished oocyte yield but not a reduction in embryo quality and pregnancy outcomes. These results suggest that serum AMH levels should not be adopted as a criterion for discouraging these patients from undergoing IVF/ICSI treatments.

Key Words:
Severe endometriosis, Anti-müllerian hormone, Oocyte maturity, Embryo quality, IVF clinical outcome.

Introduction

Ovarian aging is due to the progressive reduction of the amount and quality of the follicular ovarian reserve, which may predict the duration of a woman's reproductive lifespan. Furthermore, diminished ovarian reserve is associated to reduced fertility and poor response to ovarian stimulation during *in vitro* fertilization (IVF) treatment[1].

Anti-müllerian hormone (AMH) is a glycoprotein belonging to the transforming growth factor-b (TGF-b) superfamily. In women, AMH is primarily produced by granulosa cells, surrounding preantral and small antral follicles[2], and has a regulatory function in the activation of folliculogenesis. Peak AMH serum levels occur around at the age of 25 and subsequently decline with advancing female age, becoming low or no detectable after menopause, when the number of primordial follicles is depleted[3]. Moreover, AMH is a widely used marker of ovarian reserve and a good predictor of ovarian response to controlled ovarian stimulation during IVF treatment[4]. The measure of its levels, evaluated before stimulation, correlates well with the antral follicle count and is considered a better predictor of ovarian response than classical parameters, such as follicle stimulating hormone (FSH), estradiol and inhibin B levels[5]. The advantage to use AMH is due to its stability during and between menstrual cycles[6] and to the small intercentre variability of its determination. Despite most of the studies show a positive correlation between AMH serum levels and the number of oocytes retrieved[7], the capacity of AMH to predict also the IVF clinical outcome is still controversial. In particular, it has not yet been extensively studied whether young patients with severe endometriosis and premature depletion of the ovarian reserve,

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exhibit a decline in the quality of the oocytes with a consequent lowering of the quality of the embryos and so of the pregnancy outcome. The aim of the present study was to investigate whether patients <37 with severe endometriosis and low AMH values exhibited a diminished oocyte maturity/embryo quality and a poor pregnancy outcome in comparison with women of the same age but with normal AMH values and no evidence of endometriosis.

Patients and Methods

Patients

Patients undergoing IVF cycles at the IVF Unit of San Filippo Neri Hospital between November 2016 and July 2018 were retrospectively studied. Prior patient consent was informed and written.

Only patients <37 years old at the time of oocyte retrieval were included in the analysis. The women were included in the study if they fulfilled the following criteria: (1) diagnosis for stage IV of endometriosis (Figure 1); (2) Ca125 levels > 35 U/ml; (3) AMH levels evaluated in the three months before starting the stimulation; (4) infertility not attributable to male factor; (5) serum hormonal profile (FSH and LH <12 IU/ml) within the normal range; (6) regular ovulatory menstrual cycles; (7) presence of normal uterine cavity; (8) no evidences of polycystic ovary syndrome (PCOS); (9) no evidence of autoimmune diseases; (10) normal thyroid hormones value;

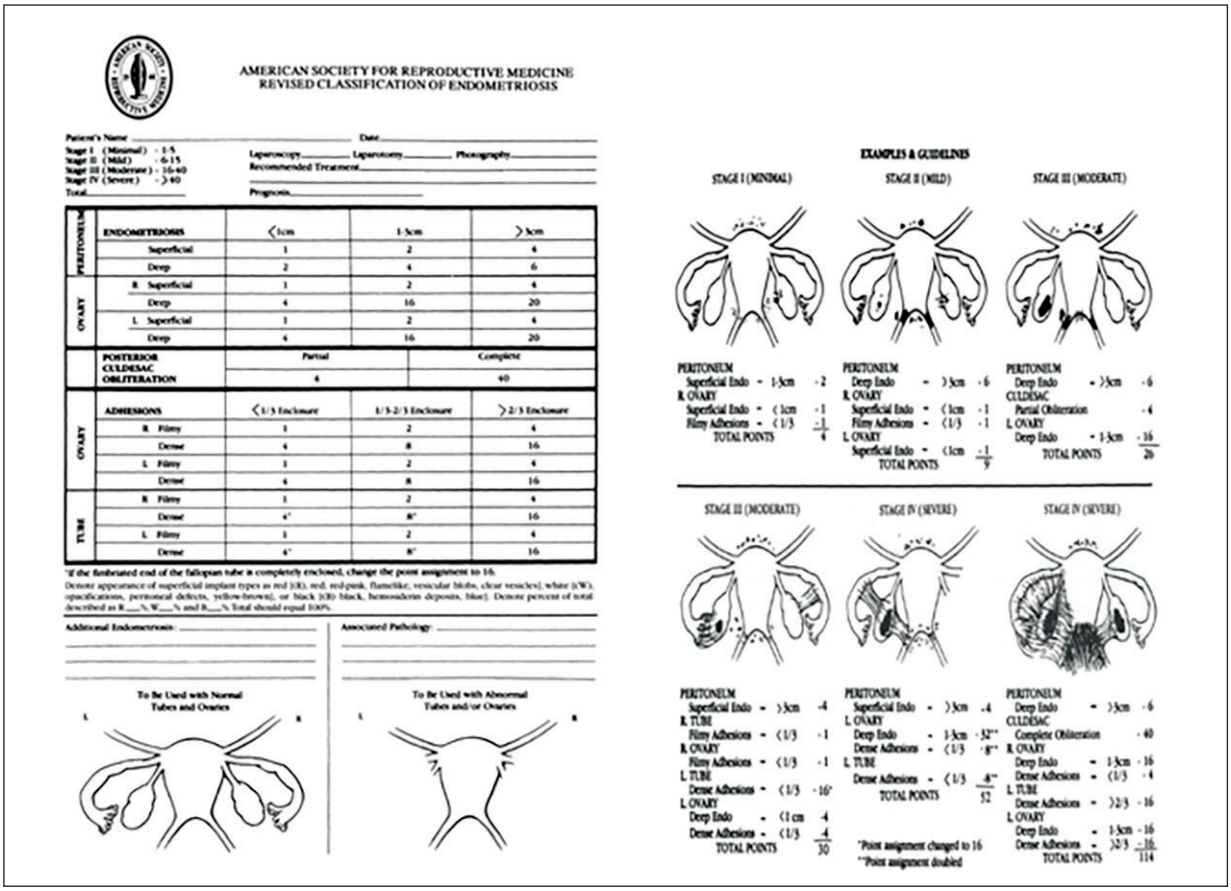

Figure 1. Determination of the stage or degree of endometrial involvement is based on a weighted point system. Distribution of points has been arbitrarily determined and may require further revision or refinement as knowledge of the disease increases. To ensure complete evaluation, inspection of the pelvis in a clockwise or counterclockwise fashion is encouraged. Number, size and location of endometrial implants, plaques, endometriomas and/or adhesions are noted. Points assigned may be circled and totaled. Aggregation of points indicates stage of disease (minimal, mild, moderate, or severe). The presence of endometriosis of the bowel, urinary tract, fallopian tube, vagina, cervix, skin etc., should be documented under "additional endometriosis." Other pathology such as tubal occlusion, leiomyoma, uterine anomaly, etc., should be documented under "associated pathology."

(11) no evidence of thrombophilic factors; (12) no evidence of infectious diseases; (13) normal genetic testing. A total of 134 patients who met the above-mentioned criteria were enrolled in this study.

Patients were divided into two groups: group A, study group, (n=50) with severe endometriosis and AMH levels ≤ 1.1 ng/ml; group B, control group, (n=84) with AMH levels >1.1 ng/ml. AMH value of 1.1 ng/ml was chosen because is the average AMH value for 37 years old women[8]. A quantitative decline in ovarian reserve is considered physiologic in women greater than 37.5 years old[9]. Therefore, considering the age of the patients (< 37 years old), the relationship between premature reduction of the ovarian reserve and reduction in oocyte quality, was investigated. AMH was measured in the Laboratory of General Pathology of San Filippo Neri Hospital, using a commercially available enzyme immunoassay kit (AMH GenII ELISA, Beckman Coulter, Brea, CA, USA), following the manufacturer's instructions. The limit of detection of the AMH GenII ELISA is 0.08 ng/ml. Reported intra-assay and inter-assay coefficients of variation are respectively < 5.4 % and < 5.6%[10].

The primary endpoints were the percentage of MII oocytes, embryo quality and pregnancy rate. The secondary endpoint was the implantation rate.

Endometriosis Classification

Over time, various classifications of endometriosis have been proposed, today the most used is the classification of the American Society of Reproductive Medicine (ASRM, 1997) which divides the disease into four stages. For the purposes of classification, a score is assigned in base of the appearance, size and depth of the peritoneal and ovarian lesions; the presence, extent and type of lesions (red, white - including peritoneal defects - and black); the presence, extent and type of adhesions at the level of the annexes and the degree of the Cul-de-Sac obliteration (Figure 1).

Based on the overall score, the four stages of the disease are identified: stage I or minimal endometriosis (points 1-5); stage II or mild endometriosis (points 6-15); stage III or moderate endometriosis (points 16-40); stage IV or severe endometriosis (points 40).

This system reflects the extent of endometriosis, but it is a partial observation point developed especially for infertility. The stage is not related to the signs and symptoms of the disease, nor to the results of the treatment and underestimates the severity of the deep lesions. In fact, there are no data showing how this classification corresponds to a clinical prognosis[11].

Ovarian Stimulation

All patients underwent a standard down-regulation protocol consisting in a dose of 0,1 mg/day of GnRH analogue hormone (triptorelin) (Decapeptyl, Ipsen, Milan, Italy) starting the first day of the cycle. Ovarian stimulation was initiated with the administration of gonadotropins, starting on day 2 of the cycle and triptorelin administration was continued up to hCG day. The patients received recombinant FSH (rFSH; Gonal-F Merck Serono, Italy and Puregon, MSD, Italy) starting with the dosage of 150 IU/day from the third day of the cycle simultaneously with 150 IU/day of human menopausal gonadotropin (hMG, Meropur, Ferring, Milan, Italy)[12-19]. After 6 days of stimulation, the FSH dosage was adjusted as necessary, according to follicular size and estradiol levels. No patients experienced excessive response to gonadotropins. Final oocyte maturation was triggered by the administration of 10000 IU of human chorionic gonadotropin (hCG) (Gonasi HP IBSA, Lugano, Switzerland) when at least half of the total follicles were 18 mm in diameter. Oocyte retrieval was performed 36 h after hCG administration. The retrieved oocytes were denuded from their cumulus cell and were assessed for their maturity. Mature metaphase II oocytes were inseminated by intracytoplasmic sperm injection (ICSI) and the resulting embryos were scored according to established criteria[20]. Ultrasound guided embryo transfer took place at the stage of blastocyst[21,22]. The luteal phase was supported by the administration of 50 mg/day of progesterone, 4000 U/day of low molecular weight heparin (clexane; Sanofi-Aventis, Surrey, UK) and 15 mg/day of prednisone (Deltacortene, Bruno Farmaceutici, Rome, Italy). The increase of serum concentration of betahCG and the presence of intrauterine gestational sac were indicators of the pregnancy.

Statistical Analysis

Statistical analysis was performed using the two tailed Student's *t*-test for independent data. The significance level was set at $p < 0.05$. Fisher's exact tests were used to compare proportions.

Results

At the time of the study, among 134 included patients, 122 underwent oocyte retrieval, 48 in group A and 74 in group B. No patients were cancelled because of excessive ovarian response leading to high risk for ovarian hyperstimulation syndrome (OHSS). Demographic data are presented in Table I. Patients in Group A were slightly older (35±2) than patients in Group B (33±3), while the two groups were comparable regarding body mass index (BMI; 24.0±5.3 in Group A *vs.* 22.5±2.8 in Group B). The mean value of total FSH administered to women of Group A (4331±1280 IU) during ovarian stimulation was about two times higher than that of women of Group B (2040±1224 IU). On the other hand, estradiol levels on the hCG day were significantly lower in Group A (1179±586 pg/ml) than in Group B (1751±928 pg/ml). Data regarding oocyte maturity, embryo morphological grades and clinical outcome are reported in Table II.

These results obtained showed that serum AMH levels were quantitatively associated with oocyte availability and this trend is in accordance with literature[4]. Indeed, the average number of retrieved oocytes was lower in Group A (3.8±2.6) than in Group B (6.9±4.6). Moreover, a lower percentage of mature MII oocytes (p = 0.001) was observed in Group A (70%) with respect to Group B (83%), whereas no statistically significant differences between the two groups were observed in terms of percentage of MI (12% *vs.* 7% respectively in Group A and Group B), GV (15% *vs.* 9% respectively in Group A and e Group B) or degenerated oocytes retrieved (3% *vs.* 1% respectively in Group A and Group B). The mean number of embryos transferred was 1.5±0.9 for Group A and 1.9±0.5 for Group B ($p > 0.05$). Despite the different percentage of mature MII oocytes retrieved between Group A and B, the differences in terms of embryo quality between the two Groups (A grade 28% *vs.* 41%; B grade 22% *vs.* 18%; C grade 22% *vs.* 26%; D grade 28% *vs.* 15% in Group A and B respectively) were not of statistical significance. According to embryo quality data, implantation (31% in Group A *vs.* 33% in Group B) and pregnancy (50% in Group A *vs.* 49% in Group B) rates were comparable between the two Groups.

Discussion

Severe endometriosis lead to a premature ovarian insufficiency with reduced blood value of AMH[23-25]. Several variables have been suggested to develop predictive models of assisted reproductive treatment success. Up to now, it has been shown that only age is able to predict embryo quality and pregnancy in IVF treatments[26].

AMH serum levels can predict quantitative oocyte retrieval after IVF/ICSI treatments[4,27], while more controversial are the available data regarding the relationship between serum AMH levels and qualitative characteristics of oocytes/embryos and clinical IVF outcomes. Some studies[7,28-30] did not find a correlation between AMH serum levels and embryo quality or pregnancy outcome in IVF cycles, whereas other reports demonstrated an association[31-33]. Three meta-analyses reported a weak predictive value of serum AMH for ongoing pregnancy[34], live birth[35], implantation rate and clinical pregnancy[36].

Only few studies investigated the relationship between AMH levels and IVF outcome by dividing patients into age groups for reducing possible age-related biases. In the study of Wang et al[37]

Table I. Demographic data and stimulation outcome.

	Group A AMH ≤ 1.1	Group B AMH > 1.1	*p*-value
Patients (n)	50	84	
Mean age (ys) ± SD	35 ± 2	33 ± 3	0.006
Mean BMI (kg/m^2) ± SD	24.0 ± 5.3	22.5 ± 2.8	0.125
Total FSH dose (IU)	4331 ± 1280	2040 ± 1224	< 0.01
Estradiol level on hCG day (pg/ml)	1179 ± 586	1751 ± 928	< 0.01
Endometrial thickness on hCG day (mm)	10.5 ± 2.3	10.8 ± 2.1	0.955
Tubal factor % (n)	32	29	0.771
Thyroid factor % (n)	0	9	0.115
Genetic factor % (n)	0	0	1

Data are expressed as mean ± SD or percentage. *Abbreviations:* AMH, anti-müllerian hormone; BMI, body mass index; FSH, follicle stimulating hormone; hCG, human chorionic gonadotropin.

Table II. Embryological characteristics and clinical outcome.

	Group A	Group B	*p*-value
N° of patients underwent egg retrieval	48	74	
Mean number of retrieved oocytes/ patient ± SD	3.8 ± 2.6	6.9 ± 4.6	0.003
Mean number of inseminated oocytes/ patient ± SD	2.7 ± 2.3	6.5 ± 3.8	0.001
Mature oocytes (MII) %	70	83	0.001
Mature oocytes (MI) %	12	7	0.949
Immature oocytes (GV) %	15	9	0.836
Degenerated oocytes %	3	1	0.845
N° of patients underwent embryo transfer	20	32	
Mean number of embryos transferred/patient ± SD	1.5 ± 0.9	1.9 ± 0.5	0.064
Grade A embryos %	28	41	0.098
Grade B embryos %	22	18	0.842
Grade C embryos %	22	26	0.341
Grade D embryos %	28	15	0.548
Pregnancy rate: CP/ET %	50	49	1
Implantation rate: GS/n° ET %	31	33	0.833

Data are expressed as mean ± SD or percentage. *Abbreviations:* CP, Clinical Pregnancy; ET, Embryo Transfer; GS, Gestational Sacs; n° ET, Total number of Embryos Transferred.

serum AMH levels were divided into tertiles (≤0.29, 0.30-1.20, ≥1.21 ng/ml) and ages into four groups. They demonstrated that clinical pregnancy rate and live birth rate for women younger than 34 years old did not differ across the three AMH groups. These results suggested that the quantitative reduction of the ovarian reserve is not correlated to the diminished oocyte quality in young patients. On the other hand, for women aged 34-37 years, they found an association between AMH concentrations and clinical pregnancy/live birth rate. Cycle cancellation risk for women displaying AMH concentrations in the lowest tertile was significantly different from that of women with AMH concentrations in the higher tertile for all age groups. The authors did not analyze embryo quality or other clinical outcomes after IVF. Reichman et al[38] reported a trend toward higher implantation rate and pregnancy rate with increasing AMH for patients aged <40. However, ROC curves for the ability of AMH to predict clinical pregnancy were low for each of the five age groups analyzed. Similarly, Gomez et al[39] failed to find an association between AMH levels and pregnancy rate in patients under 36 years of age. It was also reported[40] that AMH was not associated with live birth rate in younger women (<35 years), while it was relevant for elderly patients.

Wherever the miscarriage rate after IVF treatment can give an indirect information about embryo quality, Tarasconi et al[41], demonstrated that low anti-müllerian hormone was correlated with increased miscarriage rate in patients older than 34 years of age, but not in younger patients. Very recently, qualitative embryo characteristics as blastulation and aneuploidy rate in addition to clinical IVF outcomes were analyzed by Morin et al[42] in a large retrospective study including 3457 patients. The authors reported that women younger than 38 years old with evidence of diminished ovarian reserve (DOR) did not display an oocyte qualitative decline. Indeed, fertilized oocytes retrieved from young patients with DOR formed blastocysts of high quality, euploid and were able to produce live births as those of women of the same age with high AMH values.

In the present study, we retrospectively analyzed 134 patients under 37 years old. In young patients, low AMH levels reflected a pathological condition due to severe endometriosis and not a physiological reduction of ovarian reserve as in older women. This allowed avoiding the confounding effect due to the age.

Women displaying an AMH value ≤ 1.1 ng/ml and a story of severe endometriosis were stimulated with higher doses of total FSH and their estradiol levels on the hCG day were significantly lower than patients with pre-treatment AMH values >1.1 ng/ml. This difference reflected the worst response to gonadotropin stimulation associated with low serum AMH levels. According to previous reports[7,29], our data demonstrated that the oocyte yield and the percentage of mature oocytes obtained during IVF treatments were reduced in patients with low AMH values due

to severe endometriosis. Despite the ovarian responsiveness of women with lower AMH values was reduced, we found that neither the proportion of grade A embryos (although slightly higher in the group with AMH values >1.1 ng/ml) nor the percentage of grade B-C-D embryos were statistically different between the two groups. This result, together with the comparable rate of implantation, suggested that the quality of produced embryos is not affected by AMH serum levels in patients with endometriosis. Moreover, the probability to achieve a pregnancy after treatment was the same between the two groups.

Overall, our results suggest that patients of relatively young age with endometriosis and consequently low AMH values still have oocytes with preserved competence to produce high quality embryos and so may still obtain favorable IVF outcome. Therefore, it was hypothesized that the premature depletion of ovarian reserve, due to severe endometriosis, was not accompanied by a qualitative reduction in oocyte function. Moreover, the weak association of high AMH levels with better IVF outcomes, reported by three recent meta-analysis, reflected the quantitative advantage provided by a greater oocyte availability rather than a better oocyte quality. In conclusion, low serum AMH levels in young patients with severe endometriosis should not be used as marker to predict IVF results, because there are not differences in pregnancy rate than in patients with endometriosis and normal range of AMH.

Conclusions

This study shows that younger patients with an impairment of the ovarian reserve due to severe endometriosis displayed a diminished oocyte yield but not a reduction in embryo quality and pregnancy outcomes. Low serum AMH levels, in young patients with severe endometriosis, should not be used as marker to predict IVF results, because there are not differences in pregnancy rate than in patients with endometriosis and normal range of AMH. These results suggest that serum AMH levels should not be adopted as a criterion for discouraging these patients from undergoing IVF/ICSI treatments.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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