The role of gene polymorphisms and AMH level in prediction of poor ovarian response in Egyptian women undergoing IVF procedure

Tarek M. K. Motawi¹ · Sherine M. Rizk¹ · Nadine W. Maurice¹ ·
Ahmed Mohamed Maged^{2,3} · Ayman N. Raslan² · Ahmed H. Sawaf²

Received: 27 February 2017 / Accepted: 28 July 2017 / Published online: 19 August 2017
© Springer Science+Business Media, LLC 2017

Abstract

Objective

The aim of this study is to assess the role of AMH in prediction of poor ovarian response as well as the relation between *ESR2* (+ 1730G>A) (rs4986938) and *FSHR* p.Thr³⁰⁷Ala (c.919A>G, rs6165) SNPs and the poor ovarian response in Egyptian women undergoing IVF procedure. Discovering the genetic variants associated with ovarian response is an important step towards

individualized pharmacogenetic protocols of ovarian stimulation.

Methods

We performed a prospective study on 216 young women with unexplained infertility. Ovarian stimulation was performed according to the GnRH antagonist protocol with a fixed daily morning dose of human menopausal gonadotrophin (HMG). The estrogen receptor (*ESR2*) (+ 1730G>A) (rs4986938) and FSH receptor p.Thr307Ala (c.919A>G, rs6165) single nucleotide polymorphisms (SNPs) were detected by real-time polymerase chain reaction. Serum FSH, Estradiol (E2) and anti-Müllerian hormone (AMH) levels were measured by enzyme-linked immunosorbent assay (ELISA).

Results

This study revealed that the low AMH level was highly significantly related to the poor ovarian response ($p < 0.001$). Furthermore, the frequency of the *ESR2* (AA) genotype and the *FSHR* (Ala³⁰⁷Ala) genotypes were highly significantly associated with the poor ovarian response ($p < 0.001$).

Conclusion

The AMH level in combination with the *ESR2* and the *FSHR* gene polymorphisms predict the poor ovarian response to COH in Egyptian women.

Trial registration

[ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02640976) Identifier: NCT02640976.

Synopsis

The AMH level in combination with the *ESR2* and the *FSHR* gene polymorphisms predict the poor ovarian response to COH in Egyptian women.

✉ Ahmed Mohamed Maged
prof.ahmedmaged@gmail.com

Tarek M. K. Motawi
tarek.motawi@pharma.cu.edu.eg

Sherine M. Rizk
sherine.abdelaziz@pharma.cu.edu.eg

Nadine W. Maurice
nadine.hanna@pharma.cu.edu.eg

Ayman N. Raslan
ayman_raslan_1@yahoo.com

Ahmed H. Sawaf
hsawaf40@yahoo.com

¹ Department of Biochemistry, Faculty of Pharmacy, Cairo University, Giza, Egypt

² Obstetrics and Gynaecology Department, Cairo University, Giza, Egypt

³ 135 King Faisal Street Haram, Giza 12151, Egypt

Keywords Assisted reproductive technology · In-vitro fertilization · Controlled ovarian hyperstimulation · Follicle-stimulating hormone receptor · Estrogen receptors · Single nucleotide polymorphisms · Anti-Müllerian hormone · Infertility

Introduction

Infertility is the inability to conceive despite of having regular unprotected sexual intercourse for at least 12 months [1]. Worldwide, it affects 15% of couples of reproductive age [2]. In vitro fertilization (IVF) procedures are the reasons behind the occurrence of approximately 2–3% of childbirths in the world's developed countries [2]. The success rate of different assisted reproductive techniques is only about 30% per cycle [3].

IVF is a complicated process that involves multiple stages [4]. In order to reach the level of success necessary, the number of eggs acquired after the hormonal stimulation should be taken into account. The major and vital problem facing the patients known as “poor responders” is the insufficient number of eggs retrieved, resulting in the decrease of oocyte production, cycle cancellation and a significantly lower probability of pregnancy compared to normal responders [5].

Predicting ovarian response before starting hormonal stimulation is the only way to administer an efficient and safe treatment. The most important predictors of the ovarian response to hormonal stimulation are age, biochemical parameters as basal follicle-stimulating hormone (FSH) levels, serum anti-Müllerian hormone (AMH) and morphological characteristics as antral follicular count (AFC).

Both AFC and AMH were positively and strongly correlated [6]. However, there are concerns about the performance of the AMH assay under different conditions regarding storage of samples and handling techniques [7].

Genetic variability is also considered as an important factor. Practices of pharmacogenetics to ovarian response could predict stimulation success and could help in regulating, modifying and designing the doses to suit the treatment [8]. Estrogen receptor genes (ESRs) are of great significance in the ovarian response to exogenous FSH due to the effect of estrogen on follicle growth, maturation and oocyte release. Two subtypes of ERs have been identified in humans, ER α and ER β , encoded by ESR₁ (6q25.1) and ESR₂ (14q23.2) genes, respectively. The genetic availability of ESRs is important for improvements in the diagnosis and treatment of infertility [9]. ESR₂ (+ 1730G>A) (rs4986938) polymorphism inflects the IVF outcome by affecting the number of mature oocytes [4].

The FSH receptor gene is treated as the primary candidate gene in explaining the differences in controlled ovarian hyperstimulation (COH) results. As FSH exerts its stimulatory effect by binding to its receptor (FSHR), the efficiency of the FSH dose given to a patient is greatly related to the success of COH [10]. The FSHR gene is located at chromosome region 2p21, and consists of ten exons and nine introns [11]. Several single nucleotide polymorphisms (SNPs) in the FSHR gene have been identified; however, only minority of them are correlated with

ovarian response to FSH such as p.Thr³⁰⁷Ala (c.919A>G, rs6165) and p.Asn⁶⁸⁰Ser (c.2039A>G, rs6166) [12].

The aim of this study is to assess the role of AMH in prediction of poor ovarian response and the relation between ESR2 (+ 1730G>A) (rs4986938), FSHR p.Thr³⁰⁷Ala (c.919A>G, rs6165) SNPs and the poor response in Egyptian women undergoing IVF procedure.

Materials and methods

From June 2013 to June 2016, 234 couples with unexplained infertility, seeking the first attempt IVF treatment cycle were recruited in AL-Shark Al-Awsat fertility center. Couples fulfilling the following criteria were considered eligible for participation: women at or under the age of 35 years, with normal thyroid-stimulating hormone and prolactin levels, normal ovulatory cycles (25–35 interval days), together with proven patent fallopian tubes at hysterosalpingography or laparoscopy done within 6 months preceding the IVF cycle, absence of abnormal pelvic pathology or congenital anomalies and presence of both ovaries with normal findings as assessed by trans-vaginal ultrasound and laparoscopy. All male partners had a normal semen analysis according to WHO criteria (WHO 2010), done within 6 months preceding the IVF cycle. All participants had a thorough history taken, physical examination and further evaluation in the form of basal FSH, basal E₂ and AMH levels done at the second day of a spontaneous cycle within 3 months preceding the IVF cycle. FSH, E₂ and AMH levels were measured by enzyme-linked immunosorbent assay (ELISA).

The number of women who met our preselected inclusion criteria was 216. We excluded candidates with chronic medical disorders such as diabetes, previous inadequate response to ovulation induction, biochemical or ultrasound evidence of polycystic ovary syndrome, had performed ovarian surgery and cases of endometriosis diagnosed by laparoscopy or suspected by ultrasound or CA-125 assay.

Our research protocol was approved by the research ethics committee for experimental and clinical studies at Faculty of Pharmacy Cairo University, Cairo, Egypt (BC 705) and a written consent was obtained from all participating couples. The authors confirm that all ongoing and related trials for this drug/intervention are registered (ClinicalTrials.gov ID: NCT02640976). Neither the participants nor those administering the interventions were blinded to study condition assignment.

Ovarian stimulation was performed according to the gonadotropin releasing hormone (GnRH) antagonist protocol with a fixed daily morning dose of human menopausal gonadotropin (HMG) [Merional® 75 IU ampoules, IBSA institute] intra-muscular injection starting on cycle day 2. Based on the patient's body mass index (BMI) and hormonal profile, the

daily dose of HMG was adjusted to (225 IU) for participants with AMH levels > 1.5 ng/ml and/or FSH levels ≤ 8 mIU/ml and to (300 IU) for participants with AMH levels < 1.5 ng/ml and/or FSH levels > 8 mIU/ml, doses were increased by 75 IU in cases with BMI > 30 kg/m².

On day 2 of the menstrual cycle, the dose of HMG was commenced for all patients and was maintained for 9–15 days according to each participant individual ovarian response assessed by trans-vaginal ultrasound folliculometry starting on cycle day 7 and continued every other day until the day of human chorionic gonadotropin (hCG) injection. On cycle day 7, the GnRH antagonist Cetorelix 0.25 mg [Cetrotide® 0.25 mg syringes, Merck Serono] was introduced as daily subcutaneous injections and continued till the day of ovulation triggering. Finally, when at least two follicles reached 17 mm in diameter, ovulation was triggered by a single intra-muscular injection of 10,000 IU of hCG [Choriomon® 5000 IU ampoules, IBSA institut]. Thirty-six hours later, oocyte retrieval was performed and was guided by transvaginal ultrasound.

According to our primary outcome, the number of oocytes collected, patients were classified as poor responders (four oocytes or less) and were considered as good responders if they produced five or more oocytes. The good responders group served as the control group.

Peripheral blood was collected from each patient in an EDTA-containing tube. Genomic DNA was extracted from lymphocytes of peripheral blood by fully automated system of QIAcube using QIAamp DNA Blood Mini Kit (250) plus QIAamp DNA Blood Mini Accessory Set B, cat. no. 1043369. Detection of the polymorphisms *ESR2* (+ 1730G>A) (rs4986938) and *FSHR* p.Thr307Ala (c.919A>G, rs6165) SNPs was performed using the TaqMan system by real-time polymerase chain reaction (PCR). Primers and probes were provided as a readymade assay by Applied Biosystems, Life Technologies. cat. no. 4351379. Assays were performed with TaqMan Universal Master Mix with 20 ng of DNA per reaction. PCR conditions were holding stage at 95 °C for 10 min followed by cycling stage 40 cycles; denaturation step at 95 °C for 15 s and annealing and extension step at 60 °C for 1 min.

Data were statistically described in terms of mean and standard deviation, for quantitative data and frequencies and relative frequencies for qualitative data. Hardy-Weinberg (H-W) equilibrium for each polymorphism was tested using the Chi-square test. Comparison of quantitative variables was done using unpaired *t* test when comparing two categories and ANOVA with post hoc test when comparing more than two categories. For comparing categorical data, chi-square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. Genotype and allele frequencies were compared

between the poor responder and the control groups using chi-square tests. Odds ratio (OR) with 95% confidence intervals was calculated. A probability value (*P* value) less than 0.05 was considered statistically significant. All statistical calculations were done using SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 22.

For each genotype, the respective proportions were compared in a Stat view program (SAS institute) using Chi-2 and Fisher tests. The family wise error rate was adjusted with the sequential Bonferroni-Holm procedure.

Results

A total of 216 women, who met our preselected inclusion criteria, were included in this study, 111 were classified as good responders and 105 as poor responders. None of them were lost to follow up and they all were analysed in our results.

There was no significant difference between the two groups regarding age, BMI, duration of infertility, basal E2 and endometrial thickness at triggering but a highly significant higher FSH basal level and lower AMH levels among poor responders when compared with good responders (Table 1).

There was a highly significant difference between the two study groups regarding duration of stimulation, total dose of gonadotropins used, number of oocytes retrieved, number of embryos transferred and clinical pregnancy rate (Table 1).

Basal FSH, the total dose of gonadotropins used and duration of stimulation, were significantly lower in the good responders group. The number of oocytes retrieved, the number of embryos transferred as well as clinical pregnancy rate were significantly lower in the poor responders group (Table 1).

To nullify the effect of number of transferred embryos, we compared good responders who transferred one embryo with those poor responders who transferred one embryo. The clinical pregnancy rate was significantly higher in good responders [17/40 (42.5%)] when compared to poor responders [26/92 (28.3%)] ($P < 0.001$).

The genotype distribution and allelic frequency of both SNPs are summarized on Table 2. Genotype frequencies observed during this study followed Hardy-Weinberg equilibrium.

Studying the *ESR2* gene polymorphism, we observed a significant difference in the frequency of genotypes between the good and poor responder groups; the genotype AA was three-fold higher in the poor responders group than in the good responders group. The wild genotype GG was 1.98-fold higher in the good responders group and allele G was 1.4-fold higher in the good responders group while allele A was 1.6-fold higher in the poor responders group (Table 2).

Table 1 Baseline and cycle characteristics of the study population

	Good responders (<i>n</i> = 111)	Poor responders (<i>n</i> = 105)	<i>P</i> value
Age (years)	28.23 ± 2.93	29.43 ± 3.53	0.157
BMI (kg/m ²)	29.37 ± 3.80	30.73 ± 4.87	0.149
Duration of infertility (years)	6.23 ± 3.58	5.92 ± 3.81	0.312
Basal FSH (mIU/ml)	5.13 ± 0.62	8.82 ± 2.36	< 0.001
Basal E2 (pg/ml)	32.83 ± 6.28	38.13 ± 14.98	0.079
AMH (ng/ml)	2.19 ± 0.31	1.01 ± 0.42	< 0.001
Duration of stimulation (days)	12.65 ± 2.32	14.56 ± 3.88	< 0.001
Total Gonadotropins dose (units)	5418 ± 1081.5	7426.5 ± 1323	< 0.001
No. of oocytes retrieved	10.9 ± 1.92	1.6 ± 0.52	< 0.001
No of MII oocytes	8.7 ± 1.74	1.4 ± 0.51	< 0.001
Endometrial thickness at triggering (mm)	10.20 ± 1.76	9.82 ± 2.42	0.065
Number of embryos transferred	2.1 ± 1.57	1.1 ± 0.43	< 0.001
Clinical pregnancy rate ^a	49/111 (44.14%)	32/105 (30.47%)	< 0.001
Clinical pregnancy rate with one embryo transfer	17/40 (42.5%)	26/92 (28.3%)	< 0.001

Data are presented as mean ± SD

BMI body mass index, FSH follicle-stimulating hormone, E2 estradiol, AMH anti-Mullerian hormone

^a Data are presented as number (percentage).

Regarding the *FSHR* Thr³⁰⁷Ala gene polymorphism, there was a significant difference in the frequency of genotypes between the good and poor responders group, the genotype GG (Ala³⁰⁷Ala) was 2.5-fold higher in the poor responders group than in the good responders group. The wild genotype AA (Thr³⁰⁷Thr) was 2.8-fold higher in the good responders. Allele A was more common in the good responders group. On the other hand, allele G was 1.6-fold higher in the poor responders group (Table 2).

Regarding the association between *ESR2* genotypes and the patients' baseline characteristics, the mean AMH level

and the number of oocytes were significantly higher in the GG and the GA genotypes than in the AA genotype in good responders (Table 3). The mean number of oocytes was significantly lower in the AA genotype than in the GA genotype and in the GG genotype and the mean AMH level was significantly higher in the GG genotype than in the GA genotype and in the AA genotype in poor responders (Table 3).

Studying the association between the *FSHR* p.Thr³⁰⁷Ala genotypes and the patients' baseline characteristics, the mean age was significantly higher in the AA genotype than in the

Table 2 The genotype distribution and allelic frequency of the three single nucleotide polymorphisms

		Good responders (111)		Poor responders (105)		<i>P</i> value	OR (95% CI)
		Count	%	Count	%		
<i>ESR2</i> (+ 1730G>A)	A/A	11	9.9	32	30.5		Reference
	G/G	44	39.6	21	20.0	< 0.001*	0.164 (0.069–0.388)
	A/G	56	50.5	52	49.5	0.041*	0.514 (0.270–0.977)
	Allele A	78	35.1	116	55.2		Reference
	Allele G	144	64.9	94	44.8	< 0.001*	0.439 (0.298–0.646)
<i>FSHR</i> p.Thr ³⁰⁷ Ala (A>G)	A/A	41	36.9	14	13.3		Reference
	G/G	18	16.2	42	40.0	0.005*	0.362 (0.176–0.745)
	A/G	52	46.8	49	46.7	< 0.001*	0.146 (0.064–0.332)
	Allele A	134	60.4	77	36.7		Reference
	Allele G	88	39.6	133	63.3	< 0.001*	0.380 (0.258–0.561)

ESR2, estrogen receptor 2 gene, *FSHR* follicle-stimulating hormone receptor, OR odd ratio, CI confidence-interval

Data are presented as number (percentage)

**P* < 0.05

Table 3 Association between the *ESR2* (+ 1730 G>A) (rs4986938) genotypes and the patients' baseline characteristics

	<i>ESR2</i> (+ 1730 G>A)(rs4986938)			<i>P</i> value	<i>ESR2</i> (+ 1730 G>A)(rs4986938)			<i>P</i> value
	Good responders				Poor responders			
	<i>G/G</i>	<i>G/A</i>	<i>A/A</i>		<i>G/G</i>	<i>G/A</i>	<i>A/A</i>	
Age (years)	28.17 ± 2.92	28.47 ± 3.29	27.33 ± 0.58	0.835	30.33 ± 2.50	29.27 ± 4.53	29.11 ± 2.15	0.791
BMI (kg/m ²)	28.47 ± 3.55	29.75 ± 3.98	31.13 ± 4.36	0.496	30.13 ± 5.39	32.41 ± 4.62	31.68 ± 5.26	0.642
Basal FSH (mIU/ml)	4.98 ± 0.59	5.18 ± 0.67	5.5 ± 0.36	0.411	9.07 ± 3.04	8.25 ± 2.27	9.61 ± 2.01	0.393
Basal E2 (pg/ml)	35.33 ± 5.43	31.27 ± 6.76	30.67 ± 5.13	0.207	37.00 ± 10.60	39.80 ± 18.79	36.11 ± 10.73	0.835
AMH (ng/ml)	2.57 ± 0.47	2.46 ± 0.38	1.87 ± 0.21	0.045*	1.85 ± 0.69	1.08 ± 0.41	0.99 ± 0.74	0.018*
No. of oocytes	12.33 ± 2.90	11.80 ± 2.15	8.00 ± 1.00	0.032*	2.67 ± 0.52	2.20 ± 0.77	0.89 ± 0.93	< 0.001*

ESR2, estrogen receptor 2 gene, *BMI* body mass index, *E2* estradiol, *AMH* anti-Mullerian hormone, *SD* standard deviation

Data are presented as mean ± SD

**P* < 0.05

AG and GG genotype. The AMH level and the number of oocytes was significantly higher in the AA genotype than the AG and the GG genotypes in good responders (Table 4).

The mean age was significantly higher in the AA genotype than in the AG and GG genotype and the mean number of oocytes was significantly lower in the AG genotype than in the AA and GG genotype in poor responders group (Table 4).

Our results showed in Table 4, in the poor responders group of *FSHR* gene polymorphism, that there was no significant difference in AMH level between the three genotypes, although there was a significant difference in number of oocytes. Therefore, the genetic testing could be a better predictor of poor ovarian response, even though it is of higher cost than other markers, since it offers a possibility to proceed with a personalized approach in IVF, which reduces the incidences of treatment cancellations and complications and lowers the number of treatment cycles required to achieve pregnancy.

In a combined analysis, we observed that the most common diplotype was (A/G + G/A) (24.3%) in the good responders group and (A/G + A/A) (26.7%) in the poor responders group, as shown in Table 5.

Discussion

To the best of our knowledge, in Egypt, we are the first to demonstrate that the *ESR2* (+ 1730G>A) (rs4986938) and *FSHR* p.Thr³⁰⁷Ala (c.919A>G, rs6165) SNPs were associated with poor ovarian response to FSH stimulation in Egyptian women undergoing IVF. It is also of importance to note that, although there are studies that associate these SNPs with different disorders, there are also studies which do not provide such evidence, even for the same disease since the haplotype structure and the linkage disequilibrium vary between different populations.

Table 4 Association between the *FSHR* genotypes and the patients' characteristics

	<i>FSHR</i> p. <i>Thr</i> ³⁰⁷ <i>Ala</i> (c.919A>G, rs6165)			<i>P</i> value	<i>FSHR</i> p. <i>Thr</i> ³⁰⁷ <i>Ala</i> (c.919A>G, rs6165)			<i>P</i> value
	Good responders				Poor responders			
	<i>A/A</i>	<i>A/G</i>	<i>G/G</i>		<i>A/A</i>	<i>A/G</i>	<i>G/G</i>	
Age (years)	30.45 ± 3.08	26.71 ± 1.54	27.60 ± 2.97	0.003*	33.50 ± 2.65	29.71 ± 2.43	27.75 ± 3.84	0.012*
BMI (kg/m ²)	31.07 ± 4.20	28.55 ± 3.64	27.94 ± 2.17	0.171	34.22 ± 6.81	32.53 ± 4.39	29.98 ± 4.55	0.231
Basal FSH (mIU/ml)	5.04 ± 0.64	5.36 ± 0.57	4.72 ± 0.51	0.112	8.23 ± 0.73	9.04 ± 2.46	8.35 ± 2.69	0.835
Basal E2 (pg/ml)	35.09 ± 6.28	31.57 ± 6.43	31.20 ± 4.92	0.316	44.75 ± 15.06	38.14 ± 10.30	35.92 ± 19.57	0.610
AMH (ng/ml)	2.70 ± 0.41	2.35 ± 0.46	2.18 ± 0.16	0.039*	1.45 ± 0.35	0.99 ± 0.67	1.38 ± 0.67	0.245
No. of oocytes	13.00 ± 2.65	11.07 ± 2.73	10.20 ± 0.84	0.078	2.50 ± 0.58	1.29 ± 1.14	2.42 ± 0.51	0.005*

FSHR, follicle-stimulating hormone receptor, *BMI* body mass index, *E2* estradiol, *AMH* anti-Mullerian hormone, *SD* standard deviation

Data are presented as mean ± SD

**P* < 0.05

Table 5 Combined analysis of *FSHR* p. Thr³⁰⁷Ala (c.919A>G, rs6165) and *ESR2* (+ 1730G>A)(rs4986938) gene polymorphisms

Diplotype <i>FSHR</i> p. Thr307Ala <i>A/G</i> + <i>ESR2</i> (+ 1730G>A)	HAPLO1	HAPLO2	Group				<i>P</i> value
			Good responders (111)		Poor responders (105)		
			Count	%	Count	%	
<i>G/G</i> + <i>A/A</i>	<i>GA</i>	<i>GA</i>	0	0.0	2	1.9	0.072
<i>G/G</i> + <i>G/A</i>	<i>GG</i>	<i>GA</i>	8	7.2	27	25.7	< 0.001
<i>G/G</i> + <i>G/G</i>	<i>GG</i>	<i>GG</i>	10	9.01	13	12.38	0.045
<i>A/G</i> + <i>G/A</i>	<i>AG</i>	<i>GA</i>	27	24.3	17	16.2	0.050
<i>A/G</i> + <i>G/G</i>	<i>AG</i>	<i>GG</i>	17	15.32	4	3.8	< 0.001
<i>A/A</i> + <i>G/G</i>	<i>AG</i>	<i>AG</i>	17	15.32	4	3.8	< 0.001
<i>A/G</i> + <i>A/A</i>	<i>AA</i>	<i>GA</i>	8	7.2	28	26.7	< 0.001
<i>A/A</i> + <i>A/A</i>	<i>AA</i>	<i>AA</i>	3	2.7	2	1.9	0.137
<i>A/A</i> + <i>G/A</i>	<i>AG</i>	<i>AA</i>	21	18.9	8	7.6	< 0.001

Our clinical study observed that the AMH level was a good predictor of poor ovarian response. The mean AMH level was significantly higher in the good responders group than in the poor responders group. There was also a significant difference between the mean AMH level and the genotypes of the *ESR2* SNP in both groups: good responders and poor responders, being significantly lower in the AA genotype. Furthermore, in the good responders group, the AA genotype was significantly associated with higher AMH level in *FSHR* SNP. However, no difference was observed between the mean AMH level and the genotypes of the *FSHR* SNP in the poor responders group.

Our results were in agreement with Gleicher et al. [13]. AMH has been recognized recently in a meta-analysis as a good predictor of poor ovarian response to ovarian stimulation [14]. Furthermore, Vaze-Parab et al. in 2014 illustrated that the low serum AMH levels and the poor ovarian response in IVF cycles are highly interconnected (AMH value in poor responders was 1.20 ng/ml and in good responders it was 3.38 ng/ml and the difference was statistically significant ($P < 0.05$)). It was also correlated with high baseline FSH levels, low AFC, low pregnancy rates and high cycle cancellation rate [15].

Apart from these parameters, another aspect for determining the ovarian response in COH and IVF is the genetic variability by which changes in the human genome could occur that will change cellular and tissue effects of FSH, leading to a change in COH response [10].

The present study demonstrated that the AA genotype of the *ESR2* (+ 1730G>A) (rs4986938) polymorphism was threefold higher in the poor responders group than in the good responders group, while the GA genotype was significantly lower in the poor responders group. Therefore, the presence of allele A increased the frequency of poor ovarian response. On the other hand, the

genotype GG was twofold higher in the good responders group. Moreover, the poor responders had significantly higher basal FSH, required higher doses of FSH for COH, and were associated with lower oocyte yield; also, the AA genotype was significantly associated with lower oocyte yield compared with the other genotypes in both groups.

Our findings were in agreement with Boudjenah et al. who published a study in 2012 performed on 427 women and included 13 SNPs that reportedly influence the outcome of IVF. They have demonstrated that women who were homozygous for the G allele had a greater oocyte yield than women who were heterozygous for the A allele (8.1 ± 4.2 vs. 7.2 ± 4.0 , respectively; $P = 0.0017$) or group of women who were homozygous for the A allele (7.2 ± 3.9 ; $P = 0.0314$). Moreover, they observed that in order to achieve a similar oocyte count, higher FSH doses were given for women who were homozygous for the A allele than women who were homozygous or heterozygous for the G allele. Their results implement that the IVF outcome is modulated by the *ESR2* (+ 1730G>A) gene polymorphism by affecting the number of mature oocytes [4]. Similarly, De Mattos et al. reported a statistical relation between the GG genotype and better COH outcome [9].

Nowadays, there appears to be only two polymorphisms with positive reliable predictive value that are suitable for assessing the required rFSH dosage in COH in clinical tests, which are *FSHR* Thr³⁰⁷Ala and Asn⁶⁸⁰Ser polymorphisms [16].

Our study showed the relation between *FSHR* Thr³⁰⁷Ala polymorphism and ovarian response to COH. We observed that the GG (Ala³⁰⁷Ala) was significantly associated with higher frequency of poor ovarian response; it was threefold higher in the poor responders group; however, the genotype AG was significantly decreased in the poor responders group and the genotype AA (Thr³⁰⁷Thr) was 2.8-fold higher in the

good responders group. Therefore, the presence of Allele G significantly increased the frequency of the poor ovarian response.

Our results were in accordance with Rod et al. who noticed that position 307 of the *FSHR* SNP was more representative than position 680 of SNP. Seventy women from Caen (France) and Białystok (Poland) undergoing IVF treatment participated in his study. The oocyte yield was significantly different ($P < 0.001$) between the good responders and the poor responders (11.46 ± 3.5 and 2.67 ± 1.2 respectively). A significant difference was reported in the frequency of genotypes between the good responders and poor responders, with a higher frequency of the genotype combination Ala³⁰⁷Ala/Ser⁶⁸⁰Ser in the poor responders group. This difference was greater for variant 307 ($P = 0.012$), for which the Ala³⁰⁷Ala genotype was three times as frequent in poor responders as in good responders. As a result, the Ala³⁰⁷Ala genotype increased the risk of poor ovarian response to FSH stimulation [12].

They were also in agreement with Achyuta et al. who confirmed that women with the Ala³⁰⁷ variant were associated with reduced COH outcomes as well as lower pregnancy rates. These women had higher basal FSH serum concentrations and required higher doses of FSH for COH [15].

One of the interesting finding in our study was the significantly higher age within AA genotype when compared to AG and GG genotypes among both good and poor responders. The explanation of that is not known and never observed by previous researchers. However, that could be related to different fertility potential among different genomic expression and consequently different age of presentation for infertility and IVF treatment.

Unexpectedly, reports arose of which the association of the Ala³⁰⁷ allele with high response rather than low response emerged opposes the general findings [4, 16].

Our study is limited by a non-randomized clinical trial according to good and poor responders in order not to decrease the possibility of pregnancy rate for poor responders if accidentally randomized into the lower dose protocol of the good responders. Furthermore, not reporting the quality of follicles and/or oocytes is also a limitation to our study.

The clinical implications of the previous findings are highly important; for the final goal, in order to predict the ovarian response, the genetic markers can be considered before the ovarian stimulation. An important step ahead could be expressed in applying this for not only identifying the poor responders' patients as well as modifying the amount of FSH used in IVF protocols according to their genetic background but also, for identifying the most suitable management strategy, developing the chances of a strong outcome and sidestepping the obstacles related to the FSH stimulation. However, its routine use is limited by its costs.

Authors' role T.K.M., S.M.R., and N.W.M. conceived study design. A.M.M., A.N.R., and A.H.S. gathered the samples and performed the IVF/ICSI procedure. T.K.M., S.M.R., and N.W.M. performed the DNA extraction and real time PCR, performed statistical analysis, interpreted the results, and wrote the manuscript. A.M.M., A.N.R., and A.H.S. assisted in writing the manuscript. All authors had final approval of the submitted version.

Compliance with ethical standards

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

Funding There were not any funding sources.

References

- Lledo B, Ortiz JA, Llacer J, Bernabeu R. Pharmacogenetics of ovarian response. *Pharmacogenomics*. 2014;15:885–93.
- Gearhart J, Coutifaris C. In vitro fertilization, the Nobel Prize, and human embryonic stem cells. *Cell Stem Cell*. 2011;8:12–5.
- Macklon NS, Stouffer RL, Giudice LC, Fauser BC. The science behind 25 years of ovarian stimulation for in vitro fertilization. *Endocr Rev*. 2006;27:170–207.
- Boudjenah R, Molina-Gomes D, Torre A, Bergere M, Bailly M, Boitrelle F, et al. Genetic polymorphisms influence the ovarian response to rFSH stimulation in patients undergoing *in Vitro* fertilization programs with ICSI. *PLoS One*. 2012;7:1–16.
- Ubaldi F, Vaiarelli A, D'Anna R, Rienza L. Management of Poor Responders in IVF: is there anything new? *Bio Med Research International*. 2014;2014:1–10.
- Keane K, Cruzat VF, Wagle S, Chaudhary N, Newsholme P, Yovich J. Specific ranges of anti-Müllerian hormone and antral follicle count correlate to provide a prognostic indicator for IVF outcome. *Reprod Biol*. 2017;17(1):51–9. doi:10.1016/j.repbio.2016.12.002.
- Broer SL, Broekmans FJ, Laven JS, Fauser BC. Anti-Müllerian hormone: ovarian reserve testing and its potential clinical implications. *Hum Reprod Update*. 2014;20(5):688–701. doi:10.1093/humupd/dmu020.
- Lledo B, Guerrero J, Turienzo A, Ortiz JA, Morales R, Ten J, et al. Effect of follicle-stimulating hormone receptor N680S polymorphism on the efficacy of follicle-stimulating hormone stimulation on donor ovarian response. *Pharmacogenet Genomics*. 2013;23:262–8.
- De Mattos CS, Trevisan CM, Peluso C, Adami F, Cordts EB, Christofolini DM, et al. ESR1 and ESR2 gene polymorphisms are associated with human reproduction outcomes in Brazilian women. *Journal of Ovarian Research*. 2014;7:1–9.
- Altmäe S, Hovatta O, Stravreus-Evers A, Salumets A. Genetic predictors of controlled ovarian hyperstimulation: where do we stand today? *Hum Reprod Update*. 2011;17(6):813–28.
- Ochsenkühn R, von Schönfeldt SM. FSH receptor polymorphisms and ovarian function. *Med Reprod Gynecol Endocrinol*. 2009;11:265–70.
- Rod A, Jarzabek K, Wolczynski S, Benhaim A, Reznik Y, Denoual-Ziad C, et al. ESR1 and FSHR gene polymorphisms influence ovarian response to FSH in poor responder women with normal FSH levels. *Endocrinol Metab Syndr*. 2014;3:1–5.

13. Gleicher N, Weghofer A, Barad DH. Discordances between follicle stimulating hormone (FSH) and anti-Müllerian hormone (AMH) in female infertility. *Reprod Biol Endocrinol*. 2010;8:64.
14. Broer SL, D'olleman M, Disseldorp JV, Broeze KA, Opmeer BC, PMM B, et al. Prediction of an excessive response in in vitro fertilization from patient characteristics and ovarian reserve tests and comparison in subgroups: an individual patient data meta-analysis. *FertilSteril*. 2013;100:420–9.
15. Vaze-Parab A, Bhatt P, Parihar LM, Baldawa P. Anti-mullerian hormone, follicular phase follicle stimulating hormone and antral follicular count as predictors of ovarian response in assisted reproductive technique cycles. *IVF Lite*. 2014;1:32–5.
16. Laan M, Grigorova M, Huhtaniemi IT. Pharmacogenetics of FSH action. *Curr Opin Endocrinol Diabetes Obes*. 2012;19:220–7.