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Polymorphism FSHR (-29G/A) as a genetic agent together with ESRI (XbaIG/A) in women with poor response to controlled ovarian hyperstimulation

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Abstract.
BACKGROUND: One of the major problems in IVF is the poor response of the ovary to gonadotropins. *ESRI* and *FSHR* are two effective genes on controlled ovarian hyperstimulation (COH).
OBJECTIVES: Evaluating the correlation of alleles and genotypes of polymorphism (-29G/A) located in the FSH receptor gene and polymorphism (XbaI G/A) located in Estrogen receptor genes with the ovary's response would help to anticipate the results of ovulation in IVF cycles.
METHODS: In the present study, two hundred (200) blood samples were taken from infertile women aged 20 to 39 who were under IVF therapy. After DNA extraction from the samples, real-time PCR was performed using a specific probe-primer.
RESULTS: Statistical analysis revealed that the frequency of alleles and genotypes of polymorphisms (-29G/A) and (XbaI A/G) in women with normal to poor response did not have significant correlation.

Keywords: Polymorphism, *FSHR* gene, *ESR1* gene, IVF, infertility

1. Introduction

Infertility is one of the major problems amongst couples worldwide [1]. It is described as unsuccessful attempts to become pregnant after having regular sexual intercourse without using any contraceptive. There are different reasons for infertility; however, 15% of infertile couples have idiopathic infertility [2]. Approximately 10 to 15% of couples worldwide are infertile [3]. There are genetic and non-genetic factors responsible for the onset of infertility. Aging leads to reduction in the ovarian reserve and this can further result to infertility. In women, fertility begins to reduce after age 27 and it speeds up after age 35 [4,5]. Sin-

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Table 1
Clinical factors of FSH and LH

Characteristics	Normal ($n = 105$) mean $\pm$ std.deviation	Poor responder ($n = 97$) mean $\pm$ std.deviation	p-value
Age	32.11 ± 4.076	34.40 ± 4.053	< 0.0001*
LH[4]	4.92 ± 2.33	5.7 ± 3.76	0.78
FSH[5]	5.92 ± 2.212	5.51 ± 2.705	0.233
AMH[6]	2.569 ± 1.472	1.535 ± 1.535	< 0.0001*

LH[4]: Luteinizing hormone, FSH[5]: Follicle stimulating hormone, AMH[6]: Anti mullerian hormone ($p < 0.05$).

Table 2
Clinical factors of the poor responder in comparison with the normal group

Characteristics	Normal ($n = 105$) mean $\pm$ std.deviation	Poor responder ($n = 97$) mean $\pm$ std.deviation	p-value
Total oocyte	12.60 ± 4.215	5.05 ± 2.808	< 0.0001*
MI[1]	1.14 ± 1.704	0.63 ± 0.939	0.008*
MII[2]	9.89 ± 3.205	3.18 ± 4.22	< 0.0001*
GV[3]	1.41 ± 2.083	1.13 ± 1.929	0.338
Embryo	7.94 ± 2.732	2.94 ± 1.606	< 0.0001*

MI[1]: MetaphaseI, MII[2]: MetaphaseII, GV[3]: Germinalvesicle ($p < 0.05$).

gle nucleotide alteration in fertility related genes could initiate infertility [6].

Follicle stimulating hormone (FSH) plays different roles such as stimulating proliferation and differentiation of granulose cells, growth and maturation of follicles, estrogen production, making LH receptors on dominant follicles surface and Inhibin synthesis [7]. In women, estrogen plays the most important role in ovulation cycles [8,9].

Specific receptors are mandatory for regulating the physiological function of the female genital organ through different hormones. One of such receptors is the FSH receptor. It has been proven that the physiological function of FSH is related to the function of its receptor (FSHR), expressed by granulosa cells. *FSHR* gene is located on Chromosome 2 in region 2p21 and contains 10 exons [10].

Two sub-species of estrogen receptors exist in humans: *ESR* alpha [11] and *ESR* beta [12] encoded by *ESR1* and *ESR2* genes respectively. The *ESR1* gene including 140 kb is located on 6q25.1 and contains 8 exons that have two single nucleotide polymorphisms, Pvull and Xbal. These polymorphisms are located on intron1 [13]. The *FSH* receptor gene is the most studied factor related to controlled ovarian hyperstimulation.

2. Materials and methods

This study was cross-sectional and the formula was used was $n = z^2 p(1-p)/d^2$ that $p = 15.4\%$, $z = 5\%$, $d = 5\%$. In the present study, two hundred (200) infertile women from the age 20 to 39, who were under IVF therapy in May, 2014 till May, 2015 at Laleh, Taleghani and Erfan hospitals were chosen. Samples were taken from patients having weak response to IVF (5 ovules) and patients having strong response to IVF (5 to 20 ovules). Medical records regarding the chosen samples were investigated to make sure there are no problems such as polycystic ovary and endometriosis. The FSH and AMH levels were measured on the 3rd day of the period. Histrosalpenography was also done in order to check for any possible anatomical problems. protocol was used the same for all patient. the infertility factor was feminine and patient after one course IVF treatment had poor response to hyper ovarian stimulation (ovules < 5).

For genetic testing on the day of receiving the ovule, 5CC of the blood samples were taken in tubes containing EDTA prepared by Rabet Amin International Company. Thereafter, the blood samples were frozen at -80°C and on the test day, DNA was extracted using Gene All DNA extraction Kit, (KOREA Cat. No: 106–152). The extracted DNA was kept in -20°C for evaluation of polymorphism. The spectrophotometer Nano drop was manipulated for quantity of density (Bio Intellectual brand).

TaqMan Probe Realtime PCR was performed after DNA extraction. This step needed a proper primer-probe for each polymorphism and was ordered from Applied Biosystems California. One of the probes ordered for polymorphism rs1394205 (Cat No: 426553-10) was related to the *FSH* receptor gene and another polymorphism rs9340799 (Cat No: 3163591-10) was related to the *ESR1* gene, density of each prob-primer was 40x that was diluted with TE buffer solution. Each tube contained 2 ul DNA, 4 ul Master Mix, 13 ul dis-

Table 3
Hardy-Weinberg equilibrium stated that genotype frequencies in this population

Polymorphism	Frequency genotypes poor ($n = 97$)			Frequency genotypes normal ($n = 105$)			X^2	p-value
	AA	GA	GG	AA[3]	GA[2]	GG[1]		
rs1394205	10	43	44	16	37	52	2.188	0.335

GG[1]: Homozygot wildtype, GA[2]: Heterozygot, AA[3]: Homozygote mutant.

Table 4
Hardy-Weinberg equilibrium stated that genotype frequencies in this population

Polymorphism	Frequency genotypes poor ($n = 94$)			Frequency genotypes normal ($n = 92$)			X^2	p-value
	GG	AG	AA	GG[3]	AG[2]	AA[1]		
rs9340799	19	52	23	18	40	34	3.694	0.158

AA[1]: Homozygotwildtype, AG[2]: Heterozygot, GG[3]: Homozygote mutant.

Table 5
Frequency of rs1394205 genotype in poor responders and normal group

rs1394205 G > A	SNP association with response status ($N = 202$)				
Model	Genotype	Poor $N = 97$	Normal $N = 105$	OR (95% CI)	p-value
Codominant	G/G	44 (45.4%)	52 (49.5%)	1 (reference)	0.338
	G/A	43 (44.3%)	37 (35.2%)	1.373 (0.757–2.491)	
	A/A	10 (10.3%)	16 (15.2%)	0.739 (0.304–1.792)	
Dominant	G/G	44 (45.4%)	52 (49.5%)	1 (reference)	0.554
	G/A-AA	53 (54.6%)	53 (50.4%)	0.92 (0.698–1.213)	
Recessive	GA/GG	87 (89.7%)	89 (84.7%)	1 (reference)	0.299
	A/A	10 (10.3%)	16 (15.2%)	1.564 (0.673–3.636)	
Overdominant	G/G-A/A	54 (55.7%)	68 (64.7%)	1 (reference)	0.188
	G/A	43 (44.3%)	37 (35.2%)	1.463 (0.831–2.578)	
Allele frequency G		131 (0.6)	141 (0.6)	1 (reference)	0.9
Allele frequency A		63 (0.4)	69 (0.4)	0.13	

tilled water and 1 ul probe-primer. the realtime PCR machine was LightCycler 96 Roche.

2.1. Statistical analysis

SPSS (version 16) software was used for statistical analysis. T-test and regression logistic were used for statistical analysis. Chi-squared analysis was used to determine whether the genotype distribution at both the polymorphisms conformed to Hardy-Weinberg equilibrium. ($P \leqslant 0.05$) was considered statistically significant.

3. Results

Tables 1 and 2 show the result of the comparison between the general clinical characteristics of a poor responder and good responder with manipulated T-test. In the studied population, from 202 infertile women under IVF therapy, 97 poor responders and 105 good responders were identified.

Table 1 shows age and AMH had significant correlation between poor responder and good responder.

Table 2 shows total oocyte, MI, MII and embryo numbers had significand correlation between poor responder and good responder ($p < 0.05$).

Tables 3 and 4 show the population as allele and genotype frequencies were in Hardy-Weinberg equilibrium ($p < 0.05$).

Tables 5 and 6 show the result of comparison between all type genotypes of the poor responder and good responder had no significant correlation ($p < 0.05$).

4. Discussion

IVF is considered as the best cure for multifactorial infertile couples. The poor response of the ovary to external gonadotropins, as one of the IVF problems that obstructs the therapy process, causes decreased oocytes and infertility.

The aim of controlled ovarian stimulation is achieving multiple follicles and mature oocytes. Poor response to ovarian stimulation usually involves reduced follicle response and oocytes after COH. Incoming re-

Table 6
Frequency of ESR1 gene polymorphism in poor responders and normal group

rs9340799 A > G	SNP association with response status ($N = 186$)				
Model	Genotype	Poor $N = 94$	Normal $N = 92$	OR (95% CI)	p-value
Codominant	A/A	23 (24.5%)	34 (37%)	1 (reference)	0.883
	A/G	52 (55.3%)	40 (43.5%)	1.922 (0.983–3.758)	
	G/G	19 (20.2%)	18 (19.6%)	1.56 (0.678–3.593)	
Dominant	A/A	23 (24.5%)	34 (37%)	1 (reference)	0.066
	A/G-G/G	71 (75.5%)	58 (63.1%)	0.743 (0.542–1.020)	
Recessive	A/G-A/A	75 (79.8%)	74 (80.5%)	1 (reference)	0.912
	G/G	23 (24.5%)	18 (19.6%)	0.98 (0.684–1.405)	
Overdominant	A/A-G/G	42 (44.7%)	52 (56.6%)	1 (reference)	0.107
	A/G	52 (55.3%)	40 (43.5%)	1.610 (0.902–2.872)	
Allele frequency A		72 (0.5)	88 (0.6)	1 (reference)	0.903
Allele frequency G		64 (0.5)	56 (0.4)	0.725	

sults of studies conducted in the last two decades on infertility, has revealed the role of genetic variants of the *FSHR* gene in hormone determinations and organ response for the IVF [7].

In the present study, polymorphism rs1394205 located on *FSHR* gene and polymorphism rs9340799 located on *ESR1* gene was not effective in poor responder ovary in Iranian population. The correlation of genotypes in groups, good responder and poor responder was evaluated and it indicated that there were no effects on poor responder ovary. AMH hormone level and age increase are important factors for poor responder ovaries.

For this polymorphism, G allele is considered as an ancestral allele, GG genotype as ancestral homozygote, AG genotype as heterozygote and AA genotype as mutant homozygote. In 2005, a study conducted by Wunsch et al. in Germany and Indonesia showed that -29 positions of the *FSHR* genes SNP, in spite of higher prevalence than other SNPs in the promoter of the gene, had no association with ovarian response confirming the achieved results [14].

In 2009, a study was conducted by Ayvaz et al. on Turkish population, results showed that rs2234693 and rs9340799 polymorphisms are associated with infertility and low fetus number and low quality of fetus and ovary maturation [15]. The findings in this study revealed that A allele of rs9340799 was considered as an ancestral allele and each genotype of ancestral AA homozygotes, AG heterozygotes and GG mutant homozygotes were not observed to be associated with poor ovarian response in the two under studied group and this had no effect on the ovarian response.

Results of the study performed by Anagnostou et al. on Athenian population, showed that *FSHR* (ser680ser) and TC/SA, *ESR1* (rs9340799) compound heterozygote genotypes have the highest rate of pregnancy between poor responder women and CC/AA genotype are associated with the worst ovarian response profile, but none of the genotypes showed association with ovarian poor response themselves [11].

Desai et al. conducted a study on the Indian population to determine the relationship between genotypes of the -29 position of the *FSHR* gene and 680 position of the FSH receptor gene with considering the clinical parameters of poor ovarian responders [16].

The endocrine and clinical parameters of participants such as age, FSH level of serum, level of injected FSH for stimulating ovulation, estradiol level before the day of hCG injection and the number of primary follicles, recovered oocytes and matured oocytes were studied. Poor responders' polymorphism were studied using real time PCR technique and results showed that women with A/A-Asn/Asn genotypes were poor responders and had lower FSHR expression in comparison with women of G/G-Asn/Asn genotypes.

The A/A-Asn/Asn genotypes can be considered as ovarian poor responders to COH predicting factor proven in that study. There was no significant association between *FSHR* gene polymorphism and ovarian poor responders and rs1394205 did not prove to be an effective genetic factor on ovarian poor response in this population [16].

Another study performed by Gingold et al. in New York, investigated the relationship between number of oocytes and FSH level. The results of the study showed that by increasing FSH, the number of oocytes of metaphase I and ovarian response reduced [17]. The whole number of oocytes, the metaphase I oocytes, and the metaphase II oocytes were compared in two good responders and poor responders group. The increasing number of oocytes showed significant association with ovarian response in this study. Good responders had more oocytes in metaphase II than poor responders

with significant differentiation. Also, women with low oocyte number had few fetuses which results in low fertility, confirming the achieved results [17].

Another effective factor on fertility is the biological age of women. In 2015, Raeisi et al. conducted a study to determine the effect of age, FSH and AMH levels and their effect on predicting ovarian response. Results showed that as the age of infertile women increases, the ovarian reserve of oocytes decreases, and therefore, ovarian response reduces [18].

This study indicates that the average age of women is higher in the poor response group than the good response group. The average age of women in poor responders group was 34 and in good responders group was 32, which showed significant differentiation that implies on the effect of age on poor ovarian response and also showed association between increased age and poor response of the ovary to COH. These results confirmed the incoming results of our study [18].

Results of the study performed by Revelli et al. in Georgia proved that AMH hormone influences ovarian response but the level of FSH showed no significant differentiation between the poor responders and good responders group, which indicates that FSH cannot be considered as a predictor factor for ovarian response to induced ovulation and AMH is a better factor for predicting ovarian response [19].

In conclusion, it is recommended that polymorphism rs1394205 in the *FSHR* gene and rs9340799 in the *ESRI* gene should be investigated in other cities and populations using more samples.

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Conflict of interest

None.

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