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ARTICLE

Association of allelic combinations of *FSHR* gene polymorphisms with ovarian response

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Abstract During an IVF protocol, exogenous FSH is administered to women for ovulation induction. The ovarian response to gonadotrophin stimulation is variable and unpredictable in these women. The *FSHR* is the most studied gene in relation to ovarian response. The association of a *FSHR* gene polymorphism at position 680 (p.Asn680Ser) with ovarian response has been well documented. Recently, a polymorphism at position −29 in the 5′-untranslated region of *FSHR* (g.−29G>A) has been reported to be associated with poor ovarian response and reduced *FSHR* expression. The present study evaluated the combined effect of the polymorphisms at positions −29 and 680 of *FSHR* with type of ovarian response and receptor expression. The two *FSHR* gene polymorphisms together formed four discrete haplotypes and nine allelic combinations. Various clinical parameters revealed that 75% of the subjects with A/A–Asn/Asn genotype were poor ovarian responders (odds ratio 7.92; $P = 0.009$). The relative *FSHR* mRNA expression in granulosa cells indicated that subjects with A/A–Asn/Asn genotype express significantly lower level of *FSHR* as compared to the subjects with G/G–Asn/Ser genotype ($P = 0.029$). These results indicate that A/A–Asn/Asn genotype could be used as a potential marker to predict poor ovarian response.

KEYWORDS: FSH, FSHR, genotype analysis, poor ovarian response

Introduction

Exogenous FSH is administered to women undergoing IVF. It has been well documented that the ovarian response to the gonadotrophin stimulation is variable and unpredictable (Keay et al., 1997). Some women show a hyperresponse to the minimal dose of FSH, which may lead to a clinical condition known as ovarian hyperstimulation syndrome (OHSS). On the other hand, some women, in spite of receiving a higher dose of FSH, are poor responders, resulting in decreased number of retrieved mature oocytes. Such poor

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response may result in repeated stimulation cycles which may lead to a financial burden. Various parameters such as age and diminished ovarian reserve (Kligman and Rosenwaks, 2001), basal serum FSH concentrations (Balasch et al., 1996), poor follicular flow (Battaglia et al., 2000) and serum anti-Müllerian hormone concentrations (Nardo et al., 2009) have been proposed to predict type of ovarian response. Apart from these parameters, polymorphisms in various genes such as *ESR1*, *ESR2*, *CYP19A1*, *BMP15* and *AMH* have been studied extensively as markers to predict type of ovarian response (Altmäe et al., 2007; de-Castro et al., 2004; Morón and Ruiz, 2010).

FSH acts through binding to its specific receptor located in the plasma membrane of granulosa cells in the ovary. It has been reported that FSH receptor (FSHR) knockout mice are infertile (Dierich et al., 1998) and their phenotype was similar to the one observed in infertile women with an inactivating mutation in *FSHR* (Themmen and Huhtaniemi, 2000). These observations indicate that the normal functioning of FSHR is crucial for fertility in females. The polymorphisms g.−29G>A, p.Thr307Ala and p.Asn680Ser have been studied extensively with respect to ovarian response to FSH stimulation (Greb et al., 2005; Loutradis et al., 2006; Simoni et al., 2002; Sudo et al., 2002).

Perez-Mayorga et al. (2000) first reported the association of higher basal FSH concentrations with Ser/Ser genotype at position 680 (rs6166) of *FSHR* in women undergoing IVF. Recently, meta-analyses carried out by Morón and Ruiz (2010), Altmäe et al. (2011) and La-Marca et al. (2013) did suggest that this *FSHR* gene polymorphism can be used as a potential marker to predict poor ovarian response. However, there are reports from different populations such as the Netherlands (Klinkert et al., 2006; Laven et al., 2003), and the UK (Mohiyiddeen et al., 2012) which indicate that there is no association observed with respect to this polymorphism and poor ovarian response. On the contrary, Klinkert et al. (2006) observed the association of p.Ser680Ser genotype with a higher pregnancy rate. Our previous work carried out in Indian women undergoing IVF showed that, although not statistically significant, 50% of the subjects with p.Ser680Ser genotype developed OHSS (Achrekar et al., 2009a). These contradicting observations suggest the need to understand the competence of this polymorphism as a predictive marker for ovarian response.

Recently, a polymorphism in the 5′-untranslated region of *FSHR* at position −29 (rs1394205) has been studied to evaluate its association with ovarian response. This polymorphism has been reported to be present in the viral E26 transformation specific sequence (cETS-1) transcription factor binding site. Wunsch et al. (2005) identified the g.−29G>A polymorphism in women undergoing IVF; however, they did not find any association of this polymorphism with basal FSH or oestradiol concentrations in these women. Whereas Nakayama et al. (2006) demonstrated by an in-vitro analysis in CHO cells that the A allele at position −29 of *FSHR* expressed a significantly lower level of luciferase activity as compared to the G allele, which could be due to loss of cETS-1 transcription factor binding site. Cai et al. (2007) reported that there might be an association between reduced *FSHR* expression and poor ovarian response in women undergoing IVF. Studies carried out by our group with 50 subjects revealed that $A^{-29}A$ genotype is associated with poor ovarian response (Achrekar et al., 2009b). Analysis of an additional 100 subjects also showed similar association where 72% of the subjects with the A/A genotype were found to be poor ovarian responders. Further, the poor ovarian response observed in subjects with A/A genotype is due to reduced receptor expression at the transcript and protein levels in granulosa cells (Desai et al., 2011).

Efforts were made to study the possible combined effect of the polymorphism in the promoter region (at position −29) and the coding region (at position 680) by Wunsch et al. (2005), where they reported no association of the allelic combinations with basal FSH concentrations in women undergoing IVF from a German population. However, further analysis of the various clinical and endocrinological parameters is essential to understand its implications in predicting ovarian response.

Although the reasons for altered ovarian response observed in women are not known, the *FSHR* genotype is one of the major determinants of FSH action. Most of the studies reported previously have shown the association of altered ovarian response with *FSHR* gene polymorphisms either at position −29 or at position 680. Therefore, this study analysed the association of allelic combinations of the polymorphisms at positions −29 and 680 of *FSHR* with ovarian response to FSH stimulation in Indian women. This study also describes the association of these genotypes with the level of *FSHR* mRNA expression in granulosa cells.

Materials and methods

Study subjects

The present study analysed the association between genotypes at positions −29 and 680 of *FSHR* in combination with the clinical parameters and *FSHR* expression at the transcript level from the data reported in earlier studies (Achrekar et al., 2009a,b; Desai et al., 2011). For the clinical and endocrine parameters, age, basal FSH, amount of exogenous FSH administered for ovulation induction, oestradiol concentrations before and on the day of human chorionic gonadotrophin (HCG) administration, number of preovulatory follicles and retrieved oocytes were recorded for 150 subjects, and the number of mature oocytes was available for 100 subjects. The study was approved by the institutional ethics committee for clinical research (reference number D/IECCR/56/2009, approved 21 July 2009). Informed consent was obtained from all the subjects enrolled in this study. A total of 150 normogonadotrophic ovulatory women (menstrual cycle length 25 to 35 days) with infertility due to male or tubal factor or with unexplained infertility were retrospectively analysed. Women with polycystic ovarian syndrome, endometriosis and hyperprolactinaemia were excluded from this study. All the subjects were of Indian ethnicity.

Genotyping and quantitative real-time PCR

The genotyping for the polymorphisms at positions −29 and 680 in subjects recruited in this study was carried out as described earlier (Achrekar et al., 2009a,b; Desai et al., 2011). *FSHR* mRNA expression was quantified in granulosa