Reproductive BioMedicine Online (2013) xxx, xxx–xxx

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ARTICLE

FSH receptor genotype does not predict metaphase-II oocyte output or fertilization rates in ICSI patients

Lamiya Mohiyiddeen [a,*], William G Newman [b,c,d], Christian Cerra [a], Gregory Horne [c,d], Betselot Mulugeta [a], Helen Byers [b], Stephen A Roberts [e], Luciano G Nardo [f,g]

[a] *Department of Reproductive Medicine, St. Mary's Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom;* [b] *Centre for Genetic Medicine, Manchester Academic Health Sciences Centre (MAHSC), University of Manchester, Manchester, United Kingdom;* [c] *Genetic Medicine, Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom;* [d] *Clinical embryology, Department of Reproductive Medicine, St. Mary's Hospital, Manchester, United Kingdom;* [e] *Centre for Biostatistics, Institute of Population Health, University of Manchester, United Kingdom;* [f] *Maternal and Fetal Health Research Group, Manchester Academic Health Sciences Centre (MAHSC), St. Mary's Hospital, University of Manchester, United Kingdom;* [g] *GyneHealth, Manchester, United Kingdom*

[*] Corresponding author. *E-mail address:* lamiya.mohiyiddeen@cmft.nhs.uk (L Mohiyiddeen).

Dr Lamiya Mohiyiddeen is a consultant in reproductive medicine at St. Mary's Hospital, Manchester, United Kingdom. She obtained her MD from the University of Manchester. Her research focuses on pharmacogenetics and ovarian performance in IVF patients.

Abstract The objective of this study was to assess the role of the variant p.Asn680Ser in the FSH receptor gene (*FSHR*) in determining oocyte maturity. It also assessed the relationship between this *FSHR* variant with metaphase-II oocyte output rate (MOR) and the fertilization rate. This was a prospective observational study based at a tertiary referral centre for reproductive medicine. Women (n = 212) undergoing their first cycle of ovarian stimulation for IVF with intracytoplasmic sperm injection (ICSI) were included in the study. Baseline pelvic ultrasound and blood tests were taken on day 2 or 3 of the cycle for assessment of baseline hormones and for DNA extraction. Genotypes for *FSHR* p.Asn680Ser was determined using TaqMan allelic discrimination assay. The outcome measures were the total dose of exogenous gonadotrophins used, antral follicle count (AFC), number of mature (metaphase-II) oocytes retrieved, MOR and fertilization rate. No statistically significant differences were found between the number of mature oocytes retrieved, MOR or fertilization rates among the patients with different p.Asn680Ser *FSHR* genotypes. No significant difference was noted in the clinical pregnancy rates per transfer. There is no evidence that the p.Asn680Ser *FSHR* genotype predicts oocyte maturity.

KEYWORDS: fertilization, FSH, IVF, oocyte, polymorphisms, SNP

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http://dx.doi.org/10.1016/j.rbmo.2013.06.005

Please cite this article in press as: Mohiyiddeen, L et al. FSH receptor genotype does not predict metaphase-II oocyte output or fertilization rates in ICSI patients . Reproductive BioMedicine Online (2013), http://dx.doi.org/10.1016/j.rbmo.2013.06.005

Introduction

Ovarian stimulation using exogenous gonadotrophins is an important step during fertility treatment. FSH is a glycoprotein hormone with a key role in human reproduction (Simoni and Nieschlag, 1997). It is essential for gonadal development and it stimulates follicular maturation in the ovaries by binding to FSH receptor (FSHR), a G-coupled protein in the granulosa cells, initiating a signal transduction involving adenylate cyclase activation and elevation of intracellular cAMP (Simoni and Nieschlag, 1997). It is suggested that increasing FSH concentrations within the follicle is coincident with the generation of a positive signal necessary to complete oocyte maturation in humans (Mendoza et al., 2002).

Embryo competence is most likely due to the quality of the originating gametes (Munne, 2006). Therefore, the morphological appearance of the oocyte may indicate developmental potential of the subsequent embryo (Munne, 2006). However, morphological oocyte assessment is controversial and can only be applied to oocytes for intracytoplasmic sperm injection (ICSI). The evaluation of oocyte morphology before standard IVF is, in fact, difficult because of the presence of cumulus and corona cells. Furthermore, the quality and the degree of expansion of these cells are poor markers of oocyte maturity and mostly depend on the type of ovarian stimulation protocol (Veeck, 1990).

The use of different criteria for oocyte evaluation may be partly responsible for the discrepancies between different studies on oocyte maturity (Ebner, 2003). A study that evaluated all the current markers of ovarian reserve showed the maturity of oocytes correlated the most with antral follicle count (AFC) and age of the patients (Gallot et al., 2012). Anti-Müllerian hormone (AMH) concentrations have been used as a predictor of ovarian reserve and are a good predictive indicator of ovarian reserve; however, they do not predict the quality of oocytes retrieved (Guerif et al., 2009).

The granulosa cells displaying appropriate reactivity to FSH are not only endowed with functional FSH receptors but are also able to properly execute a cascade of specialized tasks including signal transduction, steroidogenesis and cell proliferation and differentiation. The study by Gallot et al. (2012) aimed to evaluate antral follicle responsiveness to exogenous FSH using the follicular output rate and suggested that, irrespective of AFC prior to ovarian stimulation and preovulatory follicle count, patients with a larger proportion of FSH-responsive antral follicles were more prone to become pregnant after IVF—embryo transfer. In an effort to more objectively evaluate the ovarian response, this study assessed the mature oocytes collected in relation to the intrinsic reserve reflected by AFC. This was done using the metaphase-II (MII) oocyte output rate (MOR) index, which is assessed by the ratio of MII oocytes to baseline AFC prior to ovarian stimulation.

Perez Mayorga et al. (2000) first observed that ovarian response to FSH stimulation depends on the *FSHR* genotype and noted a relationship between serum basal FSH concentrations and *FSHR* polymorphisms. Since then, studies have considered the relationship between *FSHR* gene variants and ovarian response (Mohiyiddeen and Nardo, 2010). Few studies have investigated any associations between *FSHR* genotype and outcomes such as fertilization rate, oocyte retrieval rate (Achrekar et al., 2008; Behre et al., 2005; Jun et al., 2006; Klinkert et al., 2006; Loutradis et al., 2006; Perez Mayorga et al., 2000; Sudo et al., 2002) and pregnancy rate (Behre et al., 2005; Jun et al., 2006; Klinkert et al., 2006; Loutradis et al., 2006). No studies to date have determined the effect of *FSHR* gene variants on oocyte maturity.

The aim of this study was to investigate the relationship between a common functional *FSHR* gene polymorphism and oocyte maturity. It aimed to verify whether responsiveness of AFC to ovarian stimulation assessed using MOR bears any relationship to this *FSHR* polymorphism and investigated the relationship of the *FSHR* polymorphism with fertilization and clinical pregnancy rates per transfer.

Materials and methods

Subjects and assays

A total of 212 women enrolled for ICSI treatment were prospectively recruited to the study. The inclusion criteria were: (i) first cycle of ovarian stimulation; (ii) <40 years of age; (iii) both ovaries present on transvaginal ultrasound scan; (iv) no previous history of ovarian surgery; (v) no exposure to cytotoxic drugs or pelvic radiation therapy; (vi) no hormonal therapy in the 6 months before entering the study; and (vii) body mass index 20—30 kg/m^2.

On day 2 or 3 of a spontaneous menstrual cycle within 3 months of commencing ovarian stimulation, a transvaginal ultrasound scan was performed to assess the total number of antral follicles measuring 2—5 mm in diameter (AFC) and to confirm normal anatomy of the pelvic organs. The intra-analysis coefficient of variation for follicular diameter measurements was <5% and the lower limit of detection was 0.5 mm.

Written informed consent was obtained from all the study participants. Ethical approval was given by the Local Research Ethics Committee (REC ref no. 08/81003/212; approved 9 June 2010).

Ovarian stimulation

A standard long step-down protocol was used for ovarian stimulation. The gonadotrophin-releasing hormone (GnRH) analogue (buserelin acetate; Suprecur; Aventis Pharma, West Malling, UK) was administered at a dose of 0.5 mg subcutaneously starting in the midluteal phase of the preceding menstrual cycle or approximately 7 days before menstruation. Ovarian stimulation was effected with exogenous gonadotrophins in the form of recombinant FSH (Puregon; Organon Laboratories, Cambridge, UK). The GnRH agonist was reduced to 0.25 mg from the first day of gonadotrophin stimulation. Patients on antagonist cycle had gonadotrophin stimulation initiated on day 2 of the cycle and continued up to and including the day of human chorionic gonadotrophin (HCG) administration; GnRH antagonist (Orgalutran; Organon Laboratories) at a daily dose of 0.25 mg was initiated using a fixed day-6 protocol. The starting daily dose was based on baseline AMH concentration. Transvaginal ultrasound scans were arranged on days 8 and 10 of ovarian

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stimulation and every 1 or 2 days thereafter, as required. Final oocyte maturation was induced with 5000 IU HCG (Pregnyl, Organon Laboratories), provided that there was at least one leading follicle >17 mm and two other follicles >16 mm. Transvaginal ultrasound-guided oocyte retrieval was undertaken 34–36 h after HCG injection and embryo transfer was performed 2 days later. Vaginal progesterone pessaries (Cyclogest 400 mg twice daily; Alpharma, Barnstaple, UK) were used to support the luteal phase until 12 weeks of pregnancy.

Clinical pregnancy was defined by the presence of gestational sac(s) with viable fetal heartbeat on ultrasound at 12 weeks of gestation.

Genotyping

DNA was extracted from blood samples using the Chemagen Automated DNA Separation System (Chemagen, Germany). Genotypes for *FSHR* c.2039A>G, p.Asn680Ser (rs6166) were determined using the pre-designed TaqMan allelic discrimination assays (assay id. C_2676874_10; Life Technologies, USA). Positive controls were genotyped and sequencing of a subset of samples was undertaken for quality assurance.

Calculation of rates

MOR was calculated as (MII oocytes/AFC) × 100. The fertilization rate was calculated as (2-pronuclear zygotes/MII oocytes injected) × 100. The maturity rate was calculated as (MII oocytes/total number of oocytes collected) × 100.

Statistical analysis

Statistical Package for Social Sciences version 10.1 (SPSS, Chicago, IL, USA) was used for statistical analysis. The Kruskall–Wallis test was performed to compare the groups with different genotypes. A *P*-value <0.05 was considered statistically significant.

A quantile regression on the medians as a function of the number of alleles was undertaken to quantify differences in responses between genotypes for maturity rate, maturity output rate and fertilization rate. Results are expressed as the difference in the median per allele along with a 95% confidence interval. This analysis used the R statistical environment (R Foundation for Statistical Computing, Vienna, Austria).

Results

All 212 women (mean age 33.5 years) included in the study were genotyped for the p.Asn680Ser polymorphism in *FSHR*. The genotype distribution was consistent with Hardy–Weinberg equilibrium. The minor allele frequency was consistent with the 45.7% reported in the Caucasian European population ($n = 4300$) of the exome variant server (http://evs.gs.washington.edu/EVS/). As shown in **Table 1**, there was little evidence of significant differences in the amount of gonadotrophin used for ovulation induction in individuals with different genotypes. Further, there were no significant differences between the three genotypes in terms of fertilization rate, in the median number of oocytes, mature oocytes (MII), and 2-pronuclear zygotes retrieved. This study observed a higher MOR in the heterozygous Asn/Ser group; however, this did not reach statistical significance. No significant differences in maturity ratio were observed between genotypes. A regression against allele number did not show any significant differences in maturity rate per allele (1.9, 95% CI −3.6 to 6.7), maturity output rate per allele (−7.2, 95% CI −11.6 to 7.9) and fertilization rate per allele (0, 95% CI −16 to 11).

The clinical pregnancy rate per embryo transfer was slightly lower for individuals with the heterozygous Asn/Ser genotype at 31.1%, compared with 29.3% and 29.5% for Asn/Asn and Ser/Ser genotypes, respectively. The difference in clinical pregnancy rates between the three genotypes was not statistically significant. A higher proportion of Ser/Ser patients had total fertilization failure (11% compared with 8% and 8% for Asn/Ser and Ser/Ser, respectively), although this was again not significant.

A trend towards a lower number of 2-pronuclear zygotes for the Ser/Ser genotype was observed; however, again this did not reach statistical significance.

Discussion

This prospective study investigated the relationship between genotypes for the common functional variant p.Asn680Ser in *FSHR* and oocyte maturity. There were no significant differences in the number of MII oocytes between women with different genotypes, although modest effects (~10–20%) cannot be ruled out. It is important that the number of MII oocytes is considered to measure ovarian response rather than the AFC, as it is the MII oocytes that ultimately determine the number of viable embryos. An index that helps determine the number of mature oocytes will also help determine the ovarian response thereby allowing more individualized stimulation protocols (Nardo et al., 2011).

This study is the first to investigate the association between the *FSHR* p.Asn680Ser variant and oocyte maturity (number of MII oocytes). The MOR index estimates the yield of mature oocytes and determines the actual response to ovarian stimulation with reference to the intrinsic ovarian reserve. The yield of a high or small number of oocytes may not necessarily be the best guide to the response to stimulation. A yield of mature oocytes in reference to AFC is a better reflection of response to ovarian stimulation. For example, a woman with an AFC of four who yields four oocytes will have a better response than a woman with an AFC of 20 who only yields 8 oocytes.

This study also calculated the maturity rate, MII oocytes in the context of the total number of oocytes, which did not show significant differences among the three genotypes. On comparing maturity rate with MOR rate, these were only weakly correlated (correlation coefficient 0.25).

The frequency distribution of genotype observed in this study was comparable to other studies in literature (Mohiyiddeen and Nardo, 2010). The results from the present study are consistent with three other studies showing no significant difference in the dose of exogenous gonadotrophins administered to individuals with different *FSHR* genotypes (de Castro et al., 2003; Jun et al., 2006;

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Table 1 Cycle and pregnancy outcomes for the different FSH receptor genotypes at position 680 in a subgroup of 212 patients.

	Asn/Asn (n = 72, 34%)	*Asn/Ser* (n = 106, 50%)	*Ser/Ser* (n = 34, 16%)
Age (years)	33 (26–38)	33 (25–38)	33.5 (26–38)
Gonadotrophin dose (IU)	2925 (1298–3600)	2887 (1403–3885)	2850 (1375–3525)
Oocytes retrieved	10 (3–19)	10 (3–21)	7 (2–18)
MII oocytes	8 (1–15)	7 (2–17)	6 (2–15)
2PN zygotes	4 (0–9)	4 (0–10)	2 (0–8)
Fertilization rate (%)	50 (0–100)	64 (0–100)	50 (0–100)
AFC (2–5 mm)	14 (9–24)	12 (8–24)	14 (8–24)
MOR	50 (14–107.1)	61 (11–133)	38 (11–111)
Maturity ratio	81 (37–100)	80 (52–100)	86 (50–100)
Clinical pregnancy rate per transfer	21 (29.3)	32 (31.1)	10 (29.47)

Values are median (10–90th percentiles) or *n* (%). 2PN, 2-pronuclear; AFC, antral follicle count; MII, metaphase-II; MOR, MII oocyte output rate.

Loutradis et al., 2006). Studies of maturation of isolated cumulus–oocyte complexes *in vitro* have shown that the final stage of nuclear maturation can occur independently of FSH, but it is much more efficient in the presence of a tailored dose of FSH (Anderiesz et al., 2000). A recent study that employed preimplantation genetic screening concluded that mild ovarian stimulation results in a decreased proportion of aneuploid and mosaic embryos (Baart et al., 2007). This study suggests that the dose of gonadotrophins used for ovarian stimulation for IVF affects oocyte quality and thus chromosome segregation behaviour during meiosis and early embryo development.

There were no differences in the fertilization rate among the patients with different *FSHR* genotypes, which is consistent with other groups (Behre et al., 2005; Jun et al., 2006; Klinkert et al., 2006). In agreement with Behre et al. (2005), the present results do not show significantly different clinical pregnancy rates between the three genotypes. In contrast, a significant difference in pregnancy outcomes was demonstrated by Klinkert et al. (2006), as the pregnancy rate and implantation rate in individuals with the Ser/Ser genotype were 3-times higher than in those with Asn/Asn genotype. Conversely, Jun et al. (2006) found that clinical pregnancy rate per embryo transfer was significantly higher in the Asn/Asn group. The reasons for these different results are unclear but may be due to different underlying genotype frequencies in the different populations studied.

Other outcome measures including clinical pregnancy rate, miscarriage rate and occurrence of ovarian hyperstimulation in individuals with different *FSHR* genotypes have been reported in an expanded cohort, including the population considered in this study (Mohiyiddeen et al., 2013).

The main limitation of this study is that, to assess the oocyte maturity, this study could only assess women who had ICSI treatment, (de Castro et al., 2004) where other factors, such as the presence of severe sperm abnormalities in some of the cases, may have affected the embryo quality.

It is evident from these data that *FSHR* genotype is not a useful predictor of the number of mature oocytes or fertilization rate and thereby does not have utility for determining the stimulation protocol for individual patients. Other parameters implicated in follicle responsiveness to FSH, for example polymorphisms in genes encoding proteins important in follicular growth such as *ESR1*, *ESR2*, *CYP19A1*, *BMP15*, *AMH* and *AMHR2* may influence IVF outcomes (de Castro et al., 2004) and studies to determine their utility should be undertaken.

Acknowledgements

The work of WGN and SAR is supported by the Manchester Biomedical Research Centre.

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Declaration: The authors report no financial or commercial conflicts of interest.

Received 13 July 2012; refereed 13 June 2013; accepted 13 June 2013.