Reproductive BioMedicine Online (2016) ■■, ■■–■■

www.sciencedirect.com
www.rbmonline.com

ARTICLE

# Association of a promoter polymorphism in *FSHR* with ovarian reserve and response to ovarian stimulation in women undergoing assisted reproductive treatment

Dalia Tohlob [a,b], Ekbal Abo Hashem [b], Nawal Ghareeb [b], Mohammed Ghanem [c], Reham Elfarahaty [b], Helen Byers [a], Philip Pemberton [d], Stephen A Roberts [e], William G Newman [a,1], Lamiya Mohiyiddeen [f,*,1]

[a] *Manchester Centre for Genomic Medicine, University of Manchester, Manchester, United Kingdom;* [b] *Clinical Pathology Department, Faculty of Medicine, Mansoura University Mansoura, Egypt;* [c] *Obstetrics and Gynaecology Department, Faculty of Medicine, Mansoura University Mansoura, Egypt;* [d] *Department of Clinical Biochemistry, Manchester Royal Infirmary, Manchester, United Kingdom;* [e] *Centre for Biostatistics, Institute of Population Health, Manchester Academic Health Sciences Centre (MAHSC), University of Manchester, Manchester, United Kingdom;* [f] *Department of Reproductive Medicine, Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom*

* Corresponding author. *E-mail address:* Lamiya.Mohiyiddeen@cmft.nhs.uk (L Mohiyiddeen). [1] These authors contributed equally to the study.

Dr Dalia Tohlob graduated from medical school, Mansoura, Egypt in 2004, and has a Masters degree in clinical pathology. She is currently a PhD scholar at Manchester University, Manchester Centre of Genomic Medicine. She is working in the area of genetic polymorphisms and their association with ovarian response in females undergoing assisted reproduction.

**Abstract** Previous studies have suggested an association between a variant in the promoter region of the FSHR gene and diminished response to controlled ovarian hyperstimulation (COH) in women undergoing assisted reproduction. *FSHR* -29G>A was genotyped in 559 women undergoing their first cycle of COH for IVF/intracytoplasmic sperm injection (ICSI) using TaqMan allelic discrimination assay. Correlation and regression analysis was performed to assess the relationship between *FSHR* promoter genotypes and markers of ovarian reserve and measures of response to COH, including the number of oocytes retrieved, gonadotrophin dose used and the live-birth rate. There were no statistically significant differences between the genotype frequencies and the markers of ovarian reserve or the early measures of response to COH. However, the live-birth rate was higher for women carrying the variant A allele (odds ratio [OR] 1.37; 95% confidence interval [CI] 1.02–1.84 per allele). This relationship did not reach statistical significance after adjustment

http://dx.doi.org/10.1016/j.rbmo.2016.06.001


Please cite this article in press as: Dalia Tohlob, et al., Association of a promoter polymorphism in *FSHR* with ovarian reserve and response to ovarian stimulation in women undergoing assisted reproductive treatment, Reproductive BioMedicine Online (2016), doi: 10.1016/j.rbmo.2016.06.001

for the number of embryos transferred (OR 1.33; 95% CI 0.98–1.83 per allele). Results from this study do not provide evidence that the *FSHR* -29G>A variant can be used in the individualization of the treatment protocol for women undergoing IVF/ICSI.


**KEYWORDS:** FSHR, hyperstimulation, IVF, ovarian response, polymorphism

## Introduction

The principal goal of controlled ovarian hyperstimulation (COH) is to harvest a high number of mature oocytes, which can be used for IVF (Boudjenah et al., 2012; Grady et al., 2012). Response to the drugs used in COH is variable and sometimes unexpected. Some women have a hyper-response which can be exacerbated resulting in ovarian hyperstimulation syndrome (OHSS), a life-threatening condition characterized by ovarian enlargement and abdominal fluid accumulation even with low gonadotrophin doses (Humaidan et al., 2010). In contrast, some women have a low response despite high doses of gonadotrophins and normal ovarian reserve. These situations lead to psychological and physical morbidity and have significant economic implications (Desai et al., 2013).

Individualization of treatment protocols is an attractive strategy to improve IVF outcomes. However, although many studies have been conducted to define predictors of response to COH, to date there is insufficient evidence of utility to adopt genetic biomarkers into clinical practice (Ferraretti et al., 2011).

Studies assessing biomarkers of response to COH have used different outcome measures, potentially contributing to differences in results. Predictors of inadequate ovarian response include advanced age (≥40 years) and low ovarian reserve parameters (Binder et al., 2012), including anti-Müllerian hormone (AMH) concentrations and the antral follicle count (AFC). These measures of ovarian reserve have been used to individualize the dose of gonadotrophin although they do not always improve the outcome of assisted reproduction (Trevisan et al., 2014).

Genetic variability among individuals has been studied to determine the effect on the outcome of COH, including variations in the AMH, AMH receptor, luteinizing hormone (LH), LH receptor, oestrogen receptors and folate metabolizing genes (Altmae et al., 2011). However, the most extensively studied is the *FSHR* gene, encoding the follicle stimulating hormone receptor. A number of single nucleotide polymorphisms (SNP) in *FSHR*, have been associated with measures of ovarian response (Simoni et al., 2002; Wunsch et al., 2005). Two common variants in *FSHR*, c.919G>A, p.(Thr307Ala) and c.2039A>G, p.(Asn680Ser) have been extensively studied. In the cohort described here, there was no significant association between these variants and either ovarian response or reserve parameters (Mohiyiddeen et al., 2012, 2013).

A variant in the 5′ untranslated region of *FSHR*, -29G>A (rs1394205), has been associated with changes in the receptor expression due to changes in transcriptional factor binding sites (Nakayama et al., 2006; Wunsch et al., 2005). However, no association between *FSHR* -29G>A and basal FSH or oestradiol concentrations was established in 202 females undergoing IVF treatment (Wunsch et al., 2005). In contrast, a small study of 50 Indian women reported an association between the homozygous variant genotype (AA) with decreased preovulatory follicle count, the number of oocytes retrieved and lower pregnancy rates (Achrekar et al., 2009). In a further study of 100 Indian women, homozygosity for the variant was also associated with inadequate ovarian response (Desai et al., 2011). A Turkish study of 102 infertile females found no relation between this variant and baseline FSH concentrations (Ilgaz et al., 2015).

Clinical equipoise exists as to whether the *FSHR* promoter gene variant provides useful information to indicate ovarian reserve or the outcome of IVF treatment. We therefore assessed the relationship between the -29G>A *FSHR* variant and the ovarian reserve parameters (FSH, AMH, AFC); the primary outcome measure of the number of eggs retrieved and secondary outcome measures of total gonadotrophin dose used and live-birth event in a previously collected and phenotyped cohort of 603 women undergoing assisted reproduction.

## Materials and methods

Consecutive women attending a tertiary referral centre for reproductive medicine in Manchester, UK were recruited between March 2009 and August 2010.

Inclusion criteria are as follows: (i) age <40 years; (ii) body mass index (BMI) >19 and <30 to meet eligibility criteria for government funded IVF treatment in Greater Manchester, UK; (iii) first cycle of IVF treatment; (iv) presence of two ovaries and no previous ovarian surgery or radiation therapy; and (v) no hormonal therapy was used in the six months prior to recruitment.

A total of 603 women were recruited. Of these, 19 women did not proceed with treatment. No blood sample for genotyping was available for 25 women and the analysis was confined to the remaining 559.

421 women from this study were included in a previous report of different *FSHR* variants (Mohiyiddeen et al., 2013) and 239 women were included in a study assessing the relationship between a *BMP15* variant and ovarian response and reserve parameters (Cerra et al., 2014). The protocol was approved in 2008 by the South Manchester Research Ethics Committee (REC ref no. 08/H1003/212). Written informed consent was obtained from all participants. As this study utilised a pre-existing cohort with a fixed sample size and unknown genotype frequencies, power calculations were inappropriate.

### Basic hormonal assessment

Blood samples were taken on day 3 of spontaneous menstrual cycle or after withdrawal bleeding in women with anovulatory cycles for measurement of serum FSH, serum AMH

Please cite this article in press as: Dalia Tohlob, et al., Association of a promoter polymorphism in *FSHR* with ovarian reserve and response to ovarian stimulation in women undergoing assisted reproductive treatment, Reproductive BioMedicine Online (2016), doi: 10.1016/j.rbmo.2016.06.001

and for genotyping. FSH concentrations were measured using specific immunoassay kits (Cobas, Roche Diagnostics, Mannheim, Germany) on an auto-analyser system (Roche Modular Analytics E170, Roche, USA). AMH concentrations were measured and determined by enzyme-linked immunosorbent first-generation assay (ELISA) provided by DSL (Oxford Bio Innovation, DSL LTT, Oxford, UK).

## Assessment of AFC and pelvic organs

Pelvic trans-vaginal ultrasound was performed on day 3 of a spontaneous menstrual cycle in the cycle before starting ovarian stimulation to assess the AFC, which included follicles measuring 2–5 mm in diameter and to confirm normal anatomy of pelvic organs.

## IVF treatment protocol

Women were treated with either a long down-regulated or short antagonist cycle treatment protocol. In long down-regulated cycles this study used gonadotrophin-releasing hormone (GnRH) analogues (buserelin acetate; Aventis Pharma Ltd) with exogenous recombinant FSH (Puregon; Organon Laboratories Ltd) or highly purified FSH (Menopur; Ferring Pharmaceuticals). In short cycles GnRH antagonists (Orgalutran; Organon Laboratories) were used. Individualised doses were administered based on serum AMH concentrations (Cerra et al., 2014; Mohiyiddeen et al., 2013).

Inadequate response was defined as collection of less than four oocytes or cycle cancellation, normal response was considered as having from four to 20 oocytes, and hyper-response was identified by having more than 20 oocytes (Mohiyiddeen et al., 2012). Clinical pregnancy was defined as ultrasound evidence of a gestational sac in the first trimester.

## Genotyping

Genomic DNA was extracted from peripheral blood leukocytes by the Chemagen automated extraction system. Genotyping of the -29G>A *FSHR* variant (rs1394205) was undertaken by a TaqMan allelic discrimination assay (assays ID C_27504454_10; Applied Biosystems).

## Data analysis

Statistical analysis was performed using the R environment for statistical computing, version 3.1.2 (https://www.r-project.org/). The Kruskal-Wallis test was used to compare the continuous variables and the Fisher's exact test was used to compare qualitative variables across the three different genotypes. A trend test of women's characteristics with allele number utilised the Spearman correlation test. The significance level was $P < 0.05$. For analysis of the effect of -29G>A *FSHR* variant on ovarian reserve markers, multiple linear regression analysis was performed against the number of alleles (additive genetic model) and results are presented as the change in outcome per allele with a 95% confidence interval (CI) and associated significance level (*P*-value) with adjustment for age (as cubic spline), BMI (as a linear covariate) and ethnicity. Similar regression models were used for the response outcomes (gonadotrophin dose and log of number of oocytes retrieved) with adjustment for age, BMI, ethnicity, stimulation type and serum AMH concentration. The adjustment for serum AMH concentration was undertaken as the decision to proceed to IVF was based in part on this measurement. For response markers (log oocyte number) results were additionally adjusted for total gonadotrophin doses (results not shown). Where outcome data was skewed the outcomes were log-transformed adding an offset of 1 to the oocyte numbers where these can take zero values.

Analogous logistic regression models were used to assess the effect of the -29G>A *FSHR* genotypes on late dichotomous outcome (presence or absence of live birth) and dichotomous measures of low response (<4 oocytes) and hyper response (>20 oocytes) adjusted for age, BMI, ethnicity, stimulation type and serum AMH concentrations. An additional adjustment for the number of embryos transferred was considered.

Haplotype analysis was performed by the haplo.stats package in R (http://www.mayo.edu/research/labs/statistical-genetics-genetic-epidemiology/software). Models with the same covariates were fitted using a regression approach that allows for the uncertainty in the haplotype assignments (haplo.glm) (Lake et al., 2002).

# Results

Infertility causes were assessed for all the study participants (**Table 1**) and 67 participants had clinical features consistent with a diagnosis of polycystic ovary syndrome. No significant associations were identified with the underlying cause of infertility and any of the outcome measures (**Table 1**). No cycles of COH were cancelled for hyper-response and 17 cycles were cancelled due to an inadequate response, all of which had no oocytes retrieved.

**Table 1** shows the frequency distribution of the *FSHR* -29G>A variant in the 559 women included in the analysis. The genotypes were in Hardy-Weinberg equilibrium. There were no statistically significant differences in -29G>A *FSHR* genotype frequencies with respect to BMI, treatment type, duration or causes of infertility.

**Table 2** shows that there were no significant differences in the frequencies of different genotypes for ovarian reserve markers: basal serum FSH, serum AMH, or AFC. There were also no significant associations between the genotypes and ovarian response markers: the number of oocytes retrieved or gonadotrophin dose used (**Table 3**). Women carrying the variant (A) allele were more likely to have a clinical pregnancy (odds ratio [OR] 1.32, 95% CI 1.01–1.74, $P = 0.04$) and a live birth following COH (OR 1.37, 95% CI 1.02, BMI 1.84, $P = 0.04$). However, these relationships did not retain significance when the analysis was adjusted for the number of embryos transferred (**Table 4**).

Women with a hyper-response to COH ($P = 0.005$) were more likely to carry the wild type (G) allele (**Table 5**), although the event rates of 4% (GA and AA) and 9% (GG) were low in all genotypes.

Please cite this article in press as: Dalia Tohlob, et al., Association of a promoter polymorphism in *FSHR* with ovarian reserve and response to ovarian stimulation in women undergoing assisted reproductive treatment, Reproductive BioMedicine Online (2016), doi: 10.1016/j.rbmo.2016.06.001

**Table 1** Demographic characteristics across different *FSHR* -29 G>A genotypes.

| *Parameter* | *AA (n = 51) (9.1%)* | *GA (n = 229) (41%)* | *GG (n = 279) (49.9%)* | P | $P_{trend}$ |
|---|---|---|---|---|---|
| Age (years) | 33.4 (26.1–38.3) | 32.3 (25.5–37.8) | 34 (27.3–38.3) | 0.011[a] | 0.010 |
| BMI (kg/m$^2$) | 23.9 (21.4–29.1) | 23.6 (20.1–29.3) | 23.9 (19.9–29.4) | NS | NS |
| Infertility duration in years | 3 (2–7) | 3 (2–7) | 4 (2–7) | NS | NS |
| Ethnicity | | | | | |
| White (*n* = 477) (85%) | 43 (9.1) | 196 (41) | 238 (49.9) | NS | NS |
| South Asian (*n* = 59) (11%) | 6 (10.2) | 25 (42.4) | 28 (47.5) | | |
| Black (*n* = 12) (2%) | 1 (8.3) | 5 (41.7) | 6 (50) | | |
| Others (*n* = 11) (2%) | 1 (9.1) | 3 (27.3) | 7 (63.6) | | |
| Cycle type | | | | | |
| Agonist (*n* = 191) (34%) | 18 (9.4) | 85 (44.5) | 88 (46.1) | NS | NS |
| Antagonist (*n* = 367) (66%) | 33 (9) | 144 (39.2) | 190 (51.8) | | |
| Infertility causes | | | | | |
| Male (*n* = 171) (31%) | 21 (12.3) | 70 (40.9) | 80 (46.8) | NS | NS |
| Tubal (*n* = 141) (25%) | 10 (7.1) | 63 (44.7) | 68 (48.2) | | |
| Endometriosis (*n* = 28) (5%) | 2 (7.1) | 7 (25) | 19 (67.9) | | |
| Ovarian (*n* = 57) (10%) | 4 (7) | 19 (33.3) | 34 (59.6) | | |
| Unexplained (*n* = 160) (29%) | 14 (8.7) | 70 (43.8) | 76 (47.5) | | |

Values are in median (10th–90$^{th}$ percentiles) or in numbers (percentage of women with each genotype).
$P_{trend}$ from a Spearman correlation test – for categorical variables this test compares each category with all the other categories. BMI = body mass index; NS = no statistically significant difference.
[a]Kruskal-Wallis test.

**Table 2** Ovarian reserve markers across different genotypes.

| *Parameter* | *Median (10$^{th}$–90$^{th}$ percentile) FSHR -29 G>A* | | | *Adjusted linear regression For age, BMI, ethnicity* |
|---|---|---|---|---|
| | *AA* | *GA* | *GG* | *log difference per allele (95% CI)* |
| Basal FSH (IU/l) | 7.1 (4.8–11) | 6.7 (4.6–9.8) | 6.6 (4.6–9.8) | 0.03 (–0.01–0.08) |
| AFC | 11.5 (8–19.3) | 14 (8–24) | 13 (8–22) | –0.04 (–0.09–0.02) |
| AMH (pmol/l) | 11.8 (4.7–37.1) | 16.3 (5.6–40.6) | 14.4 (4.8–44.4) | –0.07 (–0.17–0.02) |

Values are in median (10th-90th%ile).
Adjusted linear regression for age (cubic spline), BMI (linear effect) and ethnicity.
FSH, AMH, and AFC were log transformed.
No statistically significant differences were found.
AFC = antral follicle count; AMH = anti-Müllerian hormone; BMI = body mass index; CI = confidence interval.

As we had previously genotyped the *FSHR* variants c.919G>A, p.(Thr307Ala) and c.2039A>G, p.(Asn680Ser) in 421 women from the studied cohort (Mohiyiddeen et al., 2013), this study undertook an analysis to consider the effect of the -29G>A variant in combination. As c.919G>A and c.2039A>G are in complete linkage disequilibrium (Mohiyiddeen et al., 2013), c.2039A>G with the promoter variant was only considered. Four diplotypes were imputed for these two variants (**Table 6**). The diplotype analysis demonstrated that women carrying the diplotype with variant alleles for both variants (i.e. G for the coding variant and A for the promoter variant) have an increased clinical pregnancy ($P = 0.039$) and live birth ($P = 0.04$) rate. These associations were not significant when adjusted for the number of embryos transferred. Women with this diplotype were more likely to have a hyper-response to COH ($P = 0.039$, **Table 7**).

## Discussion

This large study considered the evidence for an association between -29G>A *FSHR* and measures of ovarian response and ovarian reserve in women undergoing IVF treatment. No significant associations were determined between the variant

Please cite this article in press as: Dalia Tohlob, et al., Association of a promoter polymorphism in *FSHR* with ovarian reserve and response to ovarian stimulation in women undergoing assisted reproductive treatment, Reproductive BioMedicine Online (2016), doi: 10.1016/j.rbmo.2016.06.001

**Table 3** Ovarian response markers (early outcome measures) across different genotypes.

| *Parameter* | *median (10th–90th percentile) FSHR -29 G>A* | | | *Adjusted regression analysis*[a] |
|---|---|---|---|---|
| | *AA* | *GA* | *GG* | *Difference per allele (95% CI)* |
| Total dose gonadotropin used (IU) | 3000 (1500–3825) | 2550 (1350–3765) | 2700 (1420–3750) | −4 (−124–117) |
| Oocytes retrieved | 9 (4–18) | 9 (3–18) | 8 (3–19) | 0.03 (−0.05–0.11) |

Values are in median (10th-90th%ile) for each genotype.
No statistically significant differences were found.
[a]Difference is estimated by linear regression adjusted for age, BMI, ethnicity, stimulation type and AMH. Oocyte numbers are log transformed for this analysis and difference is a difference in log (number+1).

**Table 4** Late outcome measures (clinical pregnancy and live birth) across different *FSHR* -29 G>A genotypes.

| *Parameter* | *AA* | *GA* | *GG* | *Adjusted regression analysis* | |
|---|---|---|---|---|---|
| | | | | *odds ratio per allele (95% CI)* | P |
| Clinical pregnancy rate *n* (%) | 20/51 (39) | 91/229 (40) | 87/279 (31) | 1.32 [a] (1.01–1.74) | 0.044 |
| | | | | 1.27[b] (0.95–1.71) | NS |
| Live-birth rate *n* (%) | 18/51 (35) | 67/229 (29) | 68/279 (24) | 1.37 [a] (1.02–1.84) | 0.035 |
| | | | | 1.33[b] (0.98–1.81) | NS |

AMH = anti-Müllerian hormone; BMI = body mass index; CI = confidence interval; NS = no statistically significant difference.
[a]Adjusted for age, BMI, stimulation type and AMH.
[b]Adjusted for age, BMI, stimulation type, AMH and the number of embryos transferred.

**Table 5** Type of ovarian response across different *FSHR* -29 G>A genotypes.

| *Response* | *AA* | *GA* | *GG* | *Adjusted regression analysis* [a] | |
|---|---|---|---|---|---|
| | | | | *Change per allele (odds ratio) (95% CI)* | P |
| Inadequate response (<4 oocytes retrieved) *n* (%) | 4/51 (8) | 30/229 (13) | 44/279 (16) | 0.77 (0.52–1.15) | NS |
| Hyper-response (>20 oocytes retrieved) *n* (%) | 2/51 (4) | 9/229 (4) | 24/279 (9) | 0.37 (0.18–0.74) | 0.005 |

AMH = anti-Müllerian hormone; BMI = body mass index; CI = confidence interval; NS = no statistically significant difference.
[a]Adjusted for age, BMI, stimulation type and AMH.

and measures of ovarian reserve or markers of treatment response.

Three previous studies have considered the relationship between the -29G>A *FSHR* variant and parameters associated with IVF (Achrekar et al., 2009; Desai et al., 2011; Wunsch et al., 2005). Consistent with data from this study, in all three previous reports, no significant association between basal serum FSH and the promoter variant were detected.

Both Achrekar et al., 2009 and Desai et al. 2011 reported a lower pre-ovulatory follicle count in women with the AA genotype compared with other genotypes. However, these data are based on seven and 11 women, respectively with this genotype. This study did not note any differences in the related parameter of AFC between women with different genotypes, including 51 homozygous for the A allele.

In the Achrekar and Desai studies the total gonadotrophin dose used was higher in the AA genotype groups (3069.43 ± 194.2 IU) and (4563 ± 271 IU) respectively, than in the other genotype groups. The median dose of FSH used in this study was comparable 3000 (1500–3825 IU) to these studies, but it was noted that there was no significant differences in dose between the genotype groups. Furthermore, it was determined that there were no differences in the number of oocytes retrieved in the different genotype groups, whereas in both

Please cite this article in press as: Dalia Tohlob, et al., Association of a promoter polymorphism in *FSHR* with ovarian reserve and response to ovarian stimulation in women undergoing assisted reproductive treatment, Reproductive BioMedicine Online (2016), doi: 10.1016/j.rbmo.2016.06.001

**Table 6** Diplotype frequencies for *FSHR* -29G>A and *FSHR* c.2039A>G.

| *c.2039A>G, p.(Asn680Ser)* | *-29G>A* | *diplotype* | *Frequency%* |
|---|---|---|---|
| A | A | AA | 17.5 (*n* = 98) |
| A | G | AG | 37.7 (*n* = 11) |
| G | A | GA | 12.2 (*n* = 68) |
| G | G | GG | 32.6 (*n* = 182) |

**Table 7** Adjusted linear regression analysis of diplotype of polymorphism c. 2039 A>G (p.Ser680Asn) and -29G>A.

| | *A/A* *17.6%** *(n = 98)* | *G/A* *12%** *(n = 68)* | *G/G* *32.6%** *(n = 182)* |
|---|---|---|---|
| Log FSH (IU/l) | 0.02 (–0.05–0.1) NS[a] | 0.06 (–0.02–0.14) NS[a] | 0.01 (–0.05–0.06) NS[a] |
| Log AFC | –0.07 (–0.17–0.03) NS[a] | –0.03 (–0.13–0.07) NS[a] | –0.03 (–0.1–0.04) NS[a] |
| Log AMH (pmol/l) | –0.1 (–0.24–0.05) NS[a] | –0.05 (–0.21–0.1) NS[a] | –0.02 (–0.13–0.1) NS[a] |
| Total dose gonadotropin used[b] | –0.073 (–0.258–0.112) NS[c] | 0.077 (–0.12–0.274) NS[c] | –0.021 (–0.165–0.122) NS[c] |
| Log Oocytes retrieved | 0.06 (-0.06–0.18) NS[c] | 0.03 (–0.11–0.16) NS[c] | 0.03 (–0.06–0.12) NS[c] |
| | 0.06 (–0.06–0.17) NS[d] | 0.03 (–0.1–0.16) NS[d] | 0.03 (–0.06–0.12) NS[d] |
| Clinical pregnancy rate | 1.34 (0.87–2.08) NS[d] | 1.61 (1.03–2.52) *P* = 0.039[d] | 1.2 (0.85–1.69) NS[d] |
| | 1.25 (0.78–2.01) NS[e] | 1.48 (0.91–2.4) NS[e] | 1.11 (0.77–1.6) NS[e] |
| Live birth rate | 1.39 (0.87–2.21) NS[d] | 1.65 (1.02–2.66) *P* = 0.040[d] | 1.2 (0.83–1.74) NS[d] |
| | 1.31 (0.8–2.17) NS [e] | 1.53 (0.92–2.53) NS [e] | 1.12 (0.76–1.65) NS [e] |
| Inadequate response (<4 oocytes retrieved) | 0.73 (0.41–1.3) NS[d] | 0.55 (0.27–1.12) NS[d] | 0.71 (0.45–1.11) NS[d] |
| Hyper-response (>20 oocytes retrieved) | 0.61 (0.25–1.5) NS[d] | 0.11 (0.01–0.89) P = 0.039[d] | 1.02 (0.54–1.92) NS[d] |

AFC = antral follicle count; AMH = anti-Müllerian hormone; BMI = body mass index; NS = no statistically significant difference.
Analysis of the diplotypes of the two SNP c. 2039 A>G (p.Ser680Asn) and -29G>A per diplotype and each of the diplotypes compared with the wild type A/G (A allele at position 2039/G allele at position -29) on an additive genetic model. A/A (A allele at position 2039/A allele at position -29), G/A (G allele at position 2039/A allele at position -29), G/G (G allele at position 2039/G allele at position -29).
*Are the diplotype frequencies where A/G is 37.8% (*n* = 211).
[a]Adjusted for age, BMI and ethnicity.
[b]Gonadotrophin dose is scaled (gonadotrophin dose/1000 IU).
[c]Adjusted for age, BMI, ethnicity, stimulation type, and AMH level.
[d]Adjusted for age, BMI, ethnicity, stimulation type, AMH level, and gonadotrophin dose.
[e]Adjusted for age, BMI, ethnicity, stimulation type, AMH level, gonadotrophin dose and the number of embryos transferred.

the previous Indian studies women with the AA genotype had lower numbers of retrieved mature oocytes (Achrekar et al., 2009; Desai et al., 2011).

A larger sample size would be required to determine if this promoter SNP has a smaller effect on ovarian reserve or ovarian response (beyond the limit of detection of this study). However, in practical terms, if the SNP is to have clinical utility as a biomarker, it should have a larger effect size.

This study is the first to consider the effect of this promoter SNP on the frequency of live birth between different genotypes following IVF treatment. Live birth is the most important outcome for a woman undergoing assisted reproduction and should be considered in all genomic studies of outcomes of assisted reproduction to provide women and health care providers with the information that will inform optimal treatment options. Hence, it was interesting to observe that women carrying the variant allele were more likely to have a child following IVF treatment. This association was of borderline significance after adjustment for the available prognostic factors (*P* = 0.035) but the association did not quite reach statistical significance, after additional adjustment for the number of embryos transferred. As the

Please cite this article in press as: Dalia Tohlob, et al., Association of a promoter polymorphism in *FSHR* with ovarian reserve and response to ovarian stimulation in women undergoing assisted reproductive treatment, Reproductive BioMedicine Online (2016), doi: 10.1016/j.rbmo.2016.06.001

number of embryos transferred is to a large extent determined by the ovarian response this is likely to be over-adjusting. Studies with larger numbers would be needed to investigate this complex relationship in more detail. It is interesting to note the lack of association between the variant with the early markers of ovarian response in contrast to the trend with increased live-birth rate indicating the potential pitfalls of over-reliance on intermediate outcomes.

When comparing the degree of response to COH in groups with different genotypes, this study noted that women with a hyper-response were more likely to carry the wild type (G) allele. However, the absolute hyper-response rate is low in all genotypes, indicating that this is not an absolute predictor that could be used in a clinical setting.

There was no evidence of a significant association with ovarian response or reserve markers when the diplotype, generated through consideration of c.2039 A>G and -29G>A, was used.

Similar to the results of the association analysis for the promoter variant, there was a modestly significant increased likelihood of having a clinical pregnancy and live birth in women carrying the G allele of the Ser680Asn variant and A allele of the promotor variant. However, these associations did not remain significant after correcting for the number of embryos transferred. Women with this diplotype were more likely to be hyper-responsive to COH, but this did not remain significant at a *P*-value of 0.005 after correction for multiple testing by Bonferroni.

In conclusion, the results of this study do not provide enough evidence to support the use of genotyping *FSHR* -29G>A in the individualization of treatment for women undergoing IVF, but demonstrate the importance of using live-birth rate as an outcome for studies relating to assisted reproduction.

## Acknowledgements

DT was supported by grants from the Egyptian Cultural and Educational Bureau, mission sector, Egypt.

## References

Achrekar, S.K., Modi, D.N., Desai, S.K., Mangoli, V.S., Mangoli, R.V., Mahale, S.D., 2009. Poor ovarian response to gonadotrophin stimulation is associated with FSH receptor polymorphism. Reprod. Biomed. Online 18, 509–515.

Altmae, S., Hovatta, O., Stavreus-Evers, A., Salumets, A., 2011. Genetic predictors of controlled ovarian hyperstimulation: where do we stand today? Hum. Reprod. Update 17, 813–828.

Binder, H., Strick, R., Zaherdoust, O., Dittrich, R., Hamori, M., Beckmann, M.W., Oppelt, P.G., 2012. Assessment of FSHR variants and antimullerian hormone in infertility patients with a reduced ovarian response to gonadotropin stimulation. Fertil. Steril. 97, 1169–1175.

Boudjenah, R., Molina-Gomes, D., Torre, A., Bergere, M., Bailly, M., Boitrelle, F., Taieb, S., Wainer, R., Benahmed, M., de Mazancourt, P., Selva, J., Vialard, F., 2012. Genetic polymorphisms influence the ovarian response to rFSH stimulation in patients undergoing in vitro fertilization programs with ICSI. PLoS ONE 7, e38700.

Cerra, C., Oliver, J., Roberts, S.A., Horne, G., Newman, W.G., Mohiyiddeen, L., 2014. A single nucleotide polymorphism of bone morphogenic protein-15 is not associated with ovarian reserve or response to ovarian stimulation. Hum. Reprod. 29, 2832–2837.

Desai, S.S., Achrekar, S.K., Pathak, B.R., Desai, S.K., Mangoli, V.S., Mangoli, R.V., Mahale, S.D., 2011. Follicle-stimulating hormone receptor polymorphism (G-29A) is associated with altered level of receptor expression in Granulosa cells. J. Clin. Endocrinol. Metab. 96, 2805–2812.

Desai, S.S., Achrekar, S.K., Paranjape, S.R., Desai, S.K., Mangoli, V.S., Mahale, S.D., 2013. Association of allelic combinations of FSHR gene polymorphisms with ovarian response. Reprod. Biomed. Online 27, 400–406.

Ferraretti, A.P., La Marca, A., Fauser, B.C., Tarlatzis, B., Nargund, G., Gianaroli, L., ESHRE working group on Poor Ovarian Response, 2011. ESHRE consensus on the definition of "poor response" to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum. Reprod. 26, 1616–1624.

Grady, R., Alavi, N., Vale, R., Khandwala, M., McDonald, S.D., 2012. Elective single embryo transfer and perinatal outcomes: a systematic review and meta-analysis. Fertil. Steril. 97, 324–331.

Humaidan, P., Quartarolo, J., Papanikolaou, E.G., 2010. Preventing ovarian hyperstimulation syndrome: guidance for the clinician. Fertil. Steril. 94, 389–400.

Ilgaz, N.S., Aydos, O.S., Karadag, A., Taspinar, M., Eryilmaz, O.G., Sunguroglu, A., 2015. Impact of follicle-stimulating hormone receptor variants in female infertility. J. Assist. Reprod. Genet. 32, 1659–1668.

Lake, S., Lyon, H., Silverman, E., Weiss, S., Laird, N., Schaid, D., 2002. Estimation and tests of haplotype-environment interaction when linkage phase is ambiguous. Hum. Hered. 55, 56–65.

Mohiyiddeen, L., Newman, W.G., McBurney, H., Mulugeta, B., Roberts, S.A., Nardo, L.G., 2012. Follicle-stimulating hormone receptor gene polymorphisms are not associated with ovarian reserve markers. Fertil. Steril. 97, 677–681.

Mohiyiddeen, L., Newman, W.G., Cerra, C., McBurney, H., Mulugeta, B., Roberts, S.A., Nardo, L.G., 2013. A common Asn680Ser polymorphism in the follicle-stimulating hormone receptor gene is not associated with ovarian response to gonadotropin stimulation in patients undergoing in vitro fertilization. Fertil. Steril. 99, 149–155.

Nakayama, T., Kuroi, N., Sano, M., Tabara, Y., Katsuya, T., Ogihara, T., Makita, Y., Hata, A., Yamada, M., Takahashi, N., Hirawa, N., Umemura, S., Miki, T., Soma, M., 2006. Mutation of the follicle-stimulating hormone receptor gene 5′-untranslated region associated with female hypertension. Hypertension 48, 512–518.

Simoni, M., Nieschlag, E., Gromoll, J., 2002. Isoforms and single nucleotide polymorphisms of the FSH receptor gene: implications for human reproduction. Hum. Reprod. Update 8, 413–421.

Trevisan, C.M., Peluso, C., Cordts, E.B., de Oliveira, R., Christofolini, D.M., Barbosa, C.P., Bianco, B., 2014. Ala307Thr and Asn680Ser Polymorphisms of FSHR Gene in Human Reproduction Outcomes. Cell. Physiol. Biochem. 34, 1527–1535.

Wunsch, A., Ahda, Y., Banaz-Yasar, F., Sonntag, B., Nieschlag, E., Simoni, M., Gromoll, J., 2005. Single-nucleotide polymorphisms in the promoter region influence the expression of the human follicle-stimulating hormone receptor. Fertil. Steril. 84, 446–453.

Received 21 December 2015; refereed 1 June 2016; accepted 7 June 2016.