RBMO

ELSEVIER

SHORT COMMUNICATION

FSH receptor genotype and its influence on the results of donor ovarian stimulation using corifollitropin alfa

BIOGRAPHY

Belén Lledó received her PhD in molecular biology from the University of Alicante, Spain. In 2004 she moved to Instituto Bernabeu and is now Director of the Molecular Biology Department. She has received prizes at different congresses and published tens of papers on genetic variants in infertility and preimplantation genetic diagnosis.

Belen Lledó[1,*], Jose Antonio Ortiz[1], Mónica Hortal[1], Alba Cascales[1], Ruth Morales[1], Jaime Guerrero[2], Andrea Bernabeu[1,3], Rafael Bernabeu[1,3]

ABSTRACT

Research question: Does the FSH receptor (FSHR) genotype influence the results of donor ovarian stimulation using corifollitropin alfa?

Design: A prospective cohort study was performed including 152 oocyte donor ovarian stimulations: group 1 (n = 80) using a single dose of 150 µg of corifollitropin alpha; and group 2 (n = 72) using in addition to corifollitropin alpha, continued stimulation using recombinant FSH 225 IU daily. Allelic discrimination was used to genotype the FSHR p.N680S polymorphism. Linear regression analysis was performed to study the differences between groups.

Results: No differences in clinical characteristics between genotypes were reported. Overall, the results of ovarian stimulation were better in oocyte donors with SN and NN genotypes compared with SS in terms of the number of retrieved oocytes (15.78 versus 10.83; P = 0.008) and retrieved metaphase II (MII) oocytes (12.34 versus 9.00; P = 0.032). Corresponding differences were also observed in group 1 for the number of retrieved oocytes (13.83 versus 7.50, P = 0.018) and retrieved MII oocytes (10.24 versus 5.42; P = 0.038). However, in group 2 no significant differences were found for oocytes retrieved (17.55 versus 13.06, P = 0.064) or MII oocytes (14.25 versus 11.39; P = 0.12).

Conclusions: This study suggests that ovarian stimulation protocols with corifollitropin alfa in women with the SS genotypes could be associated with fewer oocytes and MII oocytes retrieved. Despite the fact that corifollitropin alfa has a longer half-life, the results for the SS genotype do not match those for the other genotypes, so other factors must be involved. Therefore, to tailor treatments, it would be advisable to genotype women at p.N680S of the FSHR.

[1] Molecular Biology, Instituto Bernabeu Biotech, Alicante, Spain
[2] Reproductive Biology, Instituto Bernabeu of Fertility and Gynecology, Alicante, Spain
[3] Reproductive Medicine, Instituto Bernabeu of Fertility and Gynecology, Alicante, Spain

*Corresponding author. E-mail address: blledo@institutobernabeu.com (B. Lledó). https://doi.org/10.1016/j.rbmo.2022.07.013 1472-6483/
Declaration: The authors report no financial or commercial conflicts of interest.

KEY WORDS
FSHR polymorphism
Ovarian stimulation

INTRODUCTION

Genetic factors could explain the differences in drug response between individuals. The ovarian response to FSH varies widely among women undergoing ovarian stimulation. Previous studies suggest that FSH receptor (FSHR) polymorphism at position p.N680S influences the response to ovarian stimulation. Women with an SS genotype require higher doses of gonadotrophin (Perez-Mayorga et al., 2000) and have a lower number of oocytes received than for NN/SN genotypes (*Alviggi et al., 2018*). A recent Delphi consensus from a diverse international group of experts (*Conforti et al., 2022*) supports this evidence, suggesting that the p.N680S S allele is functionally ''resistant'' to FSH. Studies *in vitro* have shown lower p.N680S S genotype activation compared with the N genotype measured in terms of cell signalling, gene expression and the kinetics of progesterone production, confirming a different sensitivity to FSH (*Casarini et al., 2014*).

Corifollitropin alfa is a newly developed drug characterized by higher bioactivity and longer half-life (*Verbost et al., 2011*). The aim of this study was to elucidate whether a corifollitropin alfa-based protocol, relying on a long-acting recombinant FSH (rFSH), has the same impact on oocytes and metaphase II (MII) oocytes retrieved in a rFSH-based protocol.

MATERIALS AND METHODS

This prospective cohort study included results relating to the FSHR p.N680S polymorphism from 152 oocyte donors undergoing ovarian stimulation at the authors' clinic. The results of the stimulation were included in the present research. This study was approved by the Instituto Bernabeu Institutional Review Board on 19 September 2019 (reference 20/2019).

Egg donors were divided into two groups. In group 1 (n = 80) ovarian stimulation was carried out using a single 150 µg dose of corifollitropin alfa (Elonva; MSD, Netherlands). In group 2 (n = 72), in addition to the 150 µg dose of corifollitropin alfa, stimulation was continued with a daily dose of 225 IU rFSH (Puregon, NV Organon, Oss, The Netherlands) from the 8th day of ovarian stimulation. The gonadotrophin-releasing hormone antagonist ganirelix (Orgalutran; NV Organon, The Netherlands) 0.25 mg daily was introduced from stimulation day 5 to the day of triggering in a fixed regimen. In all cases, triggering was exclusively performed with 0.2 mg of subcutaneous triptorelin (Decapeptyl; Ipsen Pharma, France). Oocyte retrieval and manipulation was performed according to IVF laboratory guidelines.

To genotype position p.N680S of the FSHR, DNA was isolated from peripheral blood lymphocytes. Analysis of the FSHR gene polymorphism at position p.N680S was determined using the predesigned TaqMan allelic discrimination assays (rs6166, Life Technologies, USA). A real-time polymerase chain reaction was performed using StepOnePlus™ (Thermo Fisher Scientific, USA).

Donor characteristics were tested using the Wilcoxon rank sum test. Linear regression analysis was performed to study the differences between the groups by carrying out a correction for the variables anti-Müllerian hormone, age and body mass index (BMI). The statistical analysis was undertaken using SPSS version 20.0 software (SPSS, USA).

RESULTS

Regarding the frequency of FSHR variants at p.N680S in this study, 30 donors had the SS genotype (19.74%), 68 had the SN genotype (44.74%) and in 54 donors the NN genotype was detected (35.53%).

As for the clinical characteristics, in total no differences were shown between the two groups of genotypes (SN/NN versus SS) in terms of age (24.04 versus 26.43; P = 0.514), BMI (22.22 versus 22.83; P = 0.781), anti-Müllerian hormone concentration (26.40 versus, 23.58 pmol/l; P = 0.4), antral follicle count (15.57 versus 14.97; P = 0.3) or number of previous ovarian stimulations (2.71 versus 2.53; P = 0.812). In addition, no differences in clinical characteristics were reported between the FSHR genotypes for the two groups (TABLE 1).

Concerning the results of ovarian stimulation using corifollitropin alfa, for the whole set of patients a higher number of oocytes (15.78 versus 10.83; P = 0.008) and MII oocytes (12.34 versus 9.00; P = 0.032) were retrieved from donors carrying NN/SN genotypes. Moreover, when the data were analysed by treatment group, there were significant differences in group 1 in the number of oocytes (13.83 versus 7.50, P = 0.018) and MII oocytes (10.24 versus 5.42; P = 0.038) retrieved between the SN/NN and SS genotypes. On the other hand, in group 2 no significant difference was found in the number of retrieved oocytes (17.55 versus 13.06, P = 0.064) or MII oocytes (14.25 versus 11.39; P = 0.12) in donors carrying the NN/SN genotype compared with SS donors.

DISCUSSION

To the authors' knowledge, these data show for the first time the relationship between the FSHR p.N680S polymorphism and ovarian stimulation using corifollitropin alfa. The data suggest that, in the SS genotype, the use of a corifollitropin alfa-based ovarian stimulation protocol may be linked to a lower number of oocytes and MII oocytes retrieved.

Studies on pharmacogenetics have demonstrated that, in women with the SS genotype, the gonadotrophin dosage needed for ovarian stimulation is higher and the number of retrieved oocytes is lower than that for other genotypes. These findings implied that women with the SS variant of the receptor were more resistant to the action of FSH than women carrying the other variants (*Perez Mayorga et al., 2000*). A longer FSH half-life could maintain the action of FSH for longer and might overcome the resistance of the FSHR with an SS genotype.

Corifollitropin alfa is a recombinant chimeric glycoprotein, obtained by fusing rFSH with the carboxyterminal peptide of the beta subunit of human chorionic gonadotrophin. This fusion produces a half-life longer than that of FSH and increased bioactivity (*Verbost et al., 2011*). Given this, corifollitropin alfa could be considered as an alternative for ovarian stimulation in women with the SS genotype, whose receptors are more resistant to FSH. Nevertheless, the current data suggest that the outcome of ovarian stimulation using corifollitropin alfa did not improve for the SS genotype and was in fact worse than for the NN and SN genotypes, as per previous studies using rFSH. Therefore, the benefit of using long-acting rFSH in SS patients

TABLE 1 DONOR CHARACTERISTICS AND DATA FROM OVARIAN STIMULATION WITH CORIFOLLITROPIN ALFA IN RELATION TO FSH RECEPTOR P.N680S GENOTYPE

Characteristic	NN/SN (*n* = 122)[a]	SS (*n* = 30)[a]	*P*-value[b]
Total			
Age (years)	24.04 (6.27)	26.43 (4.19)	0.514
AMH (pmol/l)	26.40 (15.56)	23.58 (13.45)	0.4
Antral follicle count	15.57 (2.35)	14.97 (2.24)	0.3
Days of stimulation	9.93 (1.35)	10.00 (1.74)	0.9
Oestradiol (pg/ml)	2193.89 (1482.08)	1663.06 (1226.19)	0.074
Oocytes retrieved	15.78 (9.25)	10.83 (7.07)	0.008[c]
MII oocytes retrieved	12.34 (7.74)	9.00 (6.40)	0.032[c]
Group 1 (*n* = 80)			
Age (years)	24.17 (5.89)	26.22 (4.39)	0.2
AMH (pmol/l)	27.88 (17.13)	19.82 (13.99)	0.12
Antral follicle count	16.00 (2.44)	14.42 (2.47)	0.052
Days of stimulation	9.66 (1.36)	9.67 (1.44)	0.9
Oestradiol (pg/ml)	1981.36 (1589.69)	1273.94 (1408.78)	0.094
Oocytes retrieved	13.83 (9.33)	7.50 (6.59)	0.018[c]
MII oocytes retrieved	10.24 (7.79)	5.42 (4.40)	0.038[c]
Group 2 (*n* = 72)			
Age (years)	23.93 (6.65)	26.58 (4.18)	0.14
AMH (pmol/l)	25.06 (13.98)	26.08 (12.86)	0.6
Antral follicle count	15.19 (2.22)	15.33 (2.06)	0.7
Days of stimulation	10.19 (1.31)	10.22 (1.93)	0.6
Oestradiol (pg/ml)	2389.56 (1359.02)	1937.72 (1035.87)	0.3
Gonadotrophin dose (IU)	637.50 (312.41)	569.12 (347.82)	0.4
Oocytes retrieved	17.55 (8.88)	13.06 (6.65)	0.064[c]
MII oocytes retrieved	14.25 (7.24)	11.39 (6.49)	0.12[c]

Group 1 were treated with a single dose of 150 µg of corifollitropin alfa; in group 2, in addition to this, stimulation was continued with recombinant FSH 225 IU daily.

[a] Data are mean (SD).

[b] Mann–Whitney *U*-test.

[c] Linear regression using the SS genotype as the reference, and age, body mass index and AMH as confounding factors.

AMH, anti-Müllerian hormone; MII, metaphase II.

did not overcome the FSH resistance of the p.N680S S allele.

Moreover, a comparison between groups may show differences in ovarian stimulation outcomes according to the p.N680S genotype and ovarian stimulation protocol, but these would obviously be due to cumulative FSH doses, as previously reported. Further powered clinical studies in homogeneous patients with FSH at equal doses could demonstrate whether the doses or the ovarian stimulation protocols are the reason for these differences.

In conclusion, this investigation confirms that, in the population of fertile egg donors, the FSHR gene polymorphism at position p.N680S is associated with different ovarian responses to ovarian stimulation using corifollitropin alfa. The oocyte yield in women with the SS p.N680S genotype is lower in those undergoing ovarian stimulation with long-acting rFSH, as previously reported for daily rFSH. The FSHR genotype is an important factor in determining the prognosis of ovarian stimulation cycles in fertile women. Genotyping FSHR p.N680S together with some additional markers may therefore provide information to tailor the protocol used for ovarian stimulation in order to obtain the best outcome, mainly in women with the SS genotype, for whom the worst results have been showed.

REFERENCES

Alviggi, C., Conforti, A., Santi, D., Esteves, S.C., Andersen, C.Y., Humaidan, P., Chiodini, P., De Placido, G., Simoni, M. **Clinical relevance of genetic variants of gonadotrophins and their receptors in controlled ovarian stimulation: a systematic review and meta-analysis.** Hum. Reprod. Update 2018 Sep 1; 24: 599–614

Casarini, L., Moriondo, V., Marino, M., Adversi, F., Capodanno, F., Grisolia, C., La Marca, A., La Sala, G.B., Simoni, M. **FSHR polymorphism p.N680S mediates different responses to FSH in vitro.** Mol. Cell Endocrinol. 2014 Aug 5; 393: 83–91

Conforti, A., Tüttelmann, F., Alviggi, C., Behre, H.M., Fischer, R., Hu, L., Polyzos, N.P., Chuderland, D., Rama Raju, G.A., D'Hooghe, T., Simoni, M., Sunkara, S.K., Longobardi, S. **Effect of Genetic Variants of Gonadotropins and Their Receptors on Ovarian Stimulation Outcomes: A Delphi**

Consensus. Front Endocrinol. (Lausanne) 2022 Feb 1; 12797365

Perez Mayorga, M., Gromoll, J., Behre, H.M., Gassner, C., Nieschlag, E., Simoni, M. **Ovarian response to follicle-stimulating hormone (FSH) stimulation depends on the FSH receptor genotype.** J. Clin. Endocrinol. Metab. 2000 Sep; 85: 3365–3369

Verbost, P., Sloot, W.N., Rose, U.M., de Leeuw, R., Hanssen, R.G., Verheijden, G.F. **Pharmacologic profiling of corifollitropin alfa, the first developed sustained follicle stimulant.** Eur. J. Pharmacol. 2011 Jan 25; 651: 227–233

Received 4 March 2022; received in revised form 30 June 2022; accepted 22 July 2022.