The Evaluation of the Role of Follicle Stimulating Hormone Receptor (FSHR) Gene Polymorphism in Controlling Ovarian Hyperstimulation

Gaber, S.S.[1]; Elgindy, E[2]; Elrehany, M.A.[1]; Abd-Elghany, H.M.[1]; Okasha, A.M.[1] and Mahgoub S.S.[1]

Departments of Medical Biochemistry[1], and Obstetrics & Gynaecology[2], Faculty of Medicine, Al Minia University, Egypt
*Elrehany1963@yahoo.com

Abstract: This study was performed to investigate the association between FSH receptor (FSHR) gene polymorphism at position 680 and the outcomes of controlled ovarian stimulation (COS) for *in vitro* fertilization (IVF) in Egyptian women. The study was conducted on 41 women with age younger than 45 years old who were underwent an IVF procedure, for each one of them, the basal FSH was estimated before ovarian stimulation which was done by a subcutaneous injection of highly purified GnRH agonist at mid luteal phase and exogenous recombinant FSH was administered in daily doses, then, HMG was injected intramuscularly with repeated transvaginal sonography to monitor ovarian follicle growth, ovulation detection was and luteal support were done by administration of hCG and progesterone, respectively. Finally, intracytoplasmic sperm injection was carried out. Genomic DNA was obtained from peripheral WBCs for amplification of the fragment of DNA that contains exon 10 of FSHR in a three file program of PCR, when checking the amplicons, a single product of 520 bp was detected. The amplicons were subjected to restriction endonuclease reaction using BseNI enzyme, then, the products of the reaction were checked by 1.2% agarose gel which showed three different patterns, a 520 bp band (for 680Asn/Asn), a 520 bp and a 413 bp band (for 680 Asn/Ser), and a 413 bp band (for 680 Ser/Ser). The obtained results revealed that FSHR genotype distribution was 36.6% for Asn/Asn, 31.7% for Asn/Ser, and 31.7% for Ser/Ser FSHR genotype groups. Although there was no difference among the three genotype groups in terms of the age and infertility diagnosis of study subjects, the basal levels of FSH (day 3) were significantly different for the three groups. The Ser/Ser group tended to require a least dose of gonadotropins for COS, though these differences were not statistically significant. Upon classifying the patients according to their ovarian response, the mean levels of basal FSH in Asn/Asn and Ser/Ser subgroups showed an increasing level from poor to good and high responders. In contrast Asn/Ser subgroup showed a decreasing level of day 3 FSH from poor to good and high responders. Also, the numbers of oocytes retrieved tended to be different for the three groups. Pregnancy rate was higher in Ser/Ser versus to the other groups. The homozygous Asn/Asn genotype of FSHR polymorphism at position 680 may be associated with a reduced ovarian response to COS for IVF, while, Ser/Ser genotypes showed a higher pregnancy rate.
On conclusion, this investigation reveals that FSHR gene polymorphism at position 680 may be associated with a different ovarian response to controlled ovarian hyperstimulation (COH).
[Gaber, S.S.; Elgindy, E; Elrehany, M.A.; Abd-Elghany, H.M.; Okasha, A.M. and Mahgoub S.S. **The Evaluation of the Role of Follicle Stimulating Hormone Receptor (FSHR) Gene Polymorphism in Controlling Ovarian Hyperstimulation**] Journal of American Science 2011; 7(10): 91-100].(ISSN: 1545-1003). http://www.americanscience.org.

1. Introduction

Follicle stimulating hormone (FSH) plays a central role in oogenesis. It triggers the maturation of follicles, e.g. the proliferation of granulosa cells, and induces synthesis of the androgen-converting enzyme aromatase. Furthermore, it plays a pivotal role in the recruitment of the dominant follicle. FSH action is mediated by the FSH receptor (FSHR), a member of the family of G-protein-coupled receptors expressed solely in granulosa cells, which mediates FSH signal transduction through the cAMP pathway **(Simoni *et al*., 1997).**

Investigators have tried to evaluate the distribution of various FSHR gene polymorphisms among infertile patients. During screening for mutations of the FSHR gene which has been implemented in the search for causes of infertility, two polymorphisms were identified; one located in the extracellular domain at position 307 occupied by either alanine (Ala) or threonine (Thr), and a second one located in the intracellular domain at position 680 occupied either by asparagine (Asn) or serine (Ser) (**Aittomaki *et al*., 1995)**.

FSHR gene polymorphism at position 680 is an important prognostic factor of ovarian response to FSH stimulation. Advance identification of patients who will elicit a poor response or hyper-response to standard treatment would be of great clinical advantage and so demonstration of the FSHR genotype is a major determinant of ovarian responsiveness to FSH in ovulation induction (**Mayorga *et al*., 2000)**.

The screening of the FSH receptor gene in patients with ovarian dysgenesis, in women with hypogonadotropic hypogonadism, and in infertile men revealed that the FSH receptor is polymorphic

in at least two sites. One polymorphic site is found in the extracellular domain at position 307, which can be occupied by either Ala or Thr, whereas position 680 in the intracellular domain can be occupied by either Asn or Ser (**Liu and Dias, 1996**).

In general, the incidence of a polymorphism in a given gene ranges from 15 to 50% in the normal population. This indicates that such genetic changes have no pronounced effects on reproduction; otherwise evolution would have exerted deleterious effects on them (**Shastry, 2002**).

Ovarian Response: It is useful to identify and prospectively differentiate the poor responder from the ultra-high responder, and whenever possible to specifically tailor the stimulation protocol.

In general, women who exhibit a high response to ovarian stimulation have higher success rates than low-responders. This was confirmed by analysis of over 1,300 cycles at The Center for Reproductive Medicine and Infertility. On the other hand, these patients are at substantially higher risk for developing ovarian hyperstimulation syndrome (OHSS) and associated complications (**Sharma *et al*., 1988 and Damario *et al*., 1999**).

Ovarian hyperstimulation syndrome (OHSS): OHSS can infrequently arise spontaneously during pregnancy, but most often it is an iatrogenic complication of ovarian stimulation treatments with ovulation drugs for *in vitro* fertilization. The first genetic cause of familial recurrent spontaneous OHSS was identified as a broadening specificity of the FSHR for hCG due to naturally occurring heterozygous mutations located unexpectedly in the transmembrane domain of the FSHR (**Meduri *et al*., 2008**).

2. Patients and Methods

This study was conducted from April 2009 to February 2010 on 41 women who underwent an IVF procedure at Suzan–Mubarak University Hospital Infertility Center. The women who prospectively recruited for this study were younger than 45 years of age and underwent the procedure due to tubal, male or unexplained infertility. Patients with polycystic ovary syndrome, endometriosis, or a previous history of ovarian surgery were excluded from this study. A written informed consent was obtained from each one of the patients. The genetic analysis of this study was carried out in Medical Biochemistry Department, Faculty of Medicine, Al Minia University.

Stimulation Protocol:

Basal FSH levels (thee third of the menstrual cycle) were obtained in one of the previous cycles before ovarian stimulation. Serum levels of FSH were measured by standard specific immunoassays on the respective cycle days (Vitros EC, Ortho-Clinical Diagnostics, Schwabach, Germany).

In all cases, the controlled ovarian stimulation according to standard protocols was performed before genetic analysis. The protocol applied in this center includes a subcutaneous injection of a highly purified GnRH agonist (0.1 mg of Decapeptyl, Ferring Gmph, Wittland 11, D-24109 Kiel, Germany) at mid luteal phase (on day 21 of the preceding cycle of stimulation cycle), and continued until down regulation of pituitary ovarian axis occurs which was detected by ultrasonography in the form of thin endometrium without ovarian activity or by occurring of menstruation. In general, follicular development was monitored by transvaginal sonography, after 6 days of stimulation and then every other day. In cases of insufficient follicular growth, FSH dosage was increased gradually.

Exogenous recombinant FSH was administered in daily doses varying from three to four ampoules, depending on the patient's previous or anticipated response, age, basal FSH, antral follicle count (ANFC) and body mass index, was initiated on day 3 of the stimulation cycle by giving a daily HMG (Merional®, IBSA Institute Biochimique SA-CH 6903 Lugano) intramuscular injection. Each ampoule contains 75 IU of FSH and 75 IU of LH. Treatment was continued until appearance of leading follicle (**Jun *et al*., 2006**).

Repeated sonographic measurements of ovarian follicle growth were carefully monitored and the dose of exogenous FSH was adjusted according to the patient's individual response. Ovulation was triggered by the administration of 10,000 IU of hCG (Pregnyl®; NV Organon, Netherlands) when at least two follicles attained 18–20 mm in diameter and oocyte retrieval was performed 24–36 hours after hCG injection by transvaginal guided-ultrasound follicle aspiration under mild sedation and analgesia. Luteal support was provided with progesterone (Utrogestan®; Besins International, Montrouge, France) initiated after oocyte aspiration and extended until the 8th week of pregnancy or until the initiation of menses. The intra cytoplasmic sperm injection (ICSI) was performed according to conventional protocol (**Jun *et al*., 2006**). Up to four embryos were transferred on the second or third day after retrieval.

Relevant clinical, hormonal, and sonographic data were collected retrospectively from patients' medical records. The patients were classified according to the ovarian response into poor (produce $\leq$ 4 follicles), good (produce 5- 15 follicles), and high (produce $\geq$ 15 follicles) responders.

DNA Extraction:

Genomic DNA was obtained from peripheral blood leucocytes as described by **Medrano *et al.*, (1990).**

DNA amplification:

The fragment of DNA containing exon 10 of the FSHR (the region starting from nucleotide number 1624 and ending with nucleotide number 2143) was amplified by PCR using a pair of specific oligonucleotide primers as described by **Laven *et al.*, (2003)**, forward primer (5'-TTT GTG GTC ATC TGT GGC TGC-3') and reverse primer (5'-CAA AGG CAA GGA CTG AAT TAT CATT-3'**)** **(**supplied from Sigma Chemicals, ST. Louis, USA)**.** The PCR was performed in the thermal cycler (Progene, Duxford, Cambridge, UK) in a reaction final volume of 50 µl containing one step PCR mixture (Bio Basic INC., Torbay Road, Markham Ontario L3R 1G6 Canada), 0.3-0.4 µg DNA template and 10 pM of each primer, following this three file program : initial denaturation at 94˚C for 5 minutes; followed by 30 cycles, each one is consisted of denaturation at 94˚C for 30 seconds, annealing at 55˚C for 30 seconds and extension at 72˚C for 1 min. The amplicons were analyzed using agarose gel electrophoresis.

RFLP analysis:

The amplicons obtained from PCR were subjected to a restriction endonuclease reaction using BseNI enzyme according to the method of **Loutradis *et al.*, (2006)**. The reaction was carried out by adding 10 µl (0.1-0.5 µg of DNA) of PCR product, 6 µl of Nuclease free water, 2 µl of 10x Buffer B (10 mM Tris-HCl, 10 mM $MgCl_2$, 0.1 mg/ml BSA) and 2 µl BseNI (10 U/µl), then, incubated at 65˚C for 16 hours, thermal inactivation of the BseNI enzyme was done at 80˚C for 20 minutes. The digestion products were checked by 1.2% agarose gel and photographed. Three different patterns were obtained, a 520 bp band (for 680Asn/Asn), a 520 bp and a 413 bp band (for 680 Asn/Ser), and a 413 bp band (for 680 Ser/Ser).

Statistical analysis:

All statistical calculations were performed by using SPSS software version 13. Multiple linear regressions were used to evaluate the association of basal FSH levels, type of polymorphism, total rFSH dose, number of follicles, mature oocytes and fertilization rate. Data are presented as the mean ± SEM. P values < 0.05 were considered statistically significant.

3. Results

As shown in Table (1); the overall frequency distribution was 36.6% for the Asn/Asn variant, 31.7% for the Asn/Ser variant, and 31.7% for the Ser/Ser variant. Infertility was caused by impaired semen variables in 58.54%, by tubal pathology in 24.39%, and by unexplained factors in 17.07% of couples (Table, 1).

In table (2), it was shown that no statistically significant differences between the three groups except for basal FSH (day 3) in the homozygous Ser and Asn groups. The basal levels of FSH were significantly ($p< 0.041$) higher in the Ser/Ser group (8.42± 3.22 IU/l) versus to those of the Asn/Asn group (6.37± 1.65 IU/l), while, there was no significant difference when compared to Asn/Ser group.

The number of eggs retrieved tended to be different among FSHR genotype groups. The Asn/Asn group (5.47± 3.02) showed fewer oocytes compared to the Asn/Ser group (6.54± 7.48), and Ser/Ser group (11.23± 4.48). Pregnancy rate per embryo transfer among each group was significantly ($p< 0.05$) higher in the Ser/Ser group (23.08%) as compared to either the Asn/Ser group (7.69%) or the Asn/Asn group (0%).

By analyzing the hormonal and ovulation results according to the genotype variant, the gonadotrophin dose was correlated significantly with the observed levels of day 3 FSH (Spearman's correlation coefficient, $p< 0.05$). This correlation becomes stronger (Figs. 7, 8) if the Spearman's coefficient is calculated for each specific genotype variant subgroup (Ser/Ser: $p< 0.002$; Asn/Ser: $p = 0.907$; Asn/Asn: $p< 0.022$).

In PR, GR and HR patients; as shown in table (3); the mean levels in Asn/Asn and Ser/Ser subgroups showed an increasing level from poor to good and high responders. In contrast, Asn/Ser subgroup showed a decrease in the level of day 3 FSH from poor to good and high responders. Moreover, the numbers of preovulatory follicles and collected oocytes were higher in the Ser/Ser subgroup (Table 2). Also, pregnancy rates were higher in Ser/Ser subgroup (3/13) when compared with the other two groups (Asn/Ser: 1/13; Asn/Asn: 0/15).

The number of the obtained ovarian follicles on ultrasonography at the end of the treatment during COS were four or less, 5-15 and more than 15 in the three groups [poor responders (PR) (n= 9), good responders (GR) (n= 26) and high responders (HR) (n= 6), respectively]. All groups were nearly similar in terms of days of stimulation, dose of recombinant FSH used, and cause of infertility (Table 4). However, poor, good and high responders differed in mean age (32, 29.19, and 27.17) respectively, no significant results were shown between subgroups while, the high responders group appears among women of the young age.

As shown in table (5) the frequencies of the allelic variants among each subgroup of all types of responders are illustrated. In the PR group, it is distributed only between: Asn/Asn (40%) and Asn/Ser (23.1%). The three genotypes were equally distributed in the GR group (Asn/Asn, 53.3%; Asn/Ser, 61.5%; and Ser/Ser, 76.9%) as well. However, patients in the HR group had a higher tendency to carry the Ser /Ser variant (23.1%).

The group of HR patients (Table 3) had higher levels of basal FSH (8.62± 3.01), in comparison to the PR group (6.29± 1.68) and GR group (7.18± 2.48). Ser/Ser genotypes were distributed highly among HR group (3/6, 50%) in comparison to the other two genotypes (Asn/Ser, 2/6; Asn/Asn, 1/6). However, the Asn/Asn variant (Table 4) is distributed highly among the PR group (6/9, 66.66%) when compared to the other two variants (Asn/Ser, 3/9; Ser/Ser, 0/9).

Follicles, oocytes, eggs retrieved, and fertilization rate in PR patients showed a significant ($p< 0.001$) difference in Asn/Ser variant, and also the same parameters in GR& HR patients showed a significant ($p< 0.001$) difference among Asn/Ser variant. Furthermore, the oocytes which provide the better quality embryos appeared among Ser/Ser variants in the GR and HR groups. Therefore, oocyte production and quality of embryo appear to vary among patients with different genotypes. Pregnancies, defined by cardiac activity, were distributed as follows: two in GR Ser/Ser, one in HR Ser/Ser, and one in HR Asn/Ser (Table 6).

All products of PCR were of the expected size (520 bp) (Figure 1). RFLP analysis for the polymorphism at position 680 (exon 10) determined the genotype distribution for Ser/Ser, Asn/Ser, Asn/Asn polymorphism in patients with poor responders, good responders, and high responders (Figure 2).

Our results showed a significant ($p= 0.022$, $r= -0.583$) negative correlation between the basal and the dose of recombinant FSH in Asn/Asn genotype (Figure 7). While, in Ser/Ser variant, the results showed a positive ($p= 0.002$, $r= 0.782$) correlation between the basal and recombinant FSH (Figure 8). As regards the recombinant FSH dose used for ovarian stimulation, it showed a significant ($p= 0.0001$, $r= -0.858$), ($p= 0.001$, $r= -0.782$) and ($p= 0.001$, $r= -0.784$) negative correlation with each of eggs retrieved, number of follicles, and number of mature oocytes in Asn/Asn subgroup, respectively (Figs. 3, 5, 6). The fertilization rate in Ser/Ser variant group was significantly ($p= 0.007$, $r= 0.706$) positively correlated (Fig. 4) with the basal FSH of Ser/Ser group.

Table (1): Distribution of FSHR genotypes subdivided according to infertility factors

	Asn/Asn	Asn/Ser	Ser/Ser	Total
Tubal factor	4/15 (26.67%)	0/13 (0%)	6/13 (46.16%)	10/41 (24.39%)
Male factor	10/15 (66.67%)	9/13 (69.23%)	6/13 (46.16%)	24/41 (58.54%)
Unexplained	1/15 (6.66%)	4/13 (30.77%)	1/13 (7.70%)	7/41 (17.07%)
All patient	15/15 (100%)	13/13 (100%)	13/13 (100%)	41/41 (100%)

Table (2): the biological and clinical characteristics of patients grouped according to FSHR genotypes

Parameter	Asn/Asn	Asn/Ser	Ser/Ser
Age (years)	29.4± 4.98	32.08± 6.73	27.08± 5.42
Basal FSH (mIU/ml)	6.37± 1.65*	6.93± 2.09	8.42± 3.22*
No. of ampoules/day	4.67± 1.11	4.23± 1.3	4.00± 1.00
Duration of stimulation (days)	13.53± 2.72	10.92± 2.22	12.23± 2.52
Total rFSH dose (IU)	4908.8± 1975.78	3490.38± 1479.43	3767.31± 1340.99
No. of follicles	8.6± 5.21	10.85± 8.64	15.15± 5.99
Mature oocyte	7.27± 4.65	8.77± 7.54	12.31± 5.15
Eggs retrieved	5.47± 3.02	6.54± 7.48	11.23± 4.48
Fertilization rate%	3.33± 2.49	3.38± 2.75	8.15± 3.85
Pregnancy rate between genotypes	0/15 (0%)	1/13 (7.69%)	3/13 (23.08%)
Pregnancy outcomes	0/41 (0%)	1/41 (2.4%)	3/41 (7.3%)

* Significant if $p< 0.05$

Table (3): The mean of the basal FSH values according to the type of responders

Type of responders	All patients	Asn/Asn	Asn/Ser	Ser/Ser
Poor	6.29± 1.68	5.47± 1.66	7.93± 0.91	-----
Good	7.18± 2.48	6.71± 1.28	6.91± 2.46	8.68± 2.99
High	8.62± 3.01	9.0± 0.0	5.5± 0.99	10.57± 2.57

Table (4): The clinical profile of the PR *vs.* the GR and the HR responders undergoing COS stimulation

Variable	PR	GR	HR
No. of patient	9	26	6
Mean age (range)	32.0 (19- 45)	29.19 (19- 42)	27.17 (26- 28)
Cause of sterility (male/tubal/unexp.)	5/1/3	15/7/4	4/2/0
Days of stimulation (range)	14.33 (9- 19)	11.35 (8- 15)	12.5 (9- 16)
Mean FSH ampoules (range)	5.33 (4- 6)	4.19 (3- 6)	3.33 (3- 4)

Table (5): The distribution frequency of different allelic variants in patients according to ovarian function

HR	GR	PR	Polymorphism
1/15 (6.7%)	**8/15 (53.3%)**	**6/15 (40%)**	**Asn/Asn**
2/13 (15.4%)	**8/13 (61.5%)**	**3/13 (23.1%)**	**Asn/Ser**
3/13 (23.1%)	**10/13 (76.9%)**	**0/13 (0%)**	**Ser/Ser**

Table (6): The genetic profile of PR *vs.* GR and HR responders undergoing COS stimulation

Variants among the type of responders		No. of follicles	Mature oocyte	Eggs]retrieved	Fertilization rate%	Preg. outcome	Genotype distribution
PR group	**Asn/ Asn**	4.17± 0.75	3.17± 0.31	2.5± 0.84	1.33± 0.21	0/6	6
	Asn/ Ser	3.67± 2.08**	2.33±1.53**	2.33± 1.15**	2.00± 1.00**	0/3	3
	Ser/Ser	----	----	----	----	----	0
	Total	4.00± 1.22	2.89± 1.05	2.44± 0.88	1.56± 0.73	0/9 (0%)	9
GR group	**Asn/ Asn**	10.38± 3.38	9.00± 1.04	7.38± 2.26	5.00± 2.33	0/8	8
	Asn/ Ser	9.25± 3.62**	7.5± 1.21**	4.13± 1.64**	2.5± 0.93**	0/8	8
	Ser/Ser	12.7± 3.4	10.2± 0.93	9.4± 3.02	6.5± 2.51	2/10	10
	Total	10.92± 3.64	9.00± 3.17	7.15± 3.23	4.81± 2.62	2/26 (7.69%)	26
HR group	**Asn/ Asn**	21.00	18.00	8.00	2.00	0/1	1
	Asn/ Ser	28.0± 5.66**	23.5±4.95**	22.5± 6.36**	9.00± 2.83**	1/2	2
	Ser/Ser	23.33± 5.77	19.33± 2.85	17.33± 1.45	13.67± 1.15	1/3	3
	Total	24.5± 5.28	20.5± 4.51	17.5± 6.22	10.17± 4.83	2/6 (33.33%)	6

** Highly significant p< 0.005

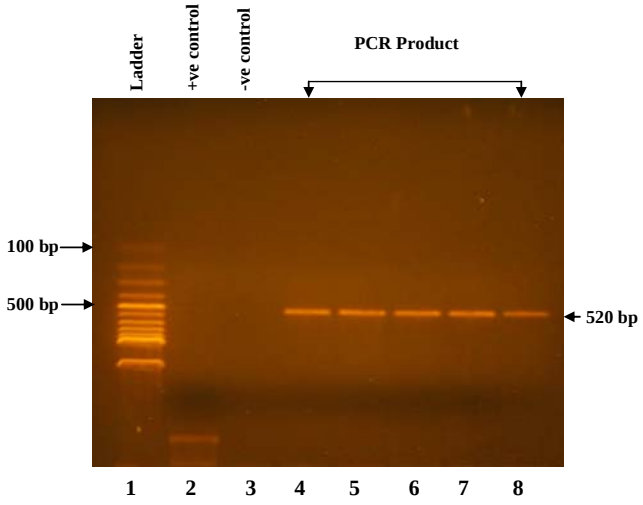


Figure (1): Agarose gel showing PCR product at 520 bp (lanes, 4-8), 100 bp ladder (lane 1), positive control (lane 2), and negative control (lane 3)

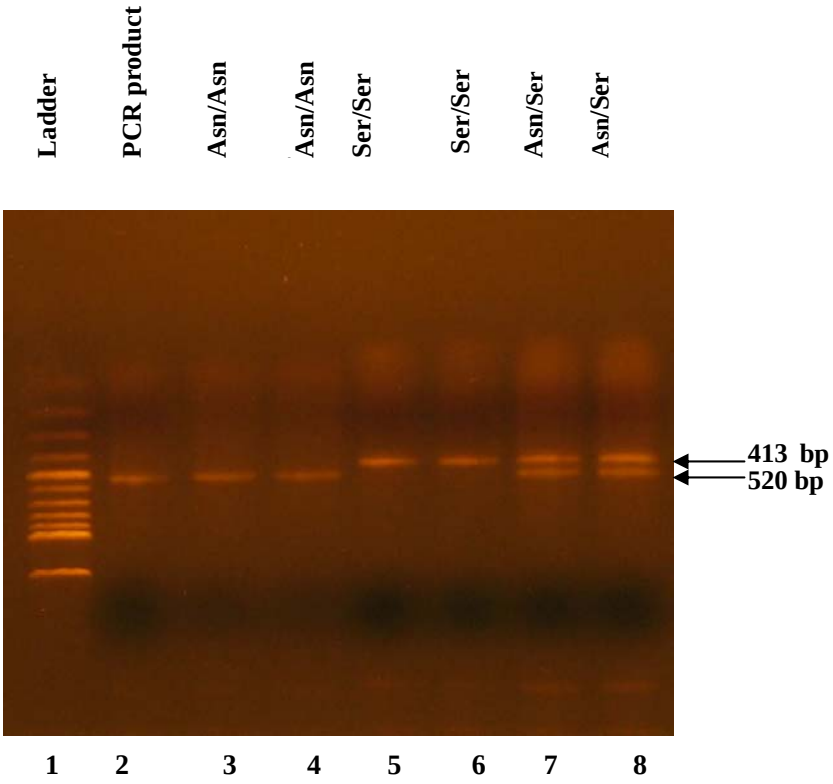


Figure (2): FSH receptor variants. Lane 3, 4 represents Asn/Asn; lane 5, 6 represents Ser/Ser; while lane 7, 8 represents Asn/Asn variant.

Figure (3): Correlation between rFSH and eggs retrieved in Asn/Asn group

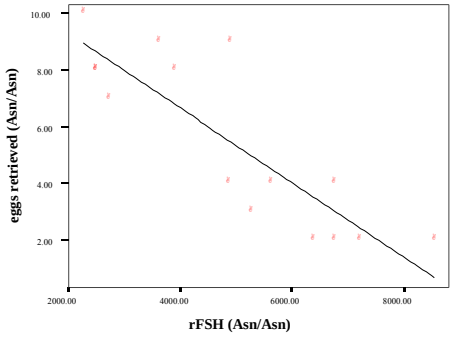


Figure (4): Correlation between basal FSH and fertilization rate in Ser/Ser group

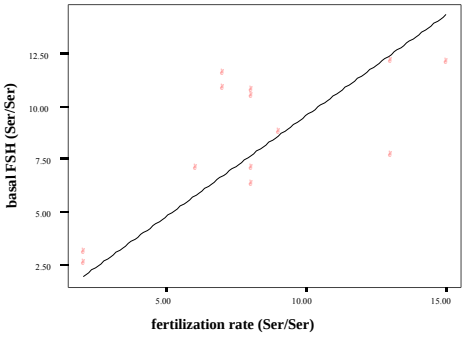


Figure (5): Correlation between No. of follicles and rFSH in Asn/Asn group

Figure (6): Correlation between No. of mature oocytes and rFSH in Asn/Asn group

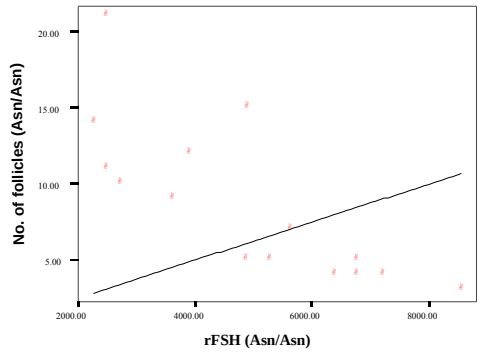


Figure (7): Correlation between basal and recombinant FSH in (Asn/Asn) genotype

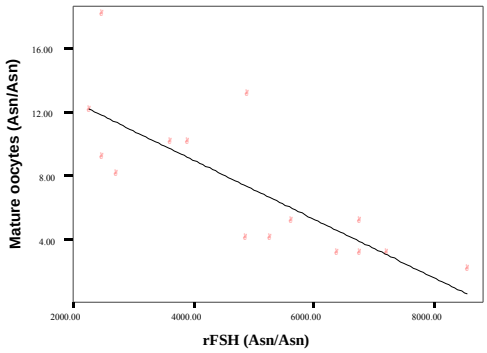
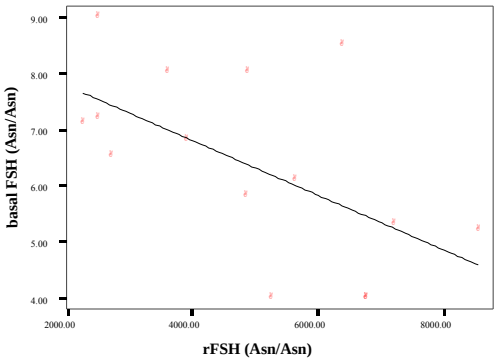
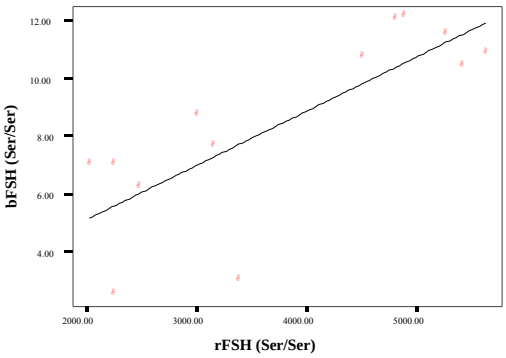


Figure (8): Correlation between basal and recombinant FSH in(Ser/Ser) genotype





4. Discussion

The distribution of several allelic variants of the FSHR gene in specific populations has been described in the past (**Laven *et al.*, 2003**). The identification of these allelic variants has led to the investigation of their potential value as predictors of ovarian response to an exogenous stimulation IVF/ICSI protocol.

Since the first report on the genotypic variance of the FSHR gene, the possibility has been considered as to whether a single nucleotide polymorphism (SNP) of FSHR gene affects the ovarian response to exogenous gonadotropins. That is to say, the ovarian responses to gonadotropins for controlled ovarian hyperstimulation (COH) in IVF cycles might differ according to FSHR gene genotypes (**Aittomaki *et al.*, 1995**).

In this study, the clinical parameters of the selected 41 patients from an IVF program were examined with correlation of the role of Asn680Ser polymorphism with various parameters including the outcome of ovulation induction, the number of oocytes, the quality of embryos and pregnancy rates.

The obtained results of PCR-RFLP analysis in the present study allows the identification of the point mutations for FSHR genes as those described by **Marson *et al.*, (2008)**, with a higher frequency of homozygous Asn genotype (15 out of 41) was recorded among cases investigated for the FSHR polymorphism.

This study demonstrates that the FSHR genotype plays a fundamental role in determining the physiological responsiveness of the target organ to FSH stimulation; also, the finding that allelic variants of the FSHR determines FSH sensitivity and helps to open a new perspective in endocrinology and indicates that subtle genetic changes might fine-tune the hormonal regulation of reproduction.

With regard to FSH, different isoforms have been described, characterized by variations in oligosaccharide structure, as well as in the number of terminal sialic acid residues. These isoforms can probably elicit different kinds of ovarian response (**Ulloa-Aguirre *et al.*, 1995**). The FSHR isoforms might further contribute to such pleotropic answers by a molecular mechanism, which could reside in the potential for glycosylation/phosphorylation that each isoform possesses, where, FSHR is capable of coupling with more than one G protein subtype, depending on the glycosylation pattern of the ligand

and on the degree of receptor transducer activation (**Arey *et al.*, 1997**).

Several reports have provided possible explanations for these molecular mechanisms that may support our findings that different receptor genotypes might show different ovarian responses to exogenous gonadotropin administration. Firstly, the expressions of cell surface receptors differ in the three genotypes, which would influence ligand/receptor interactions. Moreover, such isoform differences might lead to differences in the turnover or down regulation rates. Secondly, it should be considered that the individual receptor isoforms probably have different affinities for the different FSH isoforms (**Zambrano *et al.*, 1999**). The presence of an Asn residue at position 680 introduces a consensus sequence for glycosylation, which might be important for posttranslational receptor processing and expression at the cell surface; whereas a Ser residue contributes potential phosphorylation, which might be involved in the receptor turnover (**Davis *et al.*, 1995**). These postulations indicate that minute genetic differences in the receptors may affect the action of the hormone at target cells.

Basal day-3 FSH level has been used as a measure of ovarian reserve, with high levels predicting poor response (**Scott and Hofmann, 1995**). However, high FSH levels can also be found in fertile patients, and the sensitivity of this parameter alone has been estimated to be only 8% (**Aittomaki *et al.*, 1995**). FSH levels in our patients showed a wide variation, as was also observed in young patients with normal ovarian function (**Schipper *et al.*, 1998**). It has been suggested that this feature could be related to intraovarian modification of FSH action owing to the presence of inhibitors and/or enhancers of the binding to the receptor, principally to growth factors or to individual differences in the FSHR (**Findlay, 1994 and Fauser and Van Heusden, 1997**).

During this investigation; using standard stimulation protocols, a marked difference could be observed in the number of ampoules required to achieve ovulation. The marked difference of more than four ampoules during the duration of stimulation, between the homozygous Asn group and the homozygous Ser group is also reflected by the different FSH serum levels in these groups. However because FSH values show wide interindividual variations, even within the same FSHR group, the subdivision of the patients according to their serum FSH concentrations is not clinically useful, whereas identification of the FSHR genotype permits better classification. This underlines the clinical significance of our findings, which goes far beyond the mere description of an indirect association of two markers as shown in other polymorphism studies.

The levels of serum FSH reflect the ovarian response potential on the third day of the menstrual cycle, considering the existence of intraindividual variation from cycle to cycle and the different bioactivity of FSH measured (**Loutradis *et al.*, 2006**). Indeed, we show here that the gonadotrophin dose required for ovulation induction significantly correlates with day 3 FSH levels for specific genotype variant subgroups.

Our data show that day 3 FSH levels are statistically higher in Ser/Ser subgroup compared to Asn/Ser subgroup of patients. This variation may indicate a prompt response to FSH administration in the latter subgroup, with a consequent decrease in the basal levels of FSH. This finding is further supported by the observation that the number of days and total rFSH dose were lower in this subgroup (Asn/Ser). Similarly, other studies have suggested that ovarian response to rFSH may depend on the FSH receptor genotype of treated patients (**Mayorga *et al.*, 2000**).

Although number of preovulatory follicles and number of oocytes retrieved were similar in all allelic variants. **Mayorga *et al.* (2000),** reported increased basal serum FSH levels in the Ser/Ser subgroup. In addition, the dose of recombinant FSH needed to achieve successful stimulation was higher in Ser/Ser homozygotes than Asn/Ser genotype, a finding which is in agreement with our present data which may be explained by the suggestion that this receptor genotype might result in a mild resistance to the gonadotrophin.

Our data show that 50% of patients among the HR group tend to carry the Ser/Ser variant. However, this tendency did not prove to be statistically significant. Likewise, in other studies no specific allelic variant was found to be prominent (**Laven *et al.*, 2003**). In this study we report also, that GR patients have a tendency to carry the Ser/Ser variant while this variant does not appear in the PR group. Furthermore, fertilization rate and quality of embryos were higher among Ser/Ser genotype in the GR and HR groups. These findings may reflect a better and more rapid ovarian response to exogenous stimulation, possibly due to a more efficient FSH receptor. Thus, the allocation of a candidate for an IVF program to the Ser/Ser allelic variant might predict a better response.

In addition; our study showed that HR group lies between women of young age which needs the lowest total dose of exogenous rFSH. However the age decreased for women participated in IVF protocol, the response to the program was successful and the cost is less. Thus, we advise patients to go early to the IVF protocol for better results.

However, the Asn/Asn genotype between PR

group showed higher values in number of follicles, oocytes, eggs retrieved when compared with the Asn/Ser genotype; the fertilization rate and quality of embryos (grade I) were higher in the later genotype. This finding indicates that the heterozygous Asn genotype showed a better response among PR group.

Knowing the different sensitivity to FSH, depending on the FSHR genotype, could be important to prevent ovarian hyperstimulation syndrome, a complication with increasing incidence after the introduction of GnRH agonists, which involves the administration of higher doses of gonadotropins (**Fauser *et al*., 1999**). It is difficult to prevent this complication because of the narrow therapeutic margin of the ovulation induction agents and because of the impossibility of predicting individual response. Previous studies on FSHR genotype might provide a means of assessing this individual factor, assuming that pertinent modifications of the ovarian stimulation treatment can be made.

Among the possible benefits of adjusting stimulation protocols according to the expected response could be that the duration and the total amount of FSH needed decreases. Immediate implications are benefits not only from the economic point of view but also in terms of treatment acceptance.

During this investigation; the frequency of polymorphisms at residue 680 in Egyptian women which has indicated the differences in some clinical parameters. Clinically, the difference in ovarian response to hMG among these polymorphisms could be used not only for determination of hMG dose in an ovarian stimulating cycle, but also for prediction of OHSS.

In addition, further studies are necessary to determine whether it is possible to apply this relationship to the pre-cycle evaluation of individual genetic predisposition in terms of preventing either OHSS or a low ovarian response.

Corresponding author
Elrehany, M.A
Departments of Medical Biochemistry, Faculty of Medicine, Al Minia University, Egypt
Elrehany1963@yahoo.com

References

Simoni, M.; Gromoll, J. and Nieschlag, E. 1997: The follicle- stimulating hormone receptor: biochemistry, molecular biology, physiology, pathophysiology. Endocr. Rev., 18: 739–773.

Aittomaki, K.; Dieguez, L.L. and Pakarinen, P. 1995: Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypergonadotropic ovarian failure. Cell, 82: 959–968.

Mayorga, M.P.; Gromoll, J.; Behre, H.M.; Gassner, C.; Nieschlag, E. and Simoni, M. 2000: Ovarian Response to Follicle-Stimulating Hormone (FSH) Stimulation Depends on the FSH Receptor Genotype. J. Clin. Endocrinology & Metabolism, 85: 3365-3369.

Liu, C. and Dias, J.A.1996: Long loop residues 33–58 in the human glycoprotein hormone common alpha subunit contain structural components for subunit heterodimerization and human follitropin receptor binding. Arch. Biochem. Biophys., 329 127–135.

Shastry, B.S. 2002: SNP alleles in human disease and evolution. J. Hum. Genet., 47: 561–566.

Sharma, V.; Riddle, A.; Mason, B.A.; Pampiglione, J. and Campbell, S. 1988: An analysis of factors influencing the establishment of a clinical pregnancy in an ultrasound-based ambulatory in vitro fertilization program. Fertil, Steril., 49: 468–478.

Damario, M.A.; Davis, O.K. and Roseenwaks, Z. 1999: The role of maternal age in assisted reproductive technologies. Reprod. Med. Rev., 7: 141– 60.

Meduri, G.; Bachelot, A.; Cocca, M.P.; Vasseur, C.; Rodien, P.; Kuttenn, F.; Touraine, P. and Misrahi, M. 2008: Molecular pathology of the FSH receptor: New insights into FSH physiology. Molecular and Cellular Endocrinology, 282: 130–142.

Jun, J.K.; Yoon, J.S.; Seung-Yup Ku; Choi, Y.M.; Hwang, K.R.; Park, S.Y.; Lee, G.H.; Lee, W.D.; Kim, S.H.; Kim, J.G. and Moon, S. Y. 2006: Follicle-stimulating hormone receptor gene polymorphism and ovarian responses to controlled ovarian hyperstimulation for IVF-ET. J. Hum. Genet., 51: 665-670.

Medrano, J. F., Aasen, E. and Sharrow, L. 1990: DNA extraction from nucleated red blood cells. Biotechniques, 8:43.

Laven, J.S.; Mulders, A.G.; Fauser, B.C. and Simoni, M. 2003: Follicle-stimulating hormone receptor polymorphisms in women with normogonadotropic anovulatory infertility. Fertil. Steril., 80:986–992

Loutradis, D.; Patsoula, E.; Minas, V. and Antsaklis, A. 2006: FSH receptor gene polymorphisms have a role for different ovarian response to stimulation in patients entering IVF/ICSI-ET programs. Journal of Assisted Reproduction and Genetics, 23:177- 183.

Marson, E.P.; Ferraz, J.B.S.; Meirelles, F.V.; Balieiro, J.C.C. And Eler, J.P. 2008: Effects of polymorphisms of LHR and FSHR genes on

sexual precocity in Bos Taurus x Bos indicus beef composite population. Genetics and Molecular Research, 7: 243-251.

Ulloa-Aguirre, A.; Midgley, A.R. and Padmanabhan, V. 1995: Follicle-stimulating isohormones: characterization and physiological relevance. Endocr. Rev., 16: 765–787.

Arey, B.J.; Stevis, P.E. and Deecher, D.C. 1997: Induction of promiscuous G protein coupling of the follicle-stimulating hormone (FSH) receptor: a novel mechanism for transducing pleiotropic actions of FSH isoforms. Mol. Endocrinol., 11:517–526.

Zambrano, E.; Zarinan, T.; Olivares, A.; Barrios-de-Tomasi, J. and Ulloa-Aguirre, A. 1999: Receptor binding activity and *in vitro* biological activity of the human FSH charge isoforms as disclosed by heterologous and homologous assay systems: implications for the structure-function relationship of the FSH variants. Endocrine., 10:113–121.

Davis, D.; Liu, X. and Segaloff, D.L. 1995: Identification of the sites of N-linked glycosylation on the follicle-stimulating hormone (FSH) receptor and assessment of their role in FSH receptor function. Mol. Endocrinol., 9:159–170.

Scott, R.T. and Hofmann, G.E. 1995: Prognostic assessment of ovarian reserve. Fertil. Steril., 63:1–11.

Schipper, I.; De Jong, F.H. and Fauser, Bcjm. 1998: Lack of correlation between maximum early follicular phase serum follicle-stimulating hormone levels and menstrual cycle characteristics in women under the age of 35 years. Hum. Reprod., 13:1442–1448.

Findaly, J.K. 1994: Peripheral and local regulators of folliculogenesis. Reprod. Fertil. Dev., 6: 127–139.

Fauser, Bcjm. and Van Heusden, A.M. 1997: Manipulation of human ovarian function: physiological concepts and clinical consequences. Endocr Rev., 18: 71–106.

Fauser, Bcjm. Devroey, P. and Yen, SSC. 1999: Minimal ovarian stimulation for IVF. Appraisal of potential benefits and drawbacks. Hum. Reprod., 14: 2681–2686.