CONTROLLED OVARIAN STIMULATION IN ENDOMETRIOSIS PATIENTS CAN BE INDIVIDUALIZED BY ANTI-MÜLLERIAN HORMONE LEVELS

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Abstract

Context. Anti-Mullerian hormone (AMH) assay is becoming the best indicator of successful IVF treatment response to fertility drugs and could be a useful marker of embryo implantation potential. Various protocols are being used for controlled ovarian stimulation (COS), but there is an uncertainty regarding the implementation of the best protocol for endometriosis patients and also little evidence is available concerning the clinical value of AMH levels in endometriosis.

Objective. This study aimed to evaluate the prognostic value of serum AMH levels for pregnancy in COS using GnRH-agonist(GnRH-a) and GnRH-antagonist(GnRH-ant) protocols in endometriosis patients.

Design. This is a cross-sectional study between March 2012 and November 2015.

Subjects and Methods. Data were collected from 249 COS cycles of endometriosis patients, including 129 cycles with GnRH-a and 120 cycles with GnRH-ant. Patients in each group were classified into three subgroups based on their serum AMH levels. The outcomes of ICSI program were evaluated.

Results. The ROC curve analysis showed that embryo and oocyte counts and AMH were equally predictive for pregnancy, as demonstrated by a similar area under the curve (AUC) of 0.69, 0.66 and 0.64, respectively. The sensitivity and specificity for prediction of positive pregnancy were 70.91% and 67.01% for embryo counts, 70.91% and 67.53% for oocyte counts at the cutoff values of 5 and 7, respectively, and 83.64% and 52.58% for AMH levels at the cutoff values of 1.3ng/mL.

Conclusions. This study demonstrates that AMH as a single test has substantial accuracy in the prediction of pregnancy using the GnRH antagonist protocol for patients with endometriosis. In other words, AMH assay prior to ovarian stimulation initiation guides the clinicians to choose the antagonist stimulation protocol for the patients

with two extreme AMH levels. AMH levels can be used to individualize control ovarian stimulation in endometriosis patients.

Key words: Anti-Mullerian hormone, Ovarian induction, Endometriosis, Gonadotropin releasing hormone.

INTRODUCTION

A large number of cycles in assisted reproductive centers are cancelled due to an abnormal response to ovulation induction with gonadotropins, every year (1). Abnormal responses may be observed as poor ovarian response or ovarian hyperstimulation syndrome. Recently, a few factors such as age, third day serum FSH, antral follicle count (AFC) and Anti-Mullerian hormone (AMH) levels have been used to assess the ovarian reserve to choose the appropriate stimulation protocol (2). FSH levels and AFC have intercycle and intracycle variabilities but AMH level remains stable during the menstrual cycles (3-5).

Based on the current studies, AMH assay is becoming the best indicator of response to fertility drugs and could be a useful marker of embryo implantation potential (6-8). AMH, a member of the transforming growth factor- β (TGF- β) superfamily, is secreted by ovarian follicular granulosa cells with maximal expression in late pre-antral and small antral follicles (9, 10).

In some gynecological diseases, baseline levels of the ovarian reserve marker, AMH are reduced. As a consequence, it could be an early marker to identify the depletion of ovarian reserve in women with

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progressive disease processes such as endometriosis. Endometriosis defines as the presence of endometrium-like tissue outside of the uterine cavity, which is a very common gynecological disease and is often associated with numerous painful symptoms, dysmenorrhea and also infertility. Endometriosis presents in 20-40% of subfertile women (11). Besides that, it seems that these patients are poor responders to gonadotropin stimulation (12, 13). To improve ART cycle rate, the ovarian stimulation protocols are optimized by adopting an individualized therapy to control ovarian stimulation (COS). In this way, AMH can be helpful to define a personal specific protocol. Patients with various levels of AMH serum concentrations require different kinds of gonadotropin-releasing hormone (GnRH) analogue (5). Although ART procedures have been successful in infertile women with endometriosis, still there is a doubt about the best controlled ovarian stimulation protocols for these patients (14, 15). Ovarian stimulation with both GnRH antagonist and GnRH agonist protocols are being used in patients with endometriosis. There are no studies to identify the correlation between AMH and pregnancy to COS using GnRH-agonist and GnRH antagonist protocols in endometriosis patients. Therefore, in this study we investigated the prognostic value of serum AMH levels for the rate of the pregnancy in endometriosis patients after the use of COS with GnRH antagonist or GnRH agonist protocols.

METHODS

Study subjects and design

This is a cross-sectional study between March 2012 and November 2015. Data were collected from 249 infertile women with endometriosis who underwent their first *intracytoplasmic sperm injection* (ICSI) cycle in the Assisted Conception Unit, Laleh Hospital, Tehran, Iran. Informed consent was obtained from all participants for access to clinical and laboratory information related to their medical history for clinical research purposes. Endometriosis was diagnosed and confirmed either by laparoscopy or pathological examination according to the revised American Fertility Sterility (16) classification (rAFS). Inclusion criteria were: 1) infertility associated with endometriosis, 2) 22–39 years of age, 3) body mass index (BMI) <27 kg/m², 4) stage III/ IV endometriosis 5) absence of polycystic ovarian syndrome (PCOS) and absence of other endocrine diseases. For obtaining similar samples from patients recruited for the study, only fresh embryo transfers were selected. The patients

whose embryos were completely frozen were excluded from enrollment. Patients with severe male factor infertility, recurrent endometrioma and previous surgical resection of ovarian endometrioma (within 9 months before ovarian stimulation) were also excluded from the study.

Serum AMH levels were measured up to two months before starting the stimulation in all patients. Blood samples for AMH evaluation were taken at any stage of the menstrual cycle. Ansh Labs Ultra-Sensitive anti-Müllerian hormone (AMH) ELISA kit was the method used for detection of AMH serum levels with values presented in concentration of ng/mL (1 ng/mL = 7.14 pmol/L). The lowest detectable level of AMH at 95% probability is 0.02 ng/mL.

Ovarian stimulation

Stimulation of oocyte production was achieved using one of two different protocols (GnRH-a long protocol or GnRH-ant) according to the proper patient counseling and the clinician preferences.

GnRH agonist long protocol

All patients who underwent GnRH agonist long protocol were processed for pituitary down-Regulation on mid-luteal phase (Day 21) of the previous menstrual cycle with 0.1 mg triptorelin (decapeptyl, Ferring Pharmaceuticals, Netherlands) injection. When desensitization was achieved, as evidenced by plasma E2 levels of ≤ 50 pg/mL, the absence of ovarian follicles and endometrial thickness ≤ 6 mm, ovarian stimulation was achieved by injection (on Day 2 or 3 of menstrual period) of recombinant FSH (Gonal-F, Merck Serono, Switzerland) or purified HMG (Menopur, Ferring, Switzerland). When there were at least three follicles of 17 mm, final oocytes maturation and release was effected by withdrawal of GnRH and the injection of 5000-10000 international units (IU) of hCG.

GnRH antagonist protocol

In the GnRH antagonist protocol group, gonadotrophins (rFSH or HMG) were administered on day 2 of menstrual period, with the addition of the GnRH antagonist cetrorelix (Cetrotide, ASTA Medica, Amsterdam, The Netherlands; 0.25 mg/dL, s.c) on day 6 post stimulation. When there were at least three follicles of 17 mm, 5000-10000 IU of hCG were given. In both stimulation regimens, oocyte retrieval took place 36±2 hours after HCG injection for triggering of final follicular maturation.

Evaluation of ICSI

Cumulus-oocytes complexes were denuded. Metaphase II oocytes were injected with ejaculated sperm. Depending on the women's age, the number of embryos available for transfer, and the quality of the embryos, between one and three embryos were selected for transfer on day 3 after oocyte retrieval. Biochemical pregnancy was determined by measuring serum β -hCG level on day 14 post embryo transfer. Also, clinical pregnancy was considered as ultrasonographic visualization of an intrauterine gestational sac with fetal heartbeat. Patients were excluded if they had spontaneous miscarriage before 20 weeks' gestation.

Main outcomes

The primary endpoint of our study was to evaluate the prognostic value of serum AMH levels for pregnancy in endometriosis patients. In the second part of our study, the accuracy and clinical value of serum AMH in the prediction of pregnancy was assessed in ovarian stimulation with long GnRH agonist or GnRH antagonist subgroups in patients suffering from endometriosis.

Statistical analysis

All statistical calculations were performed using the computer program SPSS (Statistical Package for the Social Science; SPSS Inc, Chicago, IL.) software version 21 for windows. A paired t-test was used to compare the continuous variables (age, BMI, basal serum FSH, number of oocytes, number of embryos), and pregnancy rate was compared between treatments using the Chi-square test. Since pregnancy rate may be influenced by multiple factors, further analysis was performed using multivariate logistic regression analysis to determine the independent effect of age, AMH, body mass index (BMI), and basal FSH on the ability to predict pregnancy. Receiver operating characteristics (ROC) curve analysis was used to estimate the predictive accuracy of the variables. The ability of any predictors was compared by calculating

the areas under the ROC curves (ROCAUC) and their 95% confidence intervals (CIs). Areas under the ROC curves were compared using the MEDCALC software package (version 9.20; MedCalc Software, Mariakerke, Belgium). The sensitivity, specificity, positive, and negative predictive values were calculated for selected cutoff levels. $P < 0.05$ was considered statistically significant.

RESULTS

Patients' characteristics

In this study, 249 women who suffered from endometriosis were investigated. Overall, the mean age of included patients was 31.8 ± 4.12 , and the mean of AMH level was 1.89 ± 0.79 . Of samples, 129 women (51.8%) were treated with GnRH agonist long protocol and 120 women (48.2%) received GnRH antagonist protocol. Patients in each group were classified into three subgroups based on their AMH levels: (i) AMH < 1.1 ng/mL, (ii) AMH 1.1 to ≤ 2.8 ng/mL, (iii) AMH > 2.8 ng/mL (the cut off value was according to Tremellen *et al.*, 2005, La Marca *et al.*, 2011(17, 18)). About 37% of participants (93 cases) were at the AMH level less than 1.1 ng/mL, 36% (89 cases) at the AMH level equal or greater than 1.1ng/mL and less than or equal to 2.8 ng/mL, and 26.9% (67 cases) at the AMH level greater than 2.8 ng/mL.

To determine the independent effect of age, FSH, BMI, the count of embryo and oocyte, and AMH levels on the ability to predict pregnancy, multiple logistic regression analysis is used. Data demonstrated that in all endometriosis participants the only significant predictor of positive pregnancy was embryo count (Table 1).

In the next step of the study, subgroup analysis of both protocols was performed. As seen in Table 2, in the AMH <1.1 subgroup, the mean number of oocytes retrieved was significantly higher in the agonist group compared with the antagonist group ($P < 0.036$), but the mean number of the embryos was significantly

Table 1. Predictive value of variables for pregnancy in all endometriosis patients with multiple logistic regression analysis

Variable	Coefficient	Odds ratio	95% confidence interval	P-value
Age	0.08	1.08	0.998-1.18	0.05
AMH				
AMH < 1.1	-0.65	0.52	0.122-2.22	0.37
($1.1 \leq \text{AMH} \leq 2.7$ =Reference)	0.55	1.73	0.642-4.71	0.22
AMH > 2.8				
Oocyte no.	-0.14	0.86	0.682-1.09	0.81
Embryo no.	0.32	1.38	1.032-1.84	0.03
BMI (kg/m ²)	-0.1	0.9	0.810-1.00	0.05
Basal serum FSH(mIU/mL)	0.1	1.1	0.958-1.27	0.17

Table 2. Comparison of patient's characteristics in groups of the GnRH agonist and antagonist protocols in AMH subgroups

AMH category (ng/mL)	*Type of protocol (patients no)	Age (years)	FSH (mIU/mL)	BMI(kg/m ²)	Oocyte no	Embryo no	Transferred embryos no.	Transfer cancelled (no oocyte, no embryo)
AMH <1.1	Agonist (49)	33.5 (3.06)	9.004 (2.57)	23.8 (3.21)	3.04 (1.22)	1.5 (1.3)	1.3 (1.08)	13 (26.53)
	Antagonist (44)	32.4 (4.71)	9.518(2.36)	24.7 (2.5)	2.3 (1.72)	2.2 (1.5)	1.7(1.08)	8 (18.18)
	<i>P</i> -value	0.19	0.319	0.13	0.03	0.02	0.09	
1.1 ≤ AMH ≤2.7	Agonist (46)	32.5 (4.8)	7.17 (2.38)	21.9 (3.73)	8.07 (3.36)	6.1 (2.4)	1.9 (0.61)	0
	Antagonist (43)	31.3 (4.2)	7.68 (2.61)	22.8 (3.61)	6.8 (3.36)	4.9 (2.2)	1.8 (0.66)	0
	<i>P</i> -value	0.22	0.336	0.26	0.08	0.01	0.4	
AMH >2.8	Agonist (34)	30.7 (1.9)	8.17 (1.29)	23.5 (2.6)	11.3 (3.02)	7.8 (3.2)	1.8 (0.5)	0
	Antagonist (33)	29.5 (3.7)	8.14 (2.02)	24.5 (2.59)	13.5 (3.6)	11.7 (2.5)	1.8 (0.4)	0
	<i>P</i> -value	0.08	0.94	0.09	0.01	0.0001	0.8	

*Agonist: GnRH agonist long protocol, Antagonist: GnRH antagonist protocol

All values are expressed in Mean (Standard Deviation) BMI= Body Mass Index. AMH: Anti-Mullerian hormone

Table 3. Comparison of pregnancy in groups of the GnRH agonist and antagonist protocols in AMH subgroups

AMH category (ng/mL)	*Type of Protocol	Clinical pregnancy rate/ET (%)		<i>P</i> -value
		Negative n(%)	Positive n(%)	
AMH <1.1	Agonist	34/36(94.4)	2/36 (5.5)	0.2
	Antagonist	30/36 (83.3)	6/36 (13.6)	
	Total	64/72 (88.88)	8/72 (11.11)	
1.1 ≤ AMH ≤2.7	Agonist	27/46(58.7)	19/46 (41.3)	0.04
	Antagonist	34/43 (79.1)	9/43 (20.9)	
	Total	61/89 (68.5)	28/89 (31.5)	
AMH>2.8	Agonist	28/34(82.4)	6/34 (17.6)	0.06
	Antagonist	20/33(60.6)	13/33 (39.4)	
	Total	48/67 (71.6)	19/67 (28.4)	

*Agonist: GnRH agonist long protocol, Antagonist: GnRH antagonist protocol.

Table 4. Predictive value of variables for pregnancy in patients undergoing GnRH-agonist long protocol by multiple logistic regression analysis

Variable	Coefficient	Odds ratio	95% confidence interval	<i>P</i> -value
Age	0.149	1.161	0.991-1.360	0.06
AMH	0.822	2.276	0.16-32.319	0.54
(1.1 ≤ AMH ≤2.7=Reference)	1.57	4.806	0.999-23.117	0.05
AMH>2.8	-0.48	0.953	0.645-1.408	0.81
Oocyte no.	0.47	1.599	0.984-2.600	0.05
Embryo no.	-0.261	0.770	0.639-0.928	0.006
BMI (kg/m ²)	-0.213	0.808	0.621-1.051	0.11
Basal serum FSH(mIU/mL)				

higher in patients who received GnRH antagonist protocol ($P<0.026$). In the normal AMH stratum ($1.1\leq\text{AMH}\leq2.7$), the mean number of oocytes retrieved was not significantly different between the two groups of protocols ($P<0.088$), while the mean number of embryos was significantly higher in the agonist group ($P<0.014$). In the high AMH stratum, the mean number of oocytes and embryos was significantly greater in the antagonist group ($P<0.01$ and $P<0.0001$, respectively). As shown in Table 3, at the AMH<1.1, biochemical pregnancy was lower in the agonist group compared with the antagonist group, but the difference was not significant. However, at the $1.1\leq\text{AMH}\leq2.7$, there was a significant difference in pregnancy between the agonist and antagonist protocols ($P<0.043$). At the AMH

>2.8, positive pregnancy was significantly higher in the antagonist group compared to the agonist group ($P<0.061$).

Multiple logistic regression analysis of age, BMI, basal FSH, the count of the embryo and oocyte, and AMH levels in each subgroup in both protocols demonstrated that in patients who received GnRH agonist long protocol, age and BMI were significant predictors of positive pregnancy. Table 4 shows the correlation between variables and pregnancy by multiple logistic regression analysis. However, in women who underwent GnRH antagonist protocol, AMH>2.8, AMH<1.1, embryo count, and basal FSH were significant predictors of positive pregnancy (Table 5).

Table 5. Predictive value of variables for pregnancy in patients undergoing GnRH antagonist protocol by multiple logistic regression analysis

Variable	Coefficient	Odds ratio	95% confidence interval	P-value
Age	0.134	1.143	0.994-1.315	0.06
AMH				
AMH <1.1	-6.159	0.002	0.0001-0.077	0.001
(1.1 ≤ AMH ≤ 2.7=Reference)				
AMH >2.8	-4.274	0.014	0.001-0.252	0.004
Oocyte no.	0.466	1.593	0.856-2.966	0.14
Embryo no.	-0.906	.404	0.162-1.009	0.03
BMI (kg/m ²)	-0.012	.988	0.826-1.180	0.89
Basal serum FSH (mIU/mL)	0.382	1.465	1.156-1.857	0.002

Table 6. Comparison of performance of variables for pregnancy by ROC curve analysis

Variables	AUC	SE	95% CI ^b	Cut-off value	Sensitivity (%)	Specificity (%)	+LR*	-LR*
Age	0.539	0.0471	0.475 to 0.602	36	21.82	21.82	2.12	0.87
AMH	0.641	0.0365	0.578 to 0.701	1.3	83.64	52.58	1.76	0.31
FSH	0.511	0.0460	0.447 to 0.575	9.5	32.73	80.93	1.72	0.83
BMI	0.611	0.0433	0.548 to 0.672	20	36.36	82.47	2.07	0.77
Oocyte no.	0.668	0.0377	0.606 to 0.726	7	70.91	67.53	2.18	0.43
Embryo no.	0.699	0.0374	0.638 to 0.755	5	70.91	67.01	2.15	0.43

*Positive and negative likelihood ratio

Figure 1. Comparison of predictive values for positive pregnancy using the ROC curve analysis.

The ROC curve analysis for the predicting factors for positive pregnancy was performed and the results are presented in Table 6. Figure 1 shows the ROC curve for the predicting factors for positive pregnancy. The ROC curve analysis shows that embryo and oocyte counts and AMH were equally predictive for pregnancy, as demonstrated by a similar area under the curve (AUC) of 0.699, 0.66 and 0.64, respectively.

The sensitivity, specificity, positive, and negative likelihood ratio for prediction of positive pregnancy at optimum cut-off levels of variables are shown in Table 6. The sensitivity and specificity for prediction of positive pregnancy were 70.91% and 67.01% for embryo count, 70.91% and 67.53% for oocyte count at the cut-off values of 5 and 7, respectively, and 83.64% and 52.58% for AMH levels at the cut-off values of 1.3 ng/mL.

Subgroup analyses according to the different age groups in all endometriosis patients

Young women aged 29 years or less (<30 years): 26.1% (18/69) had AMH <1.1 (ng/mL); 42% (29/69) had 1.1 ≤ AMH ≤ 2.7 and 31.9% (22/69) of these women had AMH >2.8. Women aged between 30 and 35, 36.7% (47/128) had an AMH level of <1.1, 28.9% (37/128) had 1.1 ≤ AMH ≤ 2.7 and 34.4% (44/128) had AMH >2.8. Of 52 patients aged >35 years: 53% (28/52) had AMH <1.1; 44% (23/52) had 1.1 ≤ AMH ≤ 2.7 and 1.9% (1/52) of these women had AMH >2.8.

DISCUSSION

In this study, we investigated the value of AMH levels as predictor for pregnancy in infertile women with endometriosis. This study demonstrates that AMH as a single test has substantial accuracy in the prediction of pregnancy using the GnRH antagonist protocol for patients with endometriosis. In other words, AMH assay prior to ovarian stimulation initiation can be used as a guide for the clinicians to choose the antagonist stimulation protocol for the patients with two extremes AMH levels. However, the accuracy curves indicate that embryo and oocyte count are a better predictor of achieving pregnancy after oocyte pickup.

Endometriosis is a factor that contributes to subfertility in women for a variety of reasons. Altered folliculogenesis, oxidative stress, multiple hormonal, chemical and immunologic changes may affect ovulation, oocyte and embryo quality, fertilization and

implantation (19-21). The most effective technique to overcome fertilization defects in endometriosis associated infertility is *in vitro* fertilization (IVF)-ICSI (22). Although some studies that compare the rate of embryonic development of oocytes randomly inseminated by ICSI or conventional IVF in endometriosis patients suggest that ICSI may have favorable reproductive outcomes in terms of fertilization and embryo quality compared to conventional IVF and that endometriosis might be an indication for ICSI (23, 24). Therefore, in the last five years, our strategy in patients with endometriosis consists of inseminating oocytes with ICSI only. The rationale was overcoming the issues regarding the factors that may affect oocyte quality associated with endometriosis infertility (25). The options for a patient with endometriosis-associated infertility to achieve pregnancy depend on the age of the patient, sufficient ovarian reserve and the situation of endometriosis (26). Infertile endometriosis patients are not a homogeneous group of women with regards to the outcomes of ICSI. They include women with poor ovarian reserve, who behave like other poor responder patients during ovulation induction cycles and patients with adequate ovarian reserve with good response to stimulation (27).

Today a personalized fertility medicine by GnRH analogue significantly improved IVF clinical outcomes and reduced the prevalence of complications (28). Tailored treatment in IVF is based on the prediction of ovarian response for each individual patient. The starting point is to categorize women based on their ovarian reserve biomarkers such as age, FSH, AFC, inhibin β and AMH who might show a poor, normal or hyper response, and then choose the best ovarian stimulation treatment tailored based on this prediction (1). In addition, Vascular Endothelial Growth Factor serum levels are significantly lower in endometriosis patients (29). Recent studies continue to demonstrate that AMH is a better serum test for ovarian response management than the FSH, AFC, inhibin β , or estradiol and data confirm that assessment of serum AMH concentration may allow to predict both the extremes of ovarian stimulation (30, 31) and tailored stimulation strategies to reduce the clinical risk of ART (6, 32).

Although ART is the first choice for infertility associated with endometriosis, some studies demonstrated that endometriosis has adverse effects on all aspects of the reproductive process and consequently on IVF/ICSI success rates (33, 34). Selection of the best protocol for ovarian stimulation among patients having endometriosis may prevent

these negative impacts (35) but, there is no exact method to distinguish which stimulation protocol is the best for such patient to achieve the highest clinical pregnancy rate (14). In different reports it has not been certainly established whether AMH levels in patients with endometriosis would decrease or increase. Shebl *et al.* (2009) have shown that the mean level of AMH in mild endometriosis patients was comparable to that of healthy women, but serum AMH was decreased in severe endometriosis patients (36), while other studies demonstrated that endometriosis is associated with the reduction of ovarian reserve and AMH levels on day 3, thus serum AMH level is a significant predictor of poor ovarian response in such patients (12, 37). Unlike the studies mentioned above, the study of Rustamov *et al.* (2014) shows serum AMH levels in patients with endometrioma appear to be significantly higher compared to that of healthy women (38). Our data, in the present study, have shown that the patients with endometriosis have different strata of serum AMH levels. On the other hand, the prevalence of severe endometriosis in patients with low AMH level was higher than women in the other AMH categories. According to the other studies, this indicates that the severity of endometriosis is significantly related to the rate of decline of the serum AMH level (32). It has been reported that the degree of ovarian involvement, the presence of endometrioma (uni- or bilateral), surgical resection of endometrioma and the nature of the surgery undertaken (e.g. drainage, diathermy, excision) lead to a significant postoperative decline in serum AMH levels (39, 40). Thus, women with previous endometrioma resection surgery were excluded from this study.

The two protocols, GnRH-a and GnRH-ant, were used for endometriosis patients undergoing COS. Pabuccu *et al.*, in their prospective randomized trial study compared the long GnRH-a protocol to the GnRH-ant in endometriosis (grade I and II) and endometrioma in ICSI cycles, and reported that both protocols are equally effective in such patients (41). In a recent study, Bastu *et al.* showed that the outcomes of IVF in both protocols were similar in endometriosis patients (42).

To the best of our knowledge, there are no studies in literature comparing the outcomes of COS with GnRH-a and GnRH-ant protocols in patients with endometriosis on the basis of AMH strata. Although the mean number of oocytes retrieved was higher in the agonist group compared to the antagonist group, there was a trend for higher pregnancy rates and number of embryos in the antagonist group compared to the

agonist group in low AMH group. In a recent study, Hoseini *et al.* (2014) showed that the expression levels of some genes involved in the cytoplasmic maturity, antiapoptotic process and adenosine triphosphate production were higher in human oocytes of the women in the GnRH-ant protocol *versus* those of the women in the GnRH-a protocol (43). For these reasons, antagonist protocol may have a positive influence on both oocyte and embryo quality; consequently, increase the pregnancy rate. Some studies have shown that this protocol is appropriate for patients with poor response (1, 32) or severe endometriosis because the mechanism of the antagonist drug's actions may be beneficial for a restricted cohort of follicles in such patients (1, 28). The multiple logistic regression analysis of our results also suggests serum AMH level provides prognostic value to expected pregnancy to GnRH-ant stimulation and the use of GnRH-ant as a suitable protocol for endometriosis patients with low and high AMH level.

Both groups had a similar age distribution, but for further analysis, all patients were divided according to age into the following three groups: 22–29, 30–35 and 36–39 years. Then, subgroup analyses were performed according to the different stimulation protocols in the same age-related groups. It is important to emphasize that these results show that there were age-related trends in AMH level and the prevalence of endometriosis patients over the age of 35 was significantly higher in low AMH concentration subgroup. Also, some studies have reported the association between BMI and the risk for certain medical diseases, such as high blood pressure, coronary heart disease and type 2 diabetes. However, interestingly, the literature suggests an inverse correlation between endometriosis and BMI. Divya K. Shah *et al.* in the large prospective cohort study specifically designed to address the relation between body size and endometriosis reported that women with BMI higher than 40, compared to those with low normal BMI (18.5– 22.4), have 39% less susceptibility to have endometriosis (44). Another prospective study on 238 histological diagnosis of patients with endometriosis and 238 matched age and smoking controls, showed a strong association between BMI below 18.5 and deep infiltrating endometriosis (45). In the current study, BMI has reverse effect on pregnancy in all endometriosis patients and specifically in the patients undergoing GnRH-agonist long protocol (Table 1 and Table 4).

The current study was performed with a retrospective analysis, so interventions and allocation of patients in two protocols were not randomized. Based on

the clinician's decision, one of the two standard protocols of long agonist or antagonist was used for ovarian stimulation. Therefore, further prospective controlled trial investigations with more patients and stimulation cycles would have minimized potential limitations and will be more effective to confirm these results. Other limitation of the present study was the involvement of more than one clinician and their preferences for different protocols (GnRH-ant *versus* GnRH-a).

This study demonstrated that there was a trend for higher pregnancy rates with GnRH-ant compared to the GnRH-a protocol in the two extreme ranges of AMH in infertile patients with endometriosis. As an example, we can diagnose women who are at high risk of IVF cycle cancellation due to both poor and excessive ovarian responses, thus stimulation approach can be adjusted to incorporate GnRH antagonists. However, in the normal range of AMH (1.1 to 2.8), long agonist protocol is the best choice for these patients. Therefore, individualized IVF protocols, according to various ranges of AMH, may improve clinical outcomes of IVF/ICSI in patients with endometriosis.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

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