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ORIGINAL ARTICLE

The Effect of Age and AMH Level on ART Outcomes in Patients With Reduced Ovarian Reserve: A Retrospective Cross-Sectional Study

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Abstract

Background Despite many advances, patients with a poor ovarian response to stimulation are one of the most important and challenging factors of infertility. Chronological and ovarian ages are two effective factors responsible for poor response to assisted reproduction treatment. The purpose of this study was to determine the effect of age and AMH level on the in vitro fertilization (IVF) outcomes in participants with a reduced ovarian reserve.

Methods In this retrospective cross-sectional study, 210 participants with anti-Mullerian hormone (AMH) < 1.1 ng/ml were included. The effect of age and AMH on pregnancy outcomes including dominant follicle count, serum estradiol level on the day of trigger administration, number of metaphase II (MII) oocytes, number of embryos, biochemical pregnancy, clinical pregnancy, abortion and live birth rate were evaluated.

Results The number of dominant follicle ($p < 0.001$), MII oocyte ($p < 0.001$), grade A ($p < 0.001$) and B ($p < 0.001$) embryos, serum estradiol level ($p < 0.001$), gonadotropin level ($p < 0.001$), AMH ($p = 0.001$), biochemical pregnancy ($p = 0.007$), clinical ($p = 0.01$) pregnancy, and live birth rate ($p = 0.003$) were higher in participants younger than 35 years old. In univariable logistic regression, the chance of retrieving more than 3 oocytes in individuals over 35 years old was 97.1% lower than in individuals younger than 35 years old ($p < 0.001$).

Conclusion It has been concluded that the higher clinical pregnancy and live birth rate in participants younger than 35 years can be due to the higher AMH level in this group. Under the same conditions of AMH and other variables, age can affect the number of retrieved oocytes.

Keywords Ovarian reserve · Maternal age · AMH · ART Outcome

Introduction

Assisted reproductive technology (ART) is one of the most common methods in infertility treatment [1]. Despite many advances, one of the most important and challenging factors in infertility treatment is participants with a poor ovarian response (POR) to ovarian stimulation. One of the reasons for the poor response to stimulation is the decrease in the quantity and quality of oocytes [2]. It is estimated that 9–24% of infertile women have POR [3]. One of the important factors in the reduction of the quality and quantity of oocytes and poor response to stimulation is aging, which is associated with increased embryo aneuploidy, abortion, and reduced pregnancy rate [4]. This condition can also occur in young women, which in many cases is idiopathic [5]. About 10–12 years before menopause, there is a significant decrease in female fertility due to a decrease in follicular reserve and an increase in aneuploidy rate [6]. When the number of primordial follicles falls below the threshold of 25,000, the reduction in follicular reserve accelerates, which occurs at the average age of 37.5 years [7, 8].

The evaluation of ovarian reserve and individualized decision-making strategy plays an important role in the treatment and increases the ART success rate. The proper

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management of these participants can play an essential role in reducing the need for donated oocytes [3]. Among the tests to assess ovarian reserve before stimulations, AMH measurement and antral follicle count (AFC) are sensitive markers for monitoring ovarian reserve and planning individual ovarian stimulation.

AMH is a glycoprotein produced from granulosa cells in 5–8 mm ovarian follicles. Although each ovarian reserve assessment test provides useful predictive information, AMH is recognized as a good diagnostic test for ovarian reserve before IVF treatment because its level is constant throughout the menstrual cycle and has a high specificity for detecting the POR [7]. Fertility reduction has been reported in women aged 35–45 years old with AMH < 10 pmol/L [9].

Decreased ovarian reserve is a phenomenon that affects women in the middle to late third decade of life and it seems that there is a sharp decrease in follicular reserve at the age of 37–38 years [6]. This decrease in follicular reserve may also affect young women. The question now is whether a reduction in quantitative ovarian reserve is equivalent to a reduction in qualitative reserve? Whether young patients with reduced AMH should be treated in the same way as older patients with reduced AMH? Since studies are limited when AMH and age are not consistent, the purpose of this study was to determine the effect of age on the IVF outcomes in patients with a reduced ovarian reserve.

Methods

Subjects and Data Collection

The present study was a retrospective cross-sectional study that was conducted between May 2015 and July 2019 in Al-Zahra Hospital and Mehr Fertility Center, Rasht, Iran. 210 participants with infertility and AMH < 1.1 ng/ml who were candidates for treatment with GnRH antagonist protocol were included in the study. All participants had normal semen analysis report of partner. Exclusion criteria included endocrinological disorder, fertility preservation candidates, polycystic ovary syndrome (PCOS), male factor infertility, surgical history of the ovary, chemotherapy, radiotherapy, and genetic disorders.

The data were collected by reviewing participants' records and recording them in an information form. The researcher-made checklist included demographic and clinical characteristics (age, body mass index (BMI), follicle-stimulating hormone (FSH), luteinizing hormone (LH), AMH, AFC, estradiol (E2), gonadotropin dosage, duration of infertility, number of grown follicles, number of obtained MII oocytes, number of embryos, clinical and biochemical pregnancies and live births rate. Subjects were divided into two groups, over 35 years old ($n = 96$) and younger than 35 years old ($n = 114$). Hormonal tests including LH, FSH, and E2 levels, were checked for participants on day 2 to 4 of the menstrual cycle, and the AMH test performed in the last 6 months were noted.

For all participants, 4 mg of E2 was prescribed for the last 10 days of the menstrual cycle. From day 2 to 4 of the current cycle, gonadotropin (Cinnal-F, Cinnagen/ Iran and PD HOMOG, Pooyesh Darou/Iran) was started for participants in a dose of 150 units. According to the patient's response based on ultrasound evaluation, the dosage of gonadotropin was increased. The GnRH antagonist (0.25 mg cetrorelix daily, Merck, Germany) was prescribed when the follicles reached a size of 13–14 mm. Human chorionic gonadotropin trigger was given when at least two 18 mm follicles were observed (PG PREG, pooyesh Darou/ Iran 5000 IU), and oocyte puncture was performed. After intracytoplasmic sperm injection (ICSI), one to two fresh embryos at the cleavage stage were transferred to the uterus under ultrasound guidance.

Two weeks after embryo transfer, serum Beta human chorionic gonadotropin (β-hCG) levels were measured. At 7th week, an ultrasound was performed to see the fetal heartbeat and confirm clinical pregnancy. The number of dominant follicles, serum E2 level at the day of trigger administration, number of MII oocytes, number of embryos, biochemical pregnancy, clinical pregnancy, abortion and live birth rate were recorded. Since performing several IVF cycles for each patient would lead to bias in the study, only the first IVF cycle was considered in this study.

Statistical Analysis

All statistical analyses were conducted using the SPSS, version 21 for windows (SPSS Inc., Chicago, IL, USA). Mann–Whitney test was used to compare quantitative variables in two age groups, and Chi-Square and Fisher exact tests were used to evaluate qualitative variables. Univariable and multivariable regression analyses were used to determine the effective factors, especially age and AMH on study outcomes (live birth). P-value < 0.05 was considered to be significant.

Results

A total of 210 infertile women (96 patients over 35 years of age and 114 patients younger than 35 years), who were candidates for IVF, were studied. The characteristics of the study groups have been shown in Table 1. There was a statistically significant difference in the dominant follicle count ($p < 0.001$), MII oocyte ($p < 0.001$), grade A ($p < 0.001$) and B ($p < 0.001$) embryos, E2 ($p < 0.001$), gonadotropin

Table 1 The patient's characteristics in the study groups

Variable	Younger than 35 years ($N=114$)	Over 35 years ($N=96$)	P-value[a]
*Age	32 (30–34)	40 (37.5–42)	< 0.001
*BMI	26 (21–30)	26 (23–30)	0.28
*AFC	3 (2–4)	3 (2–4)	0.55
*E2 (pg/ml)	2475 (2227.5–2772)	1386 (1089–1683)	< 0.001
*FSH (IU/L)	5.8 (3.05–7.9)	5.1 (2.9–7.95)	0.37
*LH (IU/L)	5.1 (2.85–7.6)	5.2 (3.05–7.4)	0.79
*Gonadotropin dosage	2577 (2374.5–2871)	3465 (3267–3762)	< 0.001
*Infertility duration	22 (17–27)	22 (17.5–26)	0.90
*AMH (ng/m l)	82 (71–96)	66 (48.5–86.5)	0.001

*Data presented as median (interquartile range)

LH luteinizing hormone, *FSH* follicle-stimulating hormone, *AMH* anti-Mullerian hormone, *E2* estradiol, *AFC* antral follicle count

[a]Mann-Whitney

level ($p<0.001$), AMH ($p=0.001$), biochemical pregnancy ($p=0.007$) clinical ($p=0.01$) pregnancy, and live birth rate ($p=0.003$) between the two age groups (Table 2). The values of dominant follicle count, MII oocytes, grade A and B embryos, E2, AMH, biochemical and clinical pregnancy, and live birth rate was higher in participants younger than 35 years. The dosage of gonadotropin in participants over 35 years was higher than the participants younger than 35 years (Table 2).

To perform logistic regression and determine the effective factors on the outcome of the number of retrieved oocytes, less than 3 oocytes were considered abnormal and more than 3 oocytes were considered normal according to the logistic regression model. In univariable logistic regression, the odds of more than 3 oocytes in patients over 35 years were 97.1% lower than patients younger than 35 years ($p<0.001$). By a 0.01 unit increase in AMH, the chance of retrieving more than 3 oocytes increased by 2.7% ($p<0.001$). By one unit increase in E2, the chance of retrieving more than 3 oocytes increased by 0.2% ($p<0.001$). By one unit increase in gonadotropin dosage, the chance of retrieving more than 3 oocytes decreased by 0.2% ($p<0.001$) (Table 3). In multivariable logistic regression, age, AFC, AMH, E2, and gonadotropin dosage were entered into the model. AMH and age had a statistically significant relationship with more than 3 oocytes so that by one unit increase in AMH level, the chance of retrieving more than 3 oocytes in the same condition of other variables increased by 2.6% ($p=0.001$). The chance of retrieving more than 3 oocytes in patients over 35 years is 97.3% lower than patients younger than 35 years ($p<0.001$) (Table 3).

In univariable logistic regression, the chance of clinical pregnancy in patients over 35 years was 59.7% less than patients younger than 35 years ($p=0.01$). By a 0.01 unit increase in AMH level, the chance of a clinical pregnancy increased by 12% ($p<0.001$). In multivariable logistic

Table 2 Comparison of the ART outcomes in the study groups

Variable	Younger than 35 years ($N=114$)	Over 35 years ($N=96$)	P-value
*Dominant follicle count	6 (5–7)	3 (3–4)	< 0.001[a]
*Oocyte M II	4 (3–5)	2 (2–3)	< 0.001[a]
*Embryo Grade A	3 (2–3)	2 (1–2)	< 0.001[a]
*Embryo Grade B	1 (1–2)	1 (0–1)	< 0.001[a]
**Biochemical pregnancy	42 (40%)	24 (22.8%)	0.007[b]
**Clinical pregnancy	29 (27.61%)	14 (13.33%)	0.01[b]
**Live birth	28 (26.66%)	11 (10.47%)	0.003[b]
**Abortion	1 (3.4%)	3 (21.4%)	0.09[c]

*Data presented as median (interquartile range), and **Number (percentage)

ART = assisted reproductive technology

[a]Mann-Whitney, [b]Chi-suqre, [c]Fisher exact test

Table 3 The odds ratio of the studied variables in MII oocytes > 3 by controlling other variables based on the multivariable logistic regression model

*Effective variables on M II oocytes > 3	Crude odds ratio (Confidence Interval 95%)	*p*-value	Adjusted odds ratio (Confidence Interval 95%)	*p*-value
Age group	0.029 (0.01–0.06)	0.000	0.027 (0.01–0.06)	< 0.001
BMI	0.96 (0.90–1.02)	0.23	–	–
AFC	1.16 (0.95–1.42)	0.14	1.30 (0.97–1.73)	0.07
AMH (ng/ml)	1.027 (1.01–1.04)	0.000	1.026 (1.01–1.04)	0.001
E2	1.002 (1.002–1.003)	0.000	–	–
FSH	1.03 (0.94–1.13)	0.48	–	–
LH	1.02 (0.92–1.13)	0.59	–	–
Gonadotropin dosage	0.998 (0.99–0.99)	0.000	–	–
Infertility duration	0.95 (1.01–1.07)	0.68	–	–
Constant	–	–	6.03	0.04

*Logistic regression

Table 4 The odds ratio of the studied variables in clinical pregnancy by controlling other variables based on the multivariable logistic regression model

Effective variables on clinical pregnancy	Crude odds ratio (Confidence Interval 95%)	Significance level (*p*-value)	Adjusted odds ratio (Confidence Interval 95%)	Significance level (*p*-value)
Age group	0.403 (0.19–0.81)	0.01	–	–
BMI	0.927 (.85–1.002)	0.05	–	–
AFC	1.018 (0.80–1.29)	0.88	–	–
AMH (ng/ml)	1.12 (1.07–1.16)	< 0.001	1.121 (1.07–1.16)	< 0.001
E2 (pg/ml)	1.001 (1.000–1.001)	0.02	–	–
FSH (IU/L)	1.003 (0.89–1.12)	0.96	–	–
LH (IU/L)	0.942 (0.83–1.06)	0.34	–	–
Gonadotropin dosage	0.999 (0.99–1.00)	0.05	0.999 (0.99–1.00)	0.05
Infertility duration	0.965 (0.89–1.03)	0.31	–	–
Constant			0.000	< 0.001

*Logistic regression

Table 5 The odds ratio of the studied variables resulting in live birth by controlling other variables based on the multivariable logistic regression model

Effective variables on live birth	Crude odds ratio (Confidence interval 95%)	Significance level (*p*-value)	Adjusted odds ratio (Confidence interval 95%)	Significance level (*p*-value)
Group Ref: < 35 years	0.322 (0.15–0.68)	0.003	–	–
BMI	0.925 (0.85–1.002)	0.05	0.917 (0.82–1.01)	0.09
AFC	1.072 (0.83–1.37)	0.58		
AMH (ng/ml)	1.145 (1.09–1.19)	< 0.001	1.16 (1.10–1.22)	< 0.001
E2 (pg/ml)	1.001 (1.000–1.001)	0.007	–	–
FSH (IU/L)	0.993 (0.88–1.11)	0.90	–	–
LH (IU/L)	0.914 (0.80–1.04)	0.17	–	–
Gonadotropin dosage	0.999 (0.99–1.000)	0.01	0.998 (0.99–0.99)	0.005
Infertility duration	0.969(0.90–1.04)	0.39	–	–
Constant			0.001	0.009

*Logistic regression

regression, BMI, AMH, E2, gonadotropin dosage, and age were entered into the model. According to the backward method, only AMH and gonadotropin dosage remained in the model and AMH had a statistically significant relationship with clinical pregnancy so that by one unit increase in AMH level, the chance of clinical pregnancy under the same

condition of other variables increased by 12% ($p < 0.001$) (Table 4). In univariable logistic regression, the chance of live birth in patients over 35 years was 67.8% less than patients younger than 35 years ($p = 0.003$). By 0.01unit increase in AMH level, the chance of live birth increased by 14.5% ($p < 0.001$). In multivariable logistic regression, AMH and gonadotropin dosage had a statistically significant relationship with live birth rate so that by one-unit increase in AMH level, the chance of live birth under the same condition of other variables increased by 16% ($p < 0.001$). With a one unit increase in gonadotropin dosage, the chance of live birth under the same condition of other variables decreased by 0.2% ($p = 0.005$) (Table 5).

Discussion

The most important cause of POR is decreased ovarian reserve, which is usually age-dependent and the quantity and quality of oocytes decrease as age increases [5]. The effect of age on oocyte quality can be explained by an imbalance in antioxidants and the reaction of oxygen species in the follicular fluid. POR in young women may be due to chemotherapy, radiotherapy, surgery, and endometriosis, but in many cases, the cause is idiopathic [5]. Various studies have evaluated pregnancy outcomes and ovarian response in POR participants, and some of them believed that ovarian reserve assessment factors alone can predict ovarian response [10]. It has been demonstrated that women over 41 years with higher AMH levels experienced lower rates of cancelation of the IVF cycle and poor ovarian function but the live birth rate was not different [11]. The results of the present study showed that although the live birth rate in participants over 35 years is 67.8% less than participants younger than 35 years, under the same condition for AMH and other variables, age had no effect on the live birth rate and the higher live birth rate in participants younger than 35 years can be due to the high AMH level in this group. The clinical pregnancy outcome in participants over 35 years is 59.7% less than participants younger than 35 years, and under the same condition for AMH and other variables, age had no effect on the clinical pregnancy rate. Similar to the live birth rate, the higher pregnancy rate in participants younger than 35 years old can be due to the higher AMH level in this group. Contrary to the results of our study, a study reported that older women had worse quantitative and qualitative outcomes compared with younger women, and they emphasized that age is important when consulting young women with low ovarian reserve [12].

In our study, the total dosage of gonadotropin was higher in participants over 35 years, and the total E2 level was higher in women younger than 35 years, which is in line with the study of Honnma et al., that patients with higher AMH levels required less gonadotropin dosage and a shorter duration of ovarian stimulation [13]. Our results showed that the chance of having more than 3 oocytes in participants over 35 years was 97.1% less than participants younger than 35 years, and age can be effective in this outcome under the same condition for AMH and other variables entered into the regression model. Hence, having more than 3 oocytes depends on both the age (younger than 35 years group) and high AMH level. In our study, the predictive level index of AMH for live birth outcome was statistically significant and equal to 84.2% and 100% for age group younger than 35 years and over 35 years, respectively. The best cut-off point of AMH for successful live birth in participants younger than 35 years was 0.915 ng/ml with 85.7% sensitivity and 83.1% specificity. The best cut-off point for participants over 35 years was 0.995 ng/ml with 100% sensitivity and specificity.

In line with our results, in other study done in women over 40 years old, AMH was strongly associated with the pregnancy rate in ART [14]. It has been reported that ovarian age was an independent factor influencing IVF outcomes and the effect of chronological age was lower [4]. In our study, no association was found between abortion and embryo quality, and aneuploidy was not assessed. In contrary to our results, it was observed higher morphology grade of the embryo, lower abortion, and lower fetal aneuploidy was observed in young participants with POR [15]. However similar to our study, Merhi et al., concluded that an increase in AMH at an older age is associated with an improvement in pregnancy outcomes [16]. Although AMH level is a good indicator of fertility potential, it should be combined with other fertility affecting factors like AFC and ovarian volume.

Limitations

The retrospective nature of the study, the small number of subjects included in the study, and the inability to perform pre-implantation genetic disorder tests for aneuploidy evaluation of embryos were the limitations of the present study.

Conclusion

Decreased ovarian reserve is usually age-dependent which affects pregnancy outcome and live birth rate in IVF cycles. Although the clinical pregnancy and live birth rate in participants over 35 years were lower than participants younger than 35 years in the present study, under the same condition for AMH and other variables, age had no effect

on these parameters. So, it has been concluded that the higher clinical pregnancy and live birth rate in participants younger than 35 years can be due to the high AMH level in this group. However, for further confirmation of these findings, more studies in a larger population are needed.

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Authors' Contributions FH, RK., ZZS; Contributed to conception and design, data collection; NGG, and SH; Drafted the manuscript, which was revised by MM, and MMG; MG; statistical analysis; All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval Ethical approval was obtained from the ethics committee of Guilan University of Medical Sciences (approval id: IR.GUMS.REC.1399.130).

Informed consent Informed consent was taken from all participants.

Human Participants This research involved human participants. Ethical approval was obtained from the Ethics Committee of Guilan University of Medical Sciences (Approval ID: IR.GUMS.REC.1399.130). Informed consent has been obtained from all participants in adherence to the Declaration of Helsinki.

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