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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

Table 1 Clinical and endocrinological parameters for 150 subjects undergoing IVF based on the combinations of alleles at positions −29 (A or G) and 680 (Asn or Ser) of *FSHR*.

Characteristic	*1. G/G—Asn/Asn (n = 21)*	*2. G/G—Asn/Ser (n = 27)*	*3. G/G—Ser/Ser (n = 15)*	*4. G/A—Asn/Asn (n = 24)*	*5. G/A—Asn/Ser (n = 28)*	*6. G/A—Ser/Ser (n = 17)*	*7. A/A—Asn/Asn (n = 8)*	*8. A/A—Asn/Ser (n = 10)*
Age (years)	30.86 ± 1.03^{6,7}	31.11 ± 0.79^{6,7}	33.67 ± 1.18^{8}	31.96 ± 0.98	31.29 ± 0.63^{7}	33.76 ± 0.99^{1,2,8}	34.75 ± 1.52^{1,2,5,8}	30.00 ± 1.49^{3,6,7}
Basal FSH (IU/l)	5.87 ± 0.43	6.46 ± 0.41	6.75 ± 0.47	6.61 ± 0.48	6.67 ± 0.38	6.57 ± 0.48	7.91 ± 1.20	6.20 ± 0.83
Exogenous FSH administered (IU)	2754.38 ± 185.48^{6,7}	2480.56 ± 194.00^{6,7}	2639.87 ± 304.99^{6,7}	3093.33 ± 315.17^{7}	2653.68 ± 210.10^{6,7}	3562.47 ± 336.47^{1,2,3,5}	4437.50 ± 420.96^{1,2,3,4,5,8}	3280.80 ± 354.80^{7}
Oestradiol before day of HCG (pg/ml)	1808.57 ± 192.49	1789.26 ± 166.58	1622.20 ± 197.20	2034.17 ± 219.03	2001.25 ± 173.63	2027.29 ± 334.46	1451.25 ± 119.32	1644.30 ± 267.33
Oestradiol on day of HCG (pg/ml)	2130.57 ± 237.87	2056.63 ± 201.39	1999.20 ± 251.32	2433.79 ± 267.50	2390.68 ± 252.10	2286.00 ± 335.51	1560.50 ± 123.05	1994.80 ± 277.74
No. of preovulatory follicles	14.71 ± 0.94^{3,6,7,8}	13.67 ± 0.87^{3,7,8}	10.53 ± 1.12^{1,2}	13.29 ± 1.05^{7,8}	12.54 ± 0.85^{8}	11.71 ± 1.01^{1,8}	9.50 ± 1.05^{1,2,4}	7.80 ± 1.19^{1,2,4,5,6}
No. of oocytes retrieved	16.43 ± 1.50^{3,6,7,8}	15.11 ± 1.23^{8}	11.13 ± 2.13^{1}	15.04 ± 1.74^{8}	13.82 ± 1.44^{8}	10.82 ± 1.66^{1}	10.50 ± 1.19^{1}	8.10 ± 0.92^{1,2,4,5}
No. of mature oocytes retrieved ($n = 100$)	13.00 ± 1.38^{6}	11.95 ± 1.25	9.93 ± 1.87	11.14 ± 2.03	10.12 ± 1.66	8.00 ± 1.29^{1}	7.43 ± 1.21	6.25 ± 1.37

Values are mean ± SEM. One-way ANOVA tests and LSD post-hoc multiple comparisons were used for analysis of variance.
For simplicity, eight different genotypes are numbered from 1 to 8. Statistically significant difference ($P < 0.05$) observed for a given characteristic between the genotypes is indicated by number in superscript (^{1} to ^{8}).

cells collected from subjects undergoing IVF as reported earlier (Desai et al., 2011). The level of relative *FSHR* mRNA expression was compared among different allelic combinations at positions −29 and 680 by one-way ANOVA.

Statistical analysis

Chi-squared analysis was used to determine whether the genotype distribution at both the polymorphisms conformed to Hardy—Weinberg equilibrium. Odds ratio (OR) and the corresponding 95% confidence interval (CI) were calculated by Epi Info version 6 (World Health Organization, Geneva, Switzerland, USA) to measure the strength of the association of the genotypes with poor ovarian response. The clinical parameters and the level of *FSHR* mRNA expression in granulosa cells was compared among the different allelic variants at positions −29 and 680 using one-way ANOVA and the least significant difference post-hoc multiple comparisons test. The clinical parameters were compared among subjects when segregated on the basis of type of indication and type of ovarian response. Linear regression was carried out to compare various parameters as predictor of poor ovarian response, where age was used as a covariate. Statistical analysis was performed with Statistical Package for Social Sciences for Windows version 16 (SPSS, Chicago, IL, USA). $P \leq 0.05$ was considered statistically significant.

Results

Genotype frequency distributions

The frequency distribution of the *FSHR* gene polymorphisms at positions −29 and 680 in subjects undergoing IVF was analysed. In a total 150 subjects, for genotypes at position −29 the number of subjects were 63 (G/G), 69 (G/A), 18 (A/A), whereas for genotypes at position 680 the number of subjects were 53 (Asn/Asn), 65 (Asn/Ser) and 32 (Ser/Ser). The frequency distribution for the genotypes at both the positions was found to be in Hardy—Weinberg equilibrium.

As the polymorphisms at positions −29 and 680 were reported to be not in linkage disequilibrium (Ferlin et al., 2008; Wunsch et al., 2005), the two polymorphisms formed four discrete haplotypes such as A^{-29}/Asn^{680}, A^{-29}/Ser^{680}, G^{-29}/Asn^{680}, G^{-29}/Ser^{680} and nine allelic combinations. We then analysed the frequency distribution of the nine allelic variants formed. These genotypes are referred as G/G—Asn/Asn, G/G—Asn/Ser, G/G—Ser/Ser, G/A—Asn/Asn, G/A—Asn/Ser, G/A—Ser/Ser, A/A—Asn/Asn, A/A—Asn/Ser, and A/A—Ser/Ser in the present manuscript. It was interesting to note that none of the subjects showed presence of A/A—Ser/Ser genotype in the studied population (**Table 1**).

Clinical and endocrine parameters

To analyse the potential association between the genotypes at positions −29 and 680 of *FSHR* with the ovarian response during gonadotrophin stimulation, the clinical,

endocrine and ultrasonographic parameters were recorded for all the women ($n = 150$) recruited in this study (**Table 1**). Subjects were segregated on the basis of the genotypes.

Subjects with the A/A—Asn/Asn genotype were observed to have a significantly higher age (34.75 ± 1.52 years) as compared to the subjects with G/G—Asn/Asn ($P = 0.031$), G/G—Asn/Ser ($P = 0.037$), G/A—Asn/Ser ($P = 0.046$) or A/A—Asn/Ser ($P = 0.021$) genotypes. The basal FSH concentrations and peak oestradiol concentrations in serum before and on the day of HCG showed no statistically significant difference among the eight genotypes. However, it was interesting to note that the increase in oestradiol concentration post HCG treatment was minimal in subjects with the A/A—Asn/Asn genotype as compared to subjects with all the other genotypes.

In addition, the amount of exogenous FSH required for ovarian stimulation was highest in subjects with the A/A—Asn/Asn genotype (4437.50 ± 420 IU) and differed significantly as compared to subjects with the G/G—Asn/Asn ($P = 0.001$), G/G—Asn/Ser ($P < 0.001$), G/G—Ser/Ser ($P = 0.001$), G/A—Asn/Asn ($P = 0.006$), G/A—Asn/Ser ($P < 0.001$) and A/A—Asn/Ser ($P = 0.042$) genotypes. The ultrasound findings also revealed that the number of preovulatory follicles were significantly lower in subjects with the A/A—Asn/Asn genotype (9.50 ± 1.05) as compared with the G/G—Asn/Asn ($P = 0.005$), G/G—Asn/Ser ($P = 0.021$), G/A—Asn/Asn ($P = 0.038$) and genotypes (14.71 ± 0.94, 13.67 ± 0.87, and 13.29 ± 1.05 respectively). Moreover, the number of oocytes retrieved in subjects with the A/A—Asn/Asn genotype (10.50 ± 1.19) was significantly lower as compared to G/G—Asn/Asn genotype (16.43 ± 1.50; $P = 0.046$).

Subjects with the A/A—Asn/Ser genotype demonstrated significantly lower number of follicles when compared to subjects with G/G—Asn/Asn ($P < 0.001$), G/G—Asn/Ser ($P < 0.001$), G/A—Asn/Asn ($P = 0.001$), G/A—Asn/Ser ($P = 0.004$) and G/A-Ser/Ser ($P = 0.029$) genotypes. Further, the number of oocytes was significantly lower in subjects with the A/A—Asn/Ser genotype when compared to subjects with the G/G—Asn/Asn ($P = 0.003$), G/G—Asn/Ser ($P = 0.008$), G/A—Asn/Asn ($P = 0.010$) and G/A—Asn/Ser ($P = 0.030$) genotypes.

The number of mature oocytes ($n = 100$) were observed to be significantly higher in subjects with the G/G—Asn/Asn genotype (13.00 ± 1.38) as compared to the G/A—Ser/Ser genotype (8.00 ± 1.29, $P = 0.043$) (**Table 1**).

The number of subjects with different genotypes were evenly distributed among subjects with male or tubal factor or unexplained infertility (Supplementary Table S1, available online). Various clinical and endocrine parameters compared on the basis of type of indication and type of ovarian response has been provided in Supplementary Table S2. The predictive values for various parameters were evaluated by linear regression analysis, where age was considered as a covariate. Parameters such as exogenous FSH administered ($P < 0.001$), number of follicles ($P = 0.001$) and number of oocytes ($P = 0.039$) were observed to significantly influence the ovarian response, whereas age was not significantly associated with ovarian response (Supplementary Table S3).

Association of genotypes with ovarian response

To study the association of these genotypes with poor ovarian response, the total number of poor ovarian responders for each allelic variant and the OR were calculated (**Table 2**). In the study group of 150 subjects, 38 were poor ovarian responders. When these poor responders were further segregated on the basis of genotype, it was observed that six of the eight subjects (75%) with the A/A—Asn/Asn genotype and six of the 10 subjects (60%) with the A/A—Asn/Ser genotype were poor ovarian responders. For the remaining genotypes, the number of poor ovarian responders ranged from 7% to 35%. The chi-squared test was employed to study the significant association of the *FSHR* genotypes with poor ovarian response. The OR for the A/A—Asn/Asn genotype was 7.92 (95% CI 1.533—40.950; $P = 0.009$) and for the A/A—Asn/Ser genotype was 4.67 (95% CI 1.245—17.56; $P = 0.022$), whereas the OR for the G/G—Asn/Ser genotype was 0.16 (95% CI 0.037—0.736; $P = 0.008$).

FSHR mRNA expression in granulosa cells

To study the association between the level of *FSHR* expression and type of ovarian response, the relative *FSHR* mRNA expression estimated in 100 subjects undergoing IVF (Desai et al., 2011) was used. The *FSHR* mRNA expression normalized with β-actin (used as a housekeeping control) was monitored by quantitative real-time PCR and compared amongst the eight *FSHR* genotypes. The level of *FSHR* mRNA expression was observed to be variable among the genotypes. The subjects with the G/G—Asn/Asn (0.5 ± 0.1, $P = 0.039$), G/A—Ser/Ser (0.6 ± 0.2, $P = 0.050$) and A/A—Asn/Asn (0.19 ± 0.08, $P = 0.029$) genotypes expressed significantly lower levels of *FSHR* mRNA in the granulosa cells as compared with subjects with the G/G—Asn/Ser (2.06 ± 0.7) genotype. It was intriguing to find that the *FSHR* expression at the transcript level was higher in case of subjects with G/G—Asn/Ser genotype as compared to other genotypes (**Figure 1**).

Discussion

The present study evaluated the association of the allelic combinations of genotypes at positions −29 and 680 of *FSHR* with ovarian response to FSH stimulation. In the subjects studied, clinical and endocrine parameters suggest that the A/A—Asn/Asn genotype is associated with poor ovarian response. Moreover, it is interesting to note that subjects with the A/A—Asn/Asn genotype express reduced *FSHR* mRNA concentrations in granulosa cells. These findings suggest the usefulness of studying the allelic combinations of *FSHR* gene polymorphisms in predicting the type of ovarian response.

Recently, the associations of *FSHR* polymorphism at position −29 with poor ovarian response have been reviewed (Laan et al., 2012). Studies by in-vitro analysis revealed that the A allele is associated with reduced *FSHR* expression (Desai et al., 2011; Nakayama et al., 2006). In the case of the polymorphism at position 680, a number of

Table 2 Frequencies of allelic combinations at positions −29 and 680 of *FSHR* in subjects undergoing IVF protocol and their relationship with the occurrence of poor ovarian response.

	G/G—Asn/Asn (n = 21)	*G/G—Asn/Ser (n = 27)*	*G/G—Ser/Ser (n = 15)*	*G/A—Asn/Asn (n = 24)*	*G/A—Asn/Ser (n = 28)*	*G/A—Ser/Ser (n = 17)*	*A/A—Asn/Asn (n = 8)*	*A/A—Asn/Ser (n = 10)*
Poor ovarian responders	4 (19.04)	2 (7.40)	5 (33.33)	4 (16.67)	5 (17.86)	6 (35.29)	6 (75.00)	6 (60.00)
Odds ratio (95% CI)	0.52 (0.165—1.656)	0.16 (0.037—0.736)	1.23 (0.395—3.835)	0.46 (0.148—1.447)	0.46 (0.163—1.308)	1.31 (0.454—3.805)	7.92 (1.533—40.95)	4.67 (1.245—17.56)
P	NS	0.008	NS	NS	NS	NS	0.009	0.022

$P < 0.05$ calculated by chi-squared test.CI = Confidence interval; NS = not statistically significant.

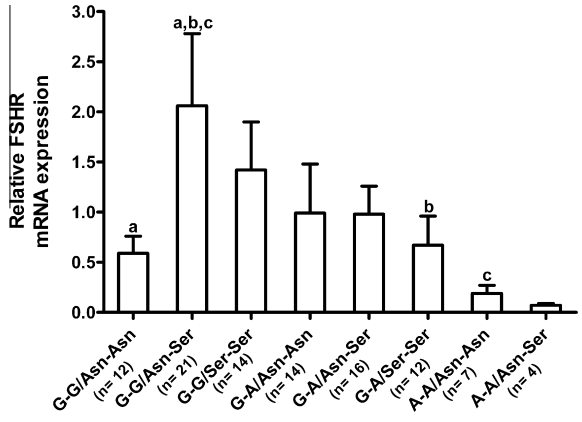

Figure 1 Level of *FSHR* mRNA expression compared among 100 subjects with different combinations of alleles at positions −29 and 680 of *FSHR* as monitored by real-time PCR. One-way ANOVA; same letters indicate statistically significant differences ($P \leq 0.05$).

association studies in different populations have been carried out extensively (Mohammad et al., 2011). Many of these suggest that the Ser680 allele is a potential marker for predicting poor ovarian response; there are few reports which suggest no association of p.Asn680Ser with ovarian response (La-Marca et al., 2013). A few studies suggest that the subjects with the Asn/Ser genotype are more associated with good response to FSH stimulation, whereas the subjects with Ser/Ser and Asn/Asn genotypes have a tendency to resist FSH stimulation and thus require more exogenous FSH for ovarian stimulation (Loutradis et al., 2012). Thus, there is a lack of consistency in the outcome of these association studies. The present study analysed both polymorphisms in combination to evaluate their effect on ovarian response. There were no subjects with the A/A—Ser/Ser genotype in this population. Previous studies have reported that the A/A genotype at position −29 was associated with poor ovarian response, whereas the Ser/Ser genotype at position 680 was associated with OHSS (Achrekar et al., 2009a,b; Desai et al., 2011). Therefore, probability of finding this combination of A/A at position −29 and Ser/Ser at position 680 is indeed rare.

When subjects were segregated on the basis of the allelic combinations, the parameters signifying ovarian response varied among these genotypes. In general, higher age is believed to be associated with poor ovarian response. It was observed that subjects with the A/A—Asn/Asn genotype show significantly higher average age as compared to the subjects with G/G—Asn/Asn, G/G—Asn/Ser, G/A—Asn/Ser and A/A—Asn/Ser genotypes. However, the basal FSH concentrations on day 3 were similar in all subjects. Similar observations were reported by Wunsch et al. (2005), wherein no significant differences in the basal serum FSH concentration among the allelic combinations were observed. The present study noted that the rise in the oestradiol concentrations post HCG treatment was minimal, although not significant, in subjects with the A/A—Asn/Asn genotype as compared to all other genotypes. This might suggest that subjects with the A/A—Asn/Asn genotype are less responsive to FSH treatment than other genotypes.

The total amount of FSH administered to the subjects ranged 2400—4500 IU among the genotypes. Subjects with the A/A—Asn/Asn genotype required significantly higher amounts of exogenous FSH for ovarian stimulation as compared with the G/G—Asn/Asn, G/G—Asn/Ser, G/G—Ser/Ser, G/A—Asn/Asn, G/A—Asn/Ser and A/A—Asn/Ser genotypes. This implies that the subjects with A/A—Asn/Asn genotype are more resistant to FSH stimulation. The number of preovulatory follicles and the number of oocytes retrieved were lower in subjects with the A/A—Asn/Asn and A/A—Asn/Ser genotypes as compared to all other genotypes, although not all differences were statistically significant. The data for the mature number of oocytes was available only for 100 women undergoing IVF. Although the mean numbers of oocytes in subjects with the A/A—Asn/Asn and A/A—Asn/Ser genotypes were 10.50 and 8.10 respectively ($n = 150$), the number of mature (MII phase) oocytes was 7.43 ± 1.21 in subjects with the A/A—Asn/Asn genotype and 6.25 ± 1.37 in subjects with the A/A—Asn/Ser genotype ($n = 100$). The above parameters clearly indicate that the A/A—Asn/Asn and A/A—Asn/Ser genotypes are associated with poor ovarian response to FSH stimulation. From this study group's previous reports, it is noteworthy that subjects with the A^{-29}/A genotype are predominantly poor ovarian responders (Desai et al., 2011). Conversely, the polymorphism at position 680 is not associated with poor ovarian response in the studied population (Achrekar et al., 2009a). However, the analysis of these polymorphisms in combination indicated that, the

680Asn allele along with −29A allele is indeed associated with poor ovarian response. As expected, parameters such as exogenous FSH administered, number of follicles and number of oocytes were all logistically related to occurrence of poor ovarian response, independently of age.

This study further calculated the OR to measure the strength of these genotypes as a biomarker to predict poor ovarian response. Among the poor responders, subjects with the A/A—Asn/Asn genotype have a higher risk of showing poor ovarian response to gonadotrophin treatment. Hence, it is tempting to speculate that the A allele at position −29 and the Asn allele at position 680 might be more susceptible to poor ovarian response. Interestingly, the clinical parameters and odds ratio suggest that the subjects with the G/G—Asn/Ser genotype are good responders. However, a larger number of subjects need to be analysed to corroborate the above findings.

The level of *FSHR* expression also impacts greatly on the extent of FSH action. Studies suggest that reduced expression affects *FSHR* function thereby affecting folliculogenesis (Oktay et al., 1997). Several *FSHR* inactivating mutations were also observed to impede receptor trafficking to the membrane, causing reduced *FSHR* expression and resulting in loss of function of the receptor (Allen et al., 2003; Beau et al., 1998; Meduri et al., 2003). Moreover, the reduced expression of *FSHR* on granulosa cells has been shown to be associated with poor ovarian response (Cai et al., 2007). Recently, this study group reported that the A allele at position −29 is associated with lower receptor expression at both the transcript and the protein levels in granulosa cells obtained from subjects undergoing IVF (Desai et al., 2011). The current study evaluated the *FSHR* mRNA expression in the eight genotype combinations. Subjects with the G/G—Asn/Ser genotype expressed significantly higher levels of *FSHR* mRNA compared to the subjects with G/G—Asn/Asn, G/A—Ser/Ser and A/A—Asn/Asn genotypes. The higher expression level of the receptor observed in the subjects with the G/G—Asn/Ser genotype supports the chi-squared analysis showing that these subjects have the lowest OR and minimal risk of exhibiting poor ovarian response. Also it is important to note that, of all the genotypes, subjects with the A/A—Asn/Asn and A/A—Asn/Ser genotypes expressed lower *FSHR* mRNA.

In conclusion, the findings from this study indicate that the subjects with the A/A—Asn/Asn genotype are associated with poor ovarian response. Moreover, the reduced level of *FSHR* mRNA expression observed in these subjects support their insensitivity to exogenous FSH treatment. Thus, the present study suggests that the 680Asn allele in combination with the −29A allele, serves as a better marker to predict poor ovarian response. These observations recommend the efficacy of these allelic combinations of *FSHR* polymorphism to be used as a biomarker to identify poor ovarian responders. However, these findings need to be confirmed in large number of subjects. To increase the specificity and sensitivity of a biomarker to predict ovarian response, along with *FSHR* other candidate genes such as *ESR1*, *ESR2*, *CYP19* and *AMH* need to be analysed together (Altmäe et al., 2007; de-Castro et al., 2004; Morón and Ruiz, 2010). Thus, such multigenic analysis would help in elucidating the cumulative effect of these genes on ovarian response.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.rbmo.2013.07.007.

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