Clinical efficacy of recombinant versus highly purified follicle-stimulating hormone according to follicle-stimulating hormone receptor genotype

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Objective **Conflicting data have been reported on the comparative doses of recombinant follicle-stimulating hormone (rFSH) and urinary highly purified follicle-stimulating hormone (HP-FSH) required for ovarian stimulation. Nothing is known about the clinical efficacy of rFSH or HP-FSH depending on the N680S follicle-stimulating hormone receptor (FSHR) polymorphism. Our aim was to investigate whether the N680S polymorphism of the *FSHR* gene affects ovarian response with different forms of FSH.**

Materials and methods **This retrospective cohort study includes 382 cycles performed at Instituto Bernabeu from 191 oocyte donors. All donors carried out two cycles: one with rFSH and the other one with HP-FSH (group 1, $n = 63$), both with HP-FSH (group 2, $n = 100$) or both with rFSH (group 3, $n = 28$). The results were compared by pairs from each patient. The main outcomes were oocyte yield, metaphase II matured oocytes (MII), days of stimulation, and gonadotropin dosage.**

Results **No significant differences were found when we compared the cycles for donors in group 1. However, according to the FSHR polymorphism, statistical differences were shown. For the SS genotype, more oocytes (16.9 vs. 18.4) and MII (12.8 vs. 15.5) were yielded in the HP-FSH cycle. For the NS genotype, more oocyte (20.1 vs. 16.9) and MII (17.4 vs. 14.2) were yielded in the rFSH cycle. For the NN genotype, no differences were found. No differences were found when we compared the cycles in groups 2 and 3 irrespective of the FSHR polymorphism.**

Conclusion **For the first time, we have shown in a population of egg donors that the N680S *FSHR* gene polymorphism affects the efficacy of HP-FSH or rFSH. The FSHR genotype is an important factor to determine the dosage and the nature of the gonadotropin selected for ovarian stimulation.** ***Pharmacogenetics and Genomics*** **26:288–293**

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Introduction

Follicle-stimulating hormone (FSH) is a key factor in ovarian function. The interaction of the hormone with its cell surface receptor (FSHR), located in granulosa cells, initiates a chain of intracellular reactions characteristic of the G-protein-coupled receptor, yielding follicular development [1].

Controlled ovarian hyperstimulation (COH) using gonadotropin FSH alone or with luteinizing hormone (LH) is a frequently used strategy in Assisted Reproductive Techniques. Ovarian response to FSH, however, varies widely among women undergoing ovarian stimulation [2]. Various predictive markers of COH outcome have been proposed such as age [3], ovarian reserve [4], and cigarette smoking [5]. Besides these parameters, genetic variability also seems to be an important factor. This variability can lead to ineffective, insufficient, or unexpected response to treatment [6]. Pharmacogenetics applied to ovarian response may predict stimulation success [7], but also adjust and design the doses before the treatment.

Almost one thousand single-nucleotide polymorphisms have been located in the *FSHR* gene, only two of which are related to ovarian response. They are located at codon 307 (T307S) and 680 (N680S) in linkage disequilibrium. These polymorphisms alter the properties of the receptor and consequently modify the cellular response to FSH [8].

Clinical studies have shown that the N680S polymorphism determines ovarian response to FSH stimulation in patients undergoing in-vitro fertilization (IVF) treatment [9–13]. Patients with the S680 allele need more FSH during the stimulation phase, suggesting a lower sensitivity to FSH for the S680 allele and a poor response to gonadotropins [14].

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Various FSH-containing products extracted and purified from urine or recombinant in-vitro technology have been developed and administered for COH. The first generation of gonadotropins was human menopausal gonadotropin, extracted from the urine of menopausal women (HMG, a combination of FSH and LH in a 1 : 1 ratio). Since the 1980s, a variety of urinary gonadotropins have been obtained, such as purified FSH, which contains less than 1 IU of LH per 75 IU of FSH. The third generation of urinary gonadotropins was highly purified follicle-stimulating hormone (HP-FSH), with less than 0.1 IU of LH per 75 IU of FSH. The fourth generation of gonadotropins was produced using recombinant DNA technology [recombinant follicle-stimulating hormone (rFSH)], which is free from LH activity. The rFSH ensure a high availability of a biochemically pure FSH preparation that is free from urinary protein contaminants and low immunogenicity, which enables subcutaneous administration. There have been some controversies in terms of the clinical efficacy of human-derived FSH versus rFSH in IVF-ICSI cycles. No clear evidence of the superiority of one preparation over the other has been found [15]. It appears that all available gonadotropins are equally effective and safe. Different FSH isoforms have diverse amounts of sialic acid and complexity of the attached oligosaccharide structures, both of which appear to influence the specific bioactivity [16]. rFSH presents a lower grade of carbohydrate-branched structures and less acidic isoforms than the wild-type FSH [17,18].

To the best of our knowledge, there are no published studies that compare the clinical efficacy of rFSH or HP-FSH depending on the N680S *FSHR* polymorphism. The aim of this study was to investigate the association between the N680S polymorphism of the *FSHR* gene and the ovarian response using HP-FSH or rFSH. We proposed evaluating the ovarian stimulation in a non confusion model such as in patients from the egg donation program because egg donors are young and fertile women with a normal ovarian reserve. The aim of this study is to show whether the N680S *FSHR* polymorphism has a predictive value for ovarian response to stimulation according to the different nature of FSH administered.

Materials and methods

Study population

The selection and recruitment of donors were carried out in our clinic following strict quality criteria, including an extensive chromosomal and genetic evaluation. All donors were Hispanic White and fulfilled the legal requirements in Spain (Spanish law 14/2006). They were between 18 and 35 years of age, healthy, and with no family history of hereditary diseases. Both ASRM and ESHRE guidelines for oocyte donors were followed. A complete gynecological examination was carried out, including follicular basal count, Pap test, and screening for infectious diseases such as HIV, hepatitis B and C, gonoccocia, and syphilis. In addition to the legal requirements, we performed karyotype and genetic screening for cystic fibrosis, fragile X, spinal medullar atrophy, and α and β thalassemia. In this study, we include the results of the *FSHR* 680 polymorphism in 191 oocyte donors. These donors performed 382 COH cycles and the stimulation results were included in this research. All donors carried out two cycles: one with rFSH and the other with HP-FSH (group 1, $n = 63$), both with HP-FSH (group 2, $n = 100$) or both with rFSH (group 3, $n = 28$).

All the participants included in the study provided their informed consent for the collection of peripheral blood samples suitable for molecular analysis. This study involved only the retrospective analysis of anonymous medical records and was approved by the Instituto Bernabeu Institutional Review Board.

Genotyping

DNA was isolated from peripheral blood lymphocytes according to the manufacturer's instructions (Wizard Genomic DNA Purification Kit; Promega, Madison, Wisconsin, USA) and stored at 4°C. Analysis of the *FSHR* gene polymorphism 2039A > G/Asn680Ser was carried out using the predesigned TaqMan allelic discrimination assays (rs6166; Life Technologies Corporation, Carlsbad, New Mexico, USA). Real-time PCR was performed using the StepOne plus system from Applied Biosystems (Waltham, Massachusetts, USA) in accordance with the manufacturer's instructions. Analysis was carried out in accordance with the instructions for the device used.

Ovarian stimulation and oocyte retrieval

After an ultrasound scan performed on day 2 or 3 of the menses showed an absence of large follicles, ovarian stimulation was initiated with 150, 225, and 300 of FSH IU/day according to donor age, BMI, and antral follicle count. Patients received either rFSH (Gonal-F; Merck-Serono, Madrid, Spain) or HP-FSH (Fostipur; Angelini, Barcelona, Spain) on the basis of availability of medication and not for clinical reasons. The gonadotropin-releasing hormone antagonist cetrorelix 0.25 mg/day (Cetrotide; Merck-Serono) was added daily, starting when the leading follicle reached 14 mm in diameter, according to a multiple-dose, flexible protocol. Ovarian response was monitored by transvaginal ultrasound and plasma estradiol concentrations when needed. To trigger the LH surge, 0.4 mg of subcutaneous triptorelin (Decapeptyl; Ipsen Pharma, Barcelona, Spain) was used. Oocytes were aspirated 36 h later by transvaginal, ultrasound-guided needle aspiration under sedation. Oocyte manipulation was performed according to IVF laboratory guidelines.

Statistical analysis

For evaluation of normal distributions, the Shapiro–Wilk's test was performed. Parametric and distribution-free tests were used to analyze the variables on the basis of their distribution. Values are presented as averages ± SD. To evaluate differences between the gonadotropin administered, a *t*-test for paired samples was performed for age, days of stimulation, dose of gonadotropin, and number of oocytes and metaphase II matured oocytes (MII) retrieved. Data were analyzed using the statistical package for the social sciences (SPSS) software (version 20.0; SPSS Inc., Chicago, Illinois, USA). A P value less than 0.05 was considered significant.

Results

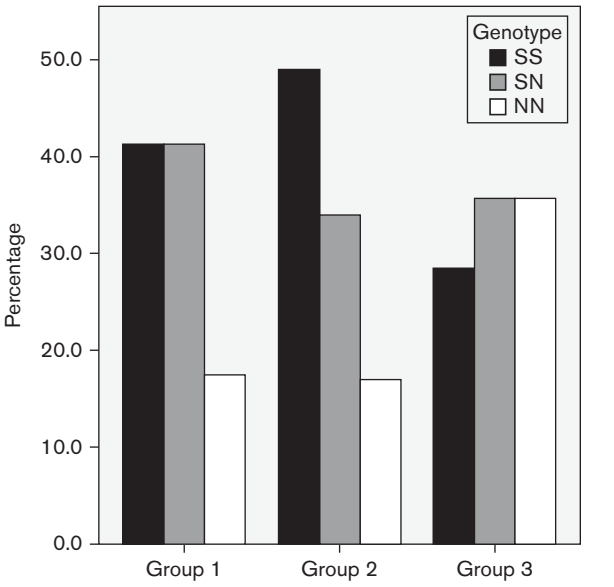
Follicle-stimulating hormone receptor N680S polymorphism genotyping

A total of 191 women were examined for the *FSHR* variant N680S in this study. In total, the results indicated that 83 (43%) patients had the SS genotype, 70 (37%) showed the NS genotype, and 38 (20%) patients had the NN genotype. In group 1, 26 (41%) patients had the SS genotype, 26 (41%) had the NS genotype, and 11 (18%) patients had the NN genotype; in group 2, 49 (49%) patients had the SS genotype, 34 (34%) had the NS genotype, and 17 (17%) patients had the NN genotype; and in group 3, eight (28%) patients had the SS genotype, 10 (36%) had the NS genotype, and 10 (36%) patients had the NN genotype (Fig. 1).

Ovarian stimulation

The 191 oocyte donors included in this study performed 382 COH cycles. To avoid the effect of age on the ovarian reserve and response, we included the closest COH. Table 1 shows the clinical characteristics and ovarian stimulation parameters in the 126 COH performed by donors in group 1. No differences were observed in any parameters between the cycle with rFSH and HP-FSH when the genotype was not taken into account. However, we found differences among cycles according to the N680S polymorphism. For the SS genotype, we found significant differences in the number of retrieved eggs and MII and the gonadotropin doses. More oocytes (16.9 vs. 18.4; $P < 0.05$) and MII (12.8 vs. 15.5; $P < 0.05$) were yielded in the HP-FSH cycle. The gonadotropin consumption for retrieved MII is higher in the rFSH cycle in the SS genotype (162 vs. 128 IU; $P < 0.05$). For the NS genotype, we found significant differences in the number of retrieved eggs and MII and the gonadotropin doses. More oocytes (20.1 vs. 16.9; $P < 0.05$) and MII (17.4 vs. 14.2; $P < 0.05$) were yielded in the rFSH cycle. In contrast with the SS genotype, in the NS genotype, the gonadotropin consumption for retrieved MII was higher in the HP-FSH cycle (133 vs. 107 IU; $P < 0.05$). Finally, as for the NN genotype, no differences were found in any parameter.

Fig. 1



Frequency comparison of N680S.

To show that the differences found in group 1 were because of the correlation between the administered gonadotropin and the genotype S680N and to determine whether there was any variability between the cycles performed by the same donor, we compared the ovarian stimulation parameters between donors using the same gonadotropin. Tables 2 and 3 shows the clinical characteristics and ovarian stimulation parameters in the 200 and 54 COH performed by donors in groups 2 and 3, respectively. No differences were observed in any parameters between cycles 1 and 2 irrespective of the genotype.

Discussion

An inadequate response to stimulation could affect the success of an IVF cycle. To improve the chances of a successful outcome, the doses should be tailored to the patient's characteristics. Several factors can predict ovarian response. Ovarian reserve is probably the most important factor in determining success rates after IVF [4]. However, a meta-analysis has shown that ovarian reserve markers have only modest value in predicting the response to gonadotropins [19]. The search for optimal biomarkers is ongoing for an accurate prognosis of the ovarian response to exogenous gonadotropins [20]. It has recently become evident that genetic factors could explain the differences among individuals in terms of response to drugs. Advanced identification of patients who may show a poor response to standard treatment would be a major clinical advantage for such patients. Moreover, these genetic factors could help us to tailor the treatment by selecting the most adequate doses and the

Table 1 Group 1 donor ovarian stimulation data in relation to the follicle-stimulating hormone receptor S680 genotype

	FSHR N680S genotypes (average ± SD)											
	Total (126)			SS (52)			NS (52)			NN (22)		
	rFSH	HP-FSH	*P*	rFSH	HP-FSH	*P*	rFSH	HP-FSH	*P*	rFSH	HP-FSH	*P*
Donor age	25.6 ± 3.9			25.4 ± 4.1			26.0 ± 3.6			25.0 ± 4.3		
Stimulation length (days)	8.8 ± 1.4	9.2 ± 1.3	0.075	9.1 ± 1.2	9.2 ± 1.2	0.743	8.5 ± 1.4	9.0 ± 1.3	0.073	8.9 ± 1.7	9.6 ± 1.3	0.278
Gonadotropin used (IU)	1979 ± 507	1965 ± 470	0.780	2071 ± 524	1988 ± 403	0.286	1866 ± 391	1884 ± 458	0.810	2030 ± 686	2107 ± 633	0.598
Number of retrieved oocytes	18.6 ± 7.7	18.2 ± 7.4	0.637	16.9 ± 6.8	18.4 ± 8.0	0.028	20.1 ± 8.6	16.9 ± 6.5	0.032	19.3 ± 7.0	20.6 ± 8.1	0.665
Number of MII oocytes	15.4 ± 6.9	15.4 ± 6.4	0.974	12.8 ± 5.2	15.5 ± 7.2	0.048	17.4 ± 7.9	14.2 ± 5.4	0.030	16.7 ± 6.6	17.9 ± 6.5	0.664
Gonadotropin/MII oocyte (IU)	129 ± 74	128 ± 74	0.895	162 ± 101	128 ± 56	0.039	107 ± 50	133 ± 85	0.035	122 ± 104	118 ± 97	0.930

Test performed for statistical analysis: *t*-Student for paired samples.
FSHR, follicle-stimulating hormone receptor; HP-FSH, highly purified follicle-stimulating hormone; MII, metaphase II matured oocytes; rFSH, recombinant follicle-stimulating hormone.

Table 2 Group 2 donor ovarian stimulation data in relation to the follicle-stimulating hormone receptor S680 genotype

	FSHR N680S genotypes (average ± SD)											
	Total (200)			SS (98)			NS (68)			NN (34)		
	HP-FSH	HP-FSH	*P*	HP-FSH	HP-FSH	*P*	HP-FSH	HP-FSH	*P*	HP-FSH	HP-FSH	*P*
Donor age	26.1 ± 3.9			26.1 ± 3.7			26.4 ± 4.0			25.2 ± 4.2		
Stimulation length (days)	9.4 ± 1.4	9.5 ± 1.3	0.509	9.5 ± 1.3	9.6 ± 1.2	0.381	9.4 ± 1.5	9.3 ± 1.4	0.753	9.4 ± 1.5	9.6 ± 1.3	0.522
Gonadotropin used (IU)	2063 ± 572	2118 ± 605	0.312	2120 ± 592	2223 ± 600	0.206	1999 ± 557	2057 ± 554	0.522	2025 ± 559	1941 ± 689	0.555
Number of retrieved oocytes	18.7 ± 7.9	18.3 ± 7.8	0.496	17.9 ± 8.9	18.2 ± 7.8	0.719	19.3 ± 7.7	18.5 ± 8.0	0.336	20.1 ± 5.3	18.4 ± 7.9	0.179
Number of MII oocytes	14.9 ± 5.9	14.5 ± 6.1	0.550	13.9 ± 5.7	14.7 ± 6.6	0.303	15.2 ± 6.4	14.5 ± 6.2	0.457	16.5 ± 5.1	14.7 ± 4.7	0.101
Gonadotropin/MII oocyte (IU)	138 ± 97	146 ± 99	0.324	153 ± 104	151 ± 91	0.993	132 ± 87	142 ± 89	0.357	123 ± 110	132 ± 147	0.329

Test performed for statistical analysis: *t*-Student for paired samples.
FSHR, follicle-stimulating hormone receptor; HP-FSH, highly purified follicle-stimulating hormone; MII, metaphase II matured oocytes.

Table 3 Group 3 donor ovarian stimulation data in relation to the follicle-stimulating hormone receptor S680 genotype

	FSHR N680S genotypes (average ± SD)											
	Total (56)			SS (16)			NS (20)			NN (20)		
	rFSH	rFSH	*P*	rFSH	rFSH	*P*	rFSH	rFSH	*P*	rFSH	rFSH	*P*
Donor age	24.9 ± 3.4			24.3 ± 3.6			25.2 ± 3.6			25.1 ± 3.5		
Stimulation length (days)	9.0 ± 1.0	9.1 ± 1.6	0.601	9.4 ± 1.0	9.6 ± 1.9	0.732	8.4 ± 1.0	9.1 ± 1.1	0.111	9.2 ± 1.1	8.8 ± 1.7	0.555
Gonadotropin used (IU)	2043 ± 485	2051 ± 547	0.930	2138 ± 183	2053 ± 529	0.715	1875 ± 495	2033 ± 517	0.052	2136 ± 620	2068 ± 643	0.674
Number of retrieved oocytes	17.3 ± 5.6	18.8 ± 8.1	0.398	16.8 ± 5.9	18.0 ± 7.0	0.555	16.5 ± 5.2	18.7 ± 9.4	0.533	18.6 ± 6.2	19.4 ± 8.4	0.797
Number of MII oocytes	14.9 ± 5.2	16.3 ± 7.8	0.321	15.1 ± 5.0	15.2 ± 7.4	0.944	13.2 ± 4.7	16.2 ± 9.1	0.340	16.4 ± 5.9	17.2 ± 7.5	0.722
Gonadotropin/MII oocyte (IU)	137 ± 93	126 ± 70	0.259	142 ± 37	135 ± 72	0.494	142 ± 105	126 ± 57	0.643	130 ± 105	120 ± 86	0.454

Test performed for statistical analysis: *t*-Student for paired samples.
FSHR, follicle-stimulating hormone receptor; MII, metaphase II matured oocytes; rFSH, recombinant follicle-stimulating hormone.

type of gonadotropin to achieve the best ovarian response.

Our data suggest that ovarian response produced by rFSH or HP-FSH is affected by the polymorphism genotype on the *FSHR* gene. The number of yielded oocytes and the gonadotropin dosage are associated with the genotype in the N680S polymorphism on the *FSHR* gene on the basis of the gonadotropin administered. For the SS genotype, HP-FSH results are more efficient for COH than rFSH in terms of MII yielded per gonadotropin consumed; however, for the NS genotype, rFSH produces more MII per gonadotropin consumed. As for the NN genotype, the same result was obtained when we used rFSH or HP-FSH. To determine whether these differences are random and caused by intercycle variability, we compared the results of cycles from donors using the same gonadotropin. No differences were found between the two cycles irrespective of the genotype when we used the same gonadotropin.

The genotypic variance of the FSHR was reported for the first time by Aittomäki *et al.* [21]. Although there is some discordance [22], compelling evidence shows that

the N680S polymorphism determines ovarian response to FSH stimulation in patients undergoing IVF treatment [10]. Furthermore, patients with the S680 allele need more FSH during the follicular phase [23,24]. Interestingly, the results of a study carried out on fertile egg donors are in agreement with the previous results. To evaluate ovarian stimulation and embryo implantation potential, oocyte donation is the best model because donors are young women of similar age with normal ovarian function. This study showed that in the SS group, the gonadotropin dosage needed is higher and the oocytes retrieved are fewer than those of the other genotype groups in COH [12]. These findings implied that women with the SS variant of the receptor were more resistant to FSH action than women carrying the other variants [24]. The only clinical trial on gene variants and COH outcome conducted so far has confirmed the previous finding of the effect of the N680S polymorphism, indicating that the lower FSH sensitivity of SS carriers may be overcome by higher FSH doses during COH [25]. Furthermore, a meta-analysis in which patients were divided into poor and good responders confirmed the role of the N680S variant in poor responders during COH [26]. Finally, the last meta-analysis evaluating the influence of genotype N680S polymorphism on poor and hyper-responders to ovarian stimulation showed confirmation of the role of the *FSHR* SS genotype in COH response [27].

In the last few decades, FSH has played a central role in ovulation induction and COH. The technology used to obtain pharmacologically available FSH has traditionally been extraction and purification from the urine of postmenopausal women. The purification process has improved progressively, yielding a HP-FSH. Finally, recombinant human FSH has been developed successfully with absolute purity, a higher batch-to-batch consistency, and no risk of transmission of infectious diseases. The comparative clinical efficacy of HP-FSH and rFSH has been investigated, but currently, there is no clear evidence showing the superiority between treatments in terms of effectiveness [15].

Different FSH isoforms have been found in different preparations. The FSH isoforms are characterized by the heterogeneity of differing amounts of sialic and the complexity of the attached oligosaccharide structures affecting the specific bioactivity [16]. A lower grade of carbohydrate-branched structures and less acidic isoforms are present in rFSH [18]. Less acidic isoforms show high in-vitro bioactivity, but they have a shorter circulating half-life than acidic FSH isoforms. The longer half-life of the acidic isoforms results in more estrogenic follicles and follicular development, maturation, and estradiol secretion [28]. Whether a specific FSH isoform composition affects the effectiveness of preparation for ovarian stimulation has been explored and there is no proof of a difference in the number of retrieved oocytes and the number of mature oocytes [29]. Molecular and structural differences also influence the interactions of different FSH isoforms with their target cell receptor in terms of inducing biological responses *in vitro* and *in vivo* [30]. However, nothing is known about the interaction between different FSH isoforms and FSHR according to the N680S polymorphism. Our results *in vivo* suggest that HP-FSH is more efficient in the FSHR SS variant than rFSH, in contrast with the NS variant, which is more efficient with rFSH. Even though the rFSH show higher binding affinity for the receptor and higher bioactivity because of their oligosaccharide type, they have a shorter half-life because they are more basic than HP-FSH. The higher gonadotropin consumption in the SS group could be explained by the fact that women with the SS variant of the receptor were more resistant to FSH action than women carrying the other variants [24]. In addition, a longer half-life in HP-FSH could maintain the action of FSH for a longer time and overcome the resistance of the FSHR with the SS genotype.

According to our results, the controversies on the clinical efficacy of rFSH or HP-FSH could be explained by the different responses to the *FSHR* genotype. To determine which gonadotropin is superior, we need to include the S680N genotype to avoid confounding factors in terms of a different response. Future trials to explore the clinical efficacy of HP-FSH or rFSH should include the S680N genotype.

In conclusion, to our knowledge, these data show for the first time the relation between the FSHR N680S polymorphism and the gonadotropin used for ovarian stimulation. This investigation indicates that in a population of fertile egg donors, the *FSHR* gene polymorphism at position 680 is associated with a different ovarian response to COH according to the nature of the gonadotropin used. The *FSHR* gene genotype is an important factor for determining the prognosis of COH cycles in fertile women with normal ovulation. Genotyping *FSHR* N680S together with some additional markers may therefore provide information to tailor the protocol used for COH to obtain the best outcome.

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Conflicts of interest

There are no conflicts of interest.

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