ORIGINAL ARTICLE

CONTENTS

The pharmacogenomic biomarkers and clinical effect of FSHR gene variants on female infertility

Hameeda Hadi Abdulwahid[1], Abeer Hussein[2], Mazin Hamid Oda[3]

[1]COLLEGE OF MEDICINE, WARITH AL ANBIYAA UNIVERSITY, KARBALA, IRAQ

[2]KARBALA HEALTH DIRECTORATE, MINISTRY OF HEALTH, IRAQ

[3]DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY, COLLEGE OF PHARMACY, UNIVERSITY OF KERBALA, IRAQ

ABSTRACT

Aim: The aims of this study are to detect the genetic polymorphisms of FSHR rs6166 (C> T) and rs6165 (C> T) gene particularly that associated with the response to FSH treatment and their effects on the pathogenesis of infertility in Iraqi women.

Materials and Methods: 210 Iraqi women, aged 20 to 34, who had just been diagnosed with infertility were included in this prospective case control research, whereas the control group consisted of 50 clinically healthy women who were free of any disorders. Following the guidelines for inclusion and exclusion in the study, each of the participating women saw a gynecologist to confirm. The time frame for this From November 2021 to June 2022, the investigation was carried out.

Results: The findings of this study in infertile women, clearly indicates that multiple genotypes of FSHR gene particularly (rs6166) (C>T) and (rs6165) (C>T), that include the homozygous wild genotype (CC), homozygous mutant (TT) and heterozygous (CT) genotype. The T allele was significantly increased ($P<0.05$) in poor responder infertile women for both rs6166 and rs6165 in FSHR which associated significantly with poor response to FSH in Iraqi infertile women.

Conclusions: Polymorphisms in FSHR gene may be associated with decrease in response to FSH treatment and it was associated with pathogenesis of infertility in Iraqi women/ Kerbala province

KEY WORDS: motivators, barriers, plans and factors

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INTRODUCTION

The World Health Organization (WHO) defines infertility as the failure to produce a clinical pregnancy after engaging in continuous, unprotected sexual activity for at least a year [1]. Infertility is a public health problem affecting 8% to 12% of couples worldwide [2]. In addition to its impact on reproductive health, female infertility also has psychological, economic, and physical repercussions it can lead to stress and trauma, particularly in cultures and countries where having children is highly valued [3]. There are two types of infertility in women: "Is unable to bear a child" refers to a woman's incapacity stress and trauma can result from it, especially in societies and nations where having children is highly regarded. This condition is known as primary infertility as well as secondary infertility, which happens when a woman has already given birth. Just 1.9 percent of couples experienced primary infertility, whereas 10.5% experienced subsequent infertility [2-4]. Every gender experiences infertility for the same reasons, despite the stereotype that women are more affected than males. Thirty percent of infertile couples have no known explanation for their infertility, a condition known as unexplained infertility. Forty percent of infertile couples attribute their infertility to the male spouse, forty percent to the female partner, and forty percent to neither of the two [5]. Recombinant DNA technology creates new genetic combinations that are used in industry, science, medicine, and agriculture by joining DNA molecules from two different species. By creating novel therapy strategies, monitoring tools, and diagnostic instruments, it also significantly contributes to the improvement of medical diseases. Two of the most prevalent applications of genetic engineering in health care are the creation of novel kinds of genetically modified bacteria to create synthetic human insulin, erythropoietin, and FSH as well as experimental mutant mice for research [6]. The treatment of infertility greatly benefits from the use of exogenous follicle stimulating hormone (FSH). Exogenous hFSH novel kinds of genetically modified bacteria to create synthetic human insulin, erythropoietin, and FSH as well as experimental mutant mice for research [7, 8]. There are several ways to

obtain follicle stimulating hormone. One technique is to extract various urinary proteins and FSH: LH activity at a 1:1 ratio from the urine of menopausal women [9-11]. In terms of amino acid sequence, glycosylation location, receptor binding capacity, and in vitro biologic activity. Comparable to urinary or pituitary FSH is recombinant FSH. Furthermore, the structures of recombinant and native carbohydrates are the same [12]. At the moment, follitropin α, β, and δ are the three r-hFSH medicines available on the market [13]. Despite having the identical amino acid sequence, follitropin α, β, and δ differ in terms of glycosylation, silica acid residue composition, and isoelectric coefficients: follitropin α has slightly altered biological activity, half-life, and metabolic clearance due to its higher acidity compared to follitropin β [14, 15]. The synthesis of FSHR is succeeded by suitable folding, post-translational modifications (PTM), and the ER and Golgi apparatus creation of highly-ordered di/oligomers. These events are followed by binding and anchoring to the cell surface, which is necessary for FSHR signaling events to function. After FSH binds, the Gas protein separates from the receptor and initiates processes that lead to Gas activation. These include protein kinase A (PKA) and extracellular signal-regulated kinase (ERK), which are triggered by phosphorylating cAMP [16, 17]we compared signaling triggered by human pituitary FSH preparations (FSH(18/21, which regulate the aromatase of downstream effectors of the mitogen-activated protein kinase pathway (MAPK) and the transcription of CREB cAMP response element-binding proteins, including phosphorylation, which are required for the processes of luteinization and ovulation. G protein-related kinases (GRKs) phosphorylated the desensitized receptor in the ILs and its C-terminal tail (Fig.1). Following phosphorylation, beta arrestin proteins interacted with the receptor to enable endocytosis coupling with clathrin-coated pits [18-19].

AIM

The aims of this study are to detect the genetic polymorphisms of FSHR rs6166 (C> T) and rs6165 (C> T) gene particularly that associated with the response to FSH treatment and their effects on the pathogenesis of infertility in Iraqi women.

MATERIALS AND METHODS

This prospective case-control study included 210 Iraqi women aged 20 to 34 years who were newly diagnosed with infertility, while the control group consisted of 50 clinically healthy women with no abnormalities. Following the guidelines for inclusion and exclusion in the study, each of the participating women saw a gynecologist to confirm. The time frame for this research is from November 2021 to June 2022, the investigation was carried out. The private clinic in the city of Kerbala provided samples. The practical part was conducted in the pharmacology and toxicology labs of the College of Pharmacy at the University of Kerbala. The Scientific and Ethical Committee approved this study, and each participant was asked to sign an informed consent form after being informed about it. A distinct patient card was made for every patient. All women were diagnosed with infertility by a consultant gynecologist and underwent laboratory tests (measurements of FSH, LH, Prolactin, TSH, E2, and AMH) as well as a physical examination and vaginal ultrasonography on the cycle's second day. Each infertile woman received subcutaneous injections of 75 IU follitropin-α on the third day of her menstrual cycle. After six days of stimulation, women who were infertile underwent vaginal ultrasound and had their E2 levels measured. Follicular growth was monitored every other day using transvaginal sonography until the minimum size of a single follicle reached eighteen millimeters. Then, to start ovulation, a single intraperitoneal injection A dose of 10,000 IU of HCG was given.

Exclusion criteria: Women are excluded if any of the following apply to them: previous ovarian surgery:

1. Systemic (diabetes mellitus, hepatic, renal, or cardiovascular illnesses) and endocrine problems
2. Infertility due to male factors
3. Ovarian polycystic syndrome
4. Ovarian Endometriosis

In this experiment, the concentration and purity of DNA were measured using a Nano-spectrophotometer, or Nano Drop.

Using the absorbance approach, the Nano drop device was utilized to assess the concentration and purity of isolated DNA. At 260 and 280 nm, absorbance readings were recorded [21]. Light is greatly absorbed by DNA at 260 nm, although it is most powerfully absorbed by protein at 280 nanometers. The A260/A280 ratio was used to determine the DNA's purity. A260/A280 ratios in the range of 1.8 to 2.0 are commonly mentioned as trustworthy indicators of DNA samples of superior quality [22]. Extremely sensitive Nano drop micro detector used as a blank. After cleaning the micro detector from blank High-quality DNA samples are often identified by their A260/A280 ratios, which are typically reported to be between 1.8 and 2.0 [23] (Fig.2).

STATISTICAL ANALYSIS

A260/A280 ratios between 1.8 and 2.0 are frequently cited as reliable markers of high-quality DNA samples.

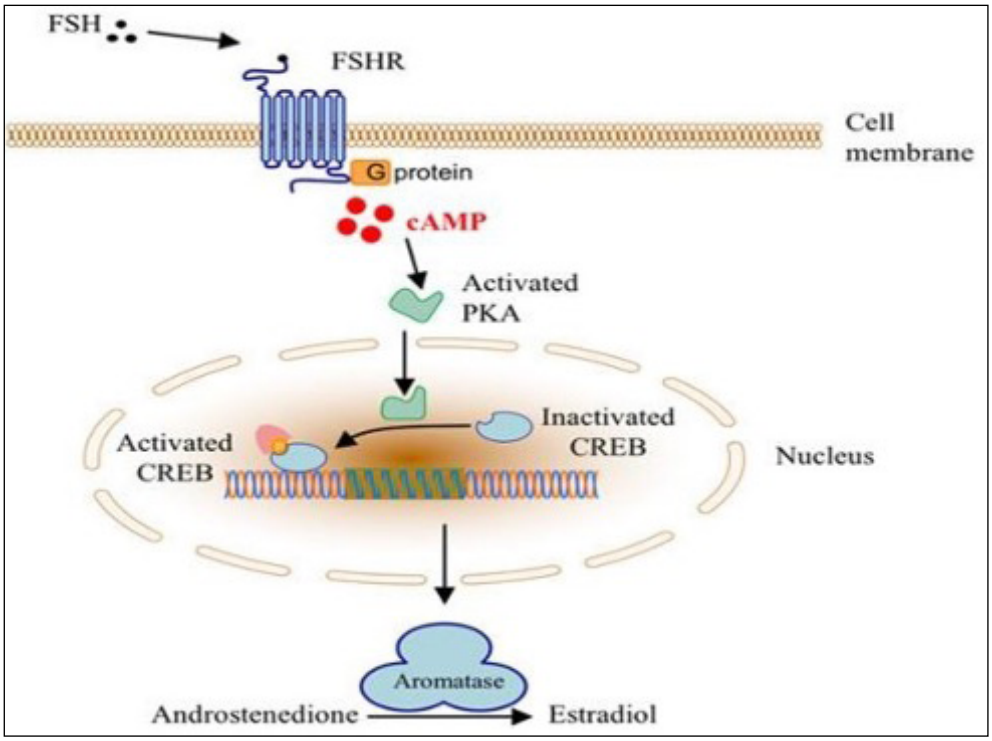

Fig. 1. Signaling mechanism (FSH and FSHR cellular activity) via the standard cyclic AMP/protein kinase An [20].

The results were examined using Pearson's correlation coefficients and the Statistical Package for the Social Sciences (SPSS) version 21.0. A P-value of less than 0.05 was considered statistically significant. Allele frequency for each genotype in the current study was estimated using the Hardy-Weinberg equilibrium online calculator; a P-value of ≤ 0.05 was deemed significant. In addition to clinical and biochemical markers, the study used the 95% confidence interval (CI 95%) and odds ratio (OR) to assess the relationship between these genotypes and the start of infertility.

RESULTS

A FEW CLINICAL AND BIOCHEMICAL CHARACTERISTICS IN THE PATIENT AND CONTROL GROUPS PRIOR TO THERAPY

Table 1 shows the biochemical marker results for this investigation. The blood readings of prolactin, AMH, TSH, LH, E2, and antral follicle count prior to treatment (for both high responder infertile women and others) were reported as mean ± standard deviation (SD). For the control group as well as the patient groups (moderate, poor) the pre-FSH, LH, TSH, prolactin, AMH, E2, and antral follicle count (for both high responder infertile women and others) therapy blood values were represented by the standard deviation ± mean (SD). The pre-treatment serum values of FSH, LH, TSH, prolactin, AMH, E2, and antral follicle count (for both high responder infertile women and others) were shown by the mean ± standard deviation (SD). It occurs in the infertile women's group with high response rates compared to the control group; among the infertile women's groups (moderate and high responder), the high responder group's mean serum FSH levels are statistically significantly lower than the poor responder group's ($P < 0.01$). For the three patient groups (poor, moderate, and high responder infertile women), the mean ± standard error of serum LH levels was 5.85 ± 0.72 mIU/mL, 8.09 ± 1.14 mIU/mL, 5.48 ± 1.52 mIU/mL, and 7.42 ± 0.66 mIU/mL, respectively. An ANOVA revealed no statistically significant difference in mean blood levels of LH between the moderate responder group and the control group ($P >0.05$). When compared to the moderate responder and control groups, the mean LH levels in the poor responder group were considerably lower (P ^ 0.001), whereas the mean LH levels in the high responder group were significantly higher (P ^ 0.001) than those in the poor responder groups. The patient groups (none of whom differed substantially from the others; $P > 0.05$ and the control group (poor, moderate, and high responder infertile women) did not exhibit significant differences in the means of their serum levels of prolactin and TSH. There were no statistically significant differences in the means of the patient groups (poor, moderate, and high responder infertile women) or in the serum levels of TSH and prolactin between the control group and the patient groups ($P > 0.05$). The results for infertile women are displayed in Table 1. Together with the serum AMH values for the patient and control groups (low, moderate, and high responder in that order, 3.24±0.96 ng/mL,

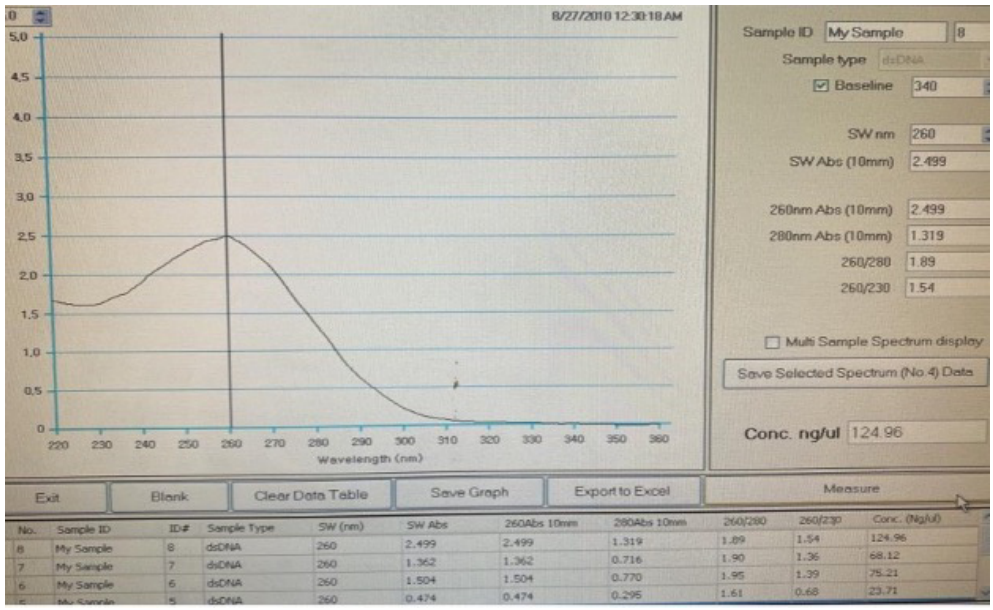

Fig. 2. DNA Nano drop results (concentration and purity).

0.63±0.12 ng/mL, 2.89±0.66 ng/mL, and 7.98±1.46 ng/mL). There was no significant difference between the moderate responder group and the control group ($P > 0.05$). The mean serum AMH level increased significantly ($P \wedge 0.001$) between the high responder group and the control group. This was seen in the subset of respondents who did not do well. Comparing the average of AMH for the high and moderate responder groups to the poor responder group and the high responder group to the moderate responder group revealed a significant increase ($P < 0.001$). Prior to therapy, the control and patient groups (poor, moderate, and high responder infertile women) had blood levels of E2 at mean±SD of 31.17±6.35 pg/mL, 38.96±6.52 pg/mL, 33.22±5.62 pg/mL, and 30.23±5.24 pg/mL. Although there was no statistically significant difference ($P>0.05$) between the high and moderate responder groups and the control group, an ANOVA comparison of the E2 before treatment results revealed that the poor responder group had very high significant increases ($P \wedge 0.001$) in the mean of E2 before treatment compared to the control group. The mean E2 before treatment for the moderate responder group exhibited very substantial significant decreases ($P \wedge 0.001$) compared to the poor responder group. Prior to treatment, the high responder group's mean E2 level in their sera was considerably lower than that of the moderate and poor responder groups ($P < 0.001$). The control and patient groups consist of infertile women who are poor, moderate, and high responders. Their respective mean ±SD values for the antral follicle counts were 11.7±1.23, 4.14±0.72, 11.16±1.01, and 16.59±2.16, respectively. The results of an ANOVA showed that the mean antral follicle count of the moderate responder group and the control group did not differ statistically significantly ($P > 0.05$). On the other hand, the average number of antral follicles was considerably lower ($P \wedge 0.001$) in the poor responder groups and significantly larger ($P \wedge 0.001$) in the high responder group compared to the control group. The mean antral follicle count for the high and moderate responder groups was significantly higher ($P \wedge 0.001$) than for the poor person group. This increase was also observed when comparing the moderate responder group to the high responder group.

The present study discovered that, in contrast to the control group, the mean serum FSH greatly increased in the poor responder group, but it sharply decreased in the infertile women groups with moderate and high responders. In contrast to the poor responder group, table 1 shows how the pituitary gland raises the quantity of FSH to maintain normal follicular growth. Therefore, the body's reaction to ovarian stimulation therapy is predicted by the basal FSH level [24]. Women with high blood FSH levels who were infertile were more likely to have a negative response to ovarian stimulation therapy, according to research by Jaiswar *et al.* These outcomes were consistent with their research, which showed that basal serum FSH levels significantly predicted ovarian reserve and response [25]. The current study's findings were in line with previous research's findings showing patients with greater serum FSH levels were less fertile, even if its discovery was distinct [26]. The results showed no significant differences in FSH levels across the several groups of ovarian stimulation treatment responders. According to table 1, the basal level of E2 in the current study revealed that the moderate and high responder infertile women experienced a very high significant reduction, while the poor responder group experienced a very high significant increase when compared to the control group. How-

Table 1. Evaluation of biochemical parameters of the control group and patient groups (women with low, medium and high response to therapy, suffering from infertility), (ANOVA test; all data were expressed as mean ±SD)

Hormonal and clinical parameters	Control	Poor responder	Moderate responder	High responder
Number	50	70	61	79
FSH (mIU/mL)	6.27 ±1.43	9.64 ±0.45 $^{a▲***}$	6.64±1.48 $^{aNS,b▼***}$	5.67 ±0.26$^{a▼**,b▼***,c▼***}$
LH (mIU/mL)	5.85 ±0.72	8.09±1.14 $^{a▲***}$	5.48±1.52 $^{aNS,b▼***}$	7.42. ±0.66 $^{a▲***,b▼***,c▲***}$
TSH (mIU/mL)	1.9 ±0.39	2.06 ±0.57 ^{NS}	2.04 ±0.41 ^{NS}	2.06 ±0.44^{NS}
Prolactin (ng/mL)	19.55±2.26	19.91 ±2.38 ^{NS}	20.08 ±2.51 ^{NS}	19.23 ±3.22 ^{NS}
AMH (ng/mL)	3.24±0.96	0.63±0.12 $^{a▼***}$	2.89±0.66 $^{aNS,b▲***}$	7.98±1.46 $^{a▲***,b▲***,c▲***}$
E2 before treatment (pg/mL)	31.17±6.35	38.96±6.52 $^{a▲***}$	33.22±5.62 $^{aNS,b▼***}$	.30.23±5.24$^{aNS,b▼***,c▼***}$
Antral follicle count	11.7±1.23	4.14±0.72$^{a▼***}$	.11.16±1.01 $^{a NS, b▲***}$	.16.59±2.16 $^{a▲*** b▲***,c▲***}$

a – ANOVA test between poor, moderate, high responder groups versus control group. b – ANOVA test between moderate, high responder groups versus poor group. c – ANOVA test between high and moderate responder groups. ▼*** – very high significant decrease (P<0.001); ▼** – high significant decrease (P<0.01); ▲*** – very high significant increase (P<0.001); NS – non-significant difference.

Table 2. The mean ±SD of E2 before and after therapy, as well as the size and quantity of Graafian follicles in the patient groups (poor, moderate, and high responder infertile women)

Clinical and hormonal parameters		Poor responder	Moderate responder	High responder
Number		70	61	79
E2 (pg/mL)	Before treatment	38.96±6.52	33.22±5.62	30.23±5.24
	After treatment	80.26±4.67 $^{c▲***}$	311.02±34.61 $^{a▲***,c▲***}$	704.78±138.85$^{a▲***,b▲***,c▲***}$
Graafian follicle size (mm)		9.76±2.23	18.31±1.16 $^{a▲***}$	21.91 ±0.9 $^{a▲***,b▲***}$
Graafian follicle count		1.04±0.2	2.07±0.25 $^{a▲***}$	3.05±0.22 $^{a▲***,b▲***}$

a – ANOVA test between moderate, high responder infertile women groups versus poor responder group. b – ANOVA test between moderate and high infertile women groups. c – Paired t-test between basal E2 and after 6 days of stimulation for poor, moderate and high responder infertile women groups. ▲*** – very high significant increase.

ever, the groups of moderate and high responders did not differ significantly from the control group. Women's baseline E2 serum levels as part of an ovarian reserve test were [27], according to studies by Carvalho et al., determining baseline FSH and E2 together may reduce the possibility of false-negative tests that just look for FSH. The ovarian response may not be sufficient if both indicators are raised too soon [28]. Prasad *et al*. found that the number of graafian follicles and the pregnancy rate increase in tandem with baseline E2 levels. Patients with high basal E2 levels should also be assessed for other stimulation options [29]. AFC measures on the second day of the menstrual cycle should show a significant increase in high responders and no appreciable changes between the moderate and control groups, as predicted by this study. The primordial follicle pool from which antral follicles are drawn determines how many antral follicles are detected by vaginal ultrasonography; the more primordial follicles available, the more follicles will proliferate. The reducing primordial follicle pool is reflected in the declining antral follicle count, which may help explain why the AFC is regarded as a predictor of ovarian response [30]. Lower AFC levels are associated with decreased ovarian reserve and response to ovarian stimulating drugs [31]. The findings showed that while the ovarian reserve of the poor responder group was considerably lower than that of the groups with moderate, high, and control responses, the ovarian reserve of the high and moderate responder groups was determined to be adequate. These results were consistent with the study's conclusions [32], Barbakadze *et al*. argue that blood AMH and AFC have a significant correlation and that combining AMH and AFC could improve the estimate of ovarian reserve, even though AFC assessment by ultrasonography is a useful way for predicting ovarian response. Groups with low AFC may be more likely to have reduced ovarian reserve [33].

SEVERAL CLINICAL AND BIOCHEMICAL MARKERS IN PATIENT GROUPS FOLLOWING THERAPY

Significant correlations have been observed between serum AMH and AFC, and combining AMH and AFC may improve the assessment of ovarian reserve. According to Barbakadze et al., low AFC and ovarian reserve can be re-

Table 3. Comparison of the mean ± SD of the studied parameters (AMH, E2 prior to therapy, and antral follicle count) in patient groups (poor, moderate, and high responder) with varying genotypes of the FSHR gene (rs6165) SNP

Biochemical parameters	Genotype	Poor responder	Moderate responder	High responder
Number		70	61	79
AMH (ng/mL)	CC	0.78±0.08	3.19±0.65 a▲***	8.51±0.32 a▲***,b▲***
	CT	0.65±0.07 c NS	3.1±0.57 a▲***, c NS	8.56±1.16 a▲***,b▲***, c NS
	TT	0.46±0.18 c ▼*, d ▼*	2.47±0.49 a▲***, c NS, d NS	7.61±0.91 a▲***,b▲***,c NS, d NS
E2 before treatment (pg/mL)	CC	36.36±10.17	33.53±5.96 a▼***	30.76±4.1 a▼***, b▼***
	CT	37.29±3.19 c NS	33.25±2.19 a ▼***, c NS	30.25±5.38 a ▼***, b ▼***, c NS
	TT	41.77±4.2 c ▲***, d ▲***	34.26±0.03 a ▼***, c NS, d NS	31.56±4.31 a ▼***, b ▼***, c NS, d NS
Antral follicle count	CC	5.41±0.61	11.7±1.13 a ▲***	17.84±1.28 a▲*** b***
	CT	4.19±0.79 c NS	10.75±0.73 a ▲***, c NS	16.44±1.52 a ▲***, b ▲***, c NS
	TT	2.9±0.75 c ▼***, d ▼***	10.7±0.48 a ▲***, c NS, d NS	16.44±1.52 a ▲***, b ▲***, c NS, d NS

c – ANOVA test between (CT, TT) and CC genotype. d – ANOVA test between TT and CT genotype. c NS, d NS ▼* – significant decrease (P<0.05); ▼*** – very high significant decrease (P<0.001); ▲*** – very high significant increase (P<0.001); NS – nonsignificant difference.

Table 4. Analyzing the mean ± SD of the investigated parameters (Graafian follicle size, Graafian follicle number, and E2 after treatment) in the patient groups (poor, moderate, and high responder) after FSH treatment according to different genotypes of the FSHR gene (rs6165)

Biochemical and clinical parameters	Genotype	Poor responder	Moderate responder	High responder
Number		70	61	79
E2 after treatment (pg/mL)	CC	85.74±5.55	319.62± 1.78 a▲***	763.89± 86.65 a▲***,b▲***
	CT	84.59±4.33 c NS	329.76± 18.57 a▲***, c NS	734.26±98.75 a▲***,b▲***, c NS
	TT	71.79±4.5 c ▼***8, d ▼***	303.66± 1.78 a▲***, c NS, d NS	695.84± 21.43 a▲*** b▲***, c NS, d NS
Size of graafian follicle (mm)	CC	11.96±1.61	18.11±1.29 a▲***	21.75±0.94 a▲***,b▲**
	CT	11.04±2.33 c NS	19.01±0.93 a ▲***, c NS	21.97±0.86 a ▲***, b ▲**, c NS
	TT	5.84±2.44 c ▼***, d ▼***	18.6±0.41 a ▲***, c NS, d NS	22.29±0.79 a ▲***, b ▲***, c NS, d NS
Number of graafian follicle	CC	1.23±0.43	2.09±1.12 a ▲*	3.17±0.53 a▲***, b▲*
	CT	1.27±0.72 c NS	2.06±0.92 a ▲*, c NS	3.11±1.13 a ▲***, b ▲*, c NS
	TT	0.65±0.49 c ▼**, d ▼**	2.01±0.44 a ▲**, c NS, d NS	3.01±0.48 a ▲***, b ▲*, c NS, d NS

c – ANOVA test between (CT, TT) and CC genotype. d – ANOVA test between TT and CT genotype. significant c NS, d NS ▼* – significant decrease (P<0.05); ▼** – high significant decrease (P<0.01); ▼*** – very high decrease (P<0.001); NS – non-significant difference.

lated. E2 results for medium, high, and poor respondents. The equivalent FSH levels for infertile women following treatment are displayed in Table 2: 311.02±34.61 pg/mL, 704.78±138.85 pg/mL, and 80.26±4.67 pg/mL comparing the moderate and high responder groups to the poor responder group. Table 2 shows that the E2 outcomes for the poor after receiving FSH treatment were 80.26±4.67 pg/mL, 311.02±34.61 pg/mL, and 704.78±138.85 pg/mL, respectively. The paired t-test showed very substantial significant increases (P ^ 0.001) in the E2 mean values when comparing the pre-treatment E2 mean values of the poor, high, and moderate responder groups with the post-treatment E2 mean values of the same groups employing FSH. Following FSH treatment, the mean ± SD Graafian follicle diameters for the three patient groups (poor, moderate, and high responder infertile women) were 9.76±2.23 mm, 18.31±1.16 mm, and (21.91±0.9) mm, respectively. The moderate and high responder groups' mean Graafian follicle size increased significantly (P ^ 0.001) in comparison to the poor responder group, according to the ANOVA test results. The mean ± SD values of Graafian follicles in infertile women in the moderate, high responder, and poor groups were 2.07±0.25, 3.05±0.22, and 1.04±0.2, respectively, after FSH treatment. When comparing the moderate responder group to the poor responder group, an ANOVA test showed a significant increase (P < 0.001) in the mean number of high responder groups and Graafian follicles.

Following six days of exogenous FSH injection, the serum levels of estradiol in the patient groups (poor, moderate, and high responder) are displayed in table 3 of the current study. It demonstrates that, in contrast to the groups of moderate and poor responders, the high

responder group experienced a very high and significant rise. Measurement of the E2 level after ovarian stimulation treatment may be useful to assess follicle maturation and predict the ovarian response to treatment. The steroid hormone E2 is released by granulose cells secreted by developing ovarian follicles. Because the primary functions of FSH are follicular development and stimulation of estradiol synthesis, a low level of estradiol indicates a reduction in the capacity of ovarian follicles to proliferate and produce estradiol in response to FSH [34]. This stimulation may be uncoupled or include distinct downstream pathways of the FSH receptor. The outcomes of this inquiry were consistent with those of study [35]. Researchers found that follicular maturation and a significantly lower pregnancy rate were associated with lower levels of estradiol in the poor responder group. Given their significant association with the size and quantity of graafian follicles after ovarian stimulation therapy, Malathi et al. claim that estradiol levels are a useful therapeutic tool in predicting maturity [36]. This stimulation may be uncoupled or include distinct downstream pathways of the FSH receptor. The results of this study were also consistent with the results of the study [35]. Researchers found that follicular maturation and a significantly lower pregnancy rate were associated with lower levels of estradiol in the poor responder group. Given their significant association with the size and quantity of graafian follicles after ovarian stimulation therapy, Malathi *et al.* claim that estradiol levels are a useful therapeutic tool in predicting maturity [36, 37]. This treatment slows down the ovaries' maturation and growth. Based on these findings, the poor responder group's poor follicle development was primarily caused by decreased granulose cell stimulation and ovarian follicle sensitivity to FSH. After this procedure, the ovaries grow and mature more slowly. Lower granulose cell stimulation and decreased ovarian follicle sensitivity to FSH were the primary causes of the poor follicle development in the poor responder group, according to the data [38].

EFFECT OF THE C>T GENETIC POLYMORPHISM IN THE FSHR GENE (rs6165) ON BIOCHEMICAL MARKERS WAS EXAMINED IN THREE PATIENT GROUPS: POOR, MODERATE, AND HIGH RESPONDERS

Table 3 presents the results of the current study, where one-way ANOVA was used to demonstrate the biochemical characteristics of each FSHR (C> T) (rs6165) genotype. To study differences between patients, it was necessary to establish a relationship between genotypes and levels of hormonal, clinical and biochemical markers, including prolactin, AMH, FSH, LH, TSH, E2 before treatment and the number of antral follicles.

In comparison to the groups with the CC and CT genotypes, the poor responder group with the TT genotype in this study showed a considerably lower AFC value for both the rs6166 and rs6165 tables 3.

THE POOR RESPONDER GROUP WITH THE TT GENOTYPE IN THIS STUDY HAD SIGNIFICANTLY LOWER AFC VALUES FOR THE rs6166 AND rs6165 TABLES THAN THE GROUP WITH THE CC AND CT GENOTYPES

Table 3 shows the E2 results after treatment in (pg/mL), the size of Graafian follicle in (mm), and the number of graafian follicles for patient groups (poor, moderate, and high responder) following an analysis of the impact of an SNP in the FSHR gene (6165) on the response to FSH through treatment with FSH. When comparing the genotypes CC, CT, and TT in the high responder group to the corresponding genotypes in the poor responder group, the averages of E2 after treatment (pg/mL) show a very high significant increase (P ^ 0.001).Additionally, the data show a highly significant increase ($p<0.001$) in the means of E2 after treatment (pg/mL) for the genotypes CC, CT, and TT in the high responder group as compared to the same genotypes in the moderate responder group. The mean ± standard deviation (SD) of serum E2 levels for the CC, CT, and TT genotypes in the poor responder group were 85.74±5.55 (pg/mL), 84.59±4.33 (pg/mL), and 71.79±4.5 (pg/mL) following the initiation of FSH treatment. The mean blood E2 levels for the (CC) and (CT) genotypes in the poor responder group do not differ significantly ($P > 0.05$), according to the results of the ANOVA test. Comparing the TT genotype to the CC and CT genotypes in the same group, however, revealed a substantial decrease ($P < 0.001$). There is no significant difference ($P > 0.05$) between the moderate and high responder groups' average E2 levels after therapy for any genotype (CC, CT, and TT). When comparing the mean size of graafian follicle (mm) findings for all patient groups with the analogues genotypes in the poor responder group, the ANOVA test showed a highly significant increase (P value < 0.001) in the (CC, CT, and TT) genotypes for the moderate and high responder groups. A highly significant increase (P value < 0.001) was observed in the (TT) genotype in the high responder group, which was linked to a similar genotype in the poor responder group. The results demonstrated a significant increase (P value < 0.01) in the case of the genotypes (CC and CT) in the Graafian follicle size when comparing the genotypes in the high responder group and the moderate responder group. The poor responder group with the CC, CT, and TT genotypes had mean ± (SD) Graafian follicle sizes in millimeters (mm) of 11.96±1.61, 11.04±2.33, and 5.84±2.44 after taking FSH medication. After FSH treatment, the poor

responder group with the CC, CT, and TT genotypes had mean ± (SD) Graafian follicle sizes in millimeters (mm) of 11.96±1.61, 11.04±2.33, and 5.84±2.44, respectively. The means of graafian follicle levels following treatment for the two groups exhibiting moderate or strong response do not differ statistically significantly ($P > 0.05$) among the genotypes (CC, CT, and TT).There are no statistically significant differences ($P > 0.05$) in the means of graafian follicle levels after treatment between the genotypes (CC, CT, and TT) for the two groups that exhibit moderate or strong response. When comparing the genotypes, the number of Graafian follicle levels increased significantly ($P < 0.001$). Compare the poor responder group with the high responder group (CC, CT, and TT). Furthermore, the genotypes in the high responder group had considerably ($P < 0.05$) more graafian follicles than the corresponding genotypes in the moderate responder group. Examine and contrast the responses of the poor responder group with those of the high responder group (CC, CT, and TT). Additionally, the TT genotype displayed a significant decrease ($P < 0.01$) in comparison to the CC and CT genotypes, while the number of graafian follicle levels for the genotypes in the high responder group showed a significant increase ($P < 0.05$) in comparison to the equivalent genotypes in the moderate responder group. Following medication, there is only a minor difference ($P>0.05$) in the average number of graafian follicles between the moderate and high responder groups across genotypes (CC, CT, and TT).

DISCUSSION

Infertile women with the TT genotype in the current study's poor responder group had somewhat lower serum levels of estradiol than women with the CC and CT genotypes after receiving the same dosage of FSH for ovarian stimulation. The special FSHR that FSH binds to in the ovary's granulose cells, which is essential in determining female reproduction. Connection between FSHR and FSH triggers an intracellular signaling mechanism that regulates granulose cell proliferation and differentiation. Granulose cells are activated by FSH to generate E2. Therefore, rs6166 and rs6165's altered FSHR activity result in lower E2 production and poor granulose cell differentiation and proliferation [38, 39]. These findings demonstrated that the T allele might be in charge of the FSH receptor's lower sensitivity to FSH, and that the FSHR polymorphisms (rs6166 and rs6165) were connected to a poor response to FSH. Both for rs6166 and rs6165, The CC, CT, and TT genotypes did not significantly differ between the moderate and high responder groups, suggesting that these genotypes and blood estradiol levels are unrelated. The current study's findings differed from those of Trevisan et al., who showed no correlation between the FSHR polymorphisms (rs6166 and rs6165) and blood levels of FSH and estradiol; nevertheless, the findings were consistent with a number of other research, such, It found When compared to other genotypes, infertile women with homozygous mutant genotypes for the two research SNPs (rs6166 and rs6165) displayed less mature oocytes and lower E2 levels [40]. In the current study, graafian follicles were considerably smaller in size and quantity in the poor responder group of FSHR (rs6166 and rs6165) TT carriers as opposed to CC and CT carriers. Estradiol synthesis, ongoing follicular development, and permanent accumulation in granulose cells, estradiol and FSH must predominate in follicular fluid. Thus, it was postulated that the FSHR's sensitivity to FSH may have been decreased by the genetic variation connected to the FSHR SNPs (rs6166 and rs6165), Due to this, Graafian follicle size and quantity have decreased, suggesting that the effect of FSH may have been diminished. Nonetheless, no appreciable variation was observed among the groups of moderate and strong responders who had CC, CT, and TT, suggesting that there was no correlation between the FSHR polymorphisms and the genotypes of these groups [41].

CONCLUSIONS

According to the study's findings, for both SNPs, the heterozygous genotype CT was more prevalent than the other genotypes, CC and TT. Additionally, FSHR polymorphisms, rs6166 and rs6165, may contribute to the genetic diversity in Iraqi infertile women's FSH responsiveness. The presence of both SNPs in the FSHR gene was linked to an increased risk of infertility in Iraqi women, according to the odd ratio. Strong ties exist between the TT genotypes of rs6166 and rs6165 and the clinical and hormonal markers of a poor ovarian response to FSH treatment. This is shown by a significant decrease in the mean of AFC, AMH, and E2, in addition to a notable rise in baseline FSH levels.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Hameeda Hadi AbdulWahid
Warith Al Anbiyaa University
Baghdad Road, Karbala, Iraq
e-mail: sgahmed1331962@outlook.com

ORCID AND CONTRIBUTIONSHIP

Hameeda Hadi AbdulWahid: 0000-0003-0628-2195 A F
Abeer Hussein: 0009-0005-8672-541X B C D
Mazin H. Oda: 0000-0002-9936-4319 D E

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

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