

The Asn680Ser Variant Negatively Impacted the Ovarian Response to Controlled Ovarian Stimulation in Thai Female Infertility

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Abstract

Background/Aim: The aim of this study was to evaluate the associations between three follicle-stimulating hormone receptor (FSHR) and FSH β subunit promoter gene polymorphisms, including Asn680Ser, -29G>A, and -211G>T with ovarian response, oocyte maturation, and embryo quality in Thai infertile women.

Materials and Methods: A total of 78 infertile Thai women undergoing *in vitro* fertilization or intracytoplasmic sperm injection were recruited. The polymorphisms were analyzed using polymerase chain reaction - restriction fragments length polymorphism.

Results: The Asn680Ser variant was significantly associated with ovarian response. The Asn/Asn genotype was more frequent in hyper-responders (66.7%), while the Asn/Ser genotype predominated among poor responders (71.4%, $p=0.038$). S-carriers combining Asn/Ser and Ser/Ser genotypes were overrepresented in the poor response group (78.6%, $p=0.029$). Logistic regression analysis revealed the association between Asn/Ser and S-carriers with poor response [crude odds ratio (OR)= 6.33, 95% confidence interval (CI)=1.56-25.66 and 5.36, 95% CI=1.36-21.10, respectively]. The associations remained after age adjustment (adjusted OR=7.71 95% CI=1.73-34.45 and 6.78, 95% CI=1.57-29.29, respectively). In contrast, -29G>A and -211G>T polymorphisms were not associated with ovarian response. There were not any associations between the three polymorphisms and oocytes maturation and embryo quality.

continued



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Conclusion: This study is the first to report the association between Asn680Ser FSHR gene polymorphism and the risk of poor response among Thai women.

Keywords: Asn680Ser, -29G>A, -211G>T, FSHR polymorphism, ovarian stimulation, poor response.

Introduction

Infertility is an increasingly prevalent global health issue, affecting approximately 17.5% of the adult population, with approximately one in six individuals expected to experience infertility at some point in their lives, according to a 2023 report by the World Health Organization (1). In Thailand, the fertility rate has been dramatically declining and forecasts show that the rate would decline to 1.36 children per women in 2035 (2). Assisted Reproductive Technology (ART) is very important procedure helping couples to have children. A crucial step of ART is controlled ovarian stimulation (COS), which aims to increase the number of mature follicles and oocytes. Follicle-stimulating hormone (FSH) plays a central role in this process, promoting folliculogenesis and stimulating estrogen production (3). In clinical practice, recombinant FSH (rFSH) is widely used to support follicular development and oocyte maturation through its interaction with the FSH receptor (FSHR) expressed by granulosa cells.

Certain single nucleotide polymorphisms (SNPs) affect FSHR signaling. The Asn680Ser (rs6166) polymorphism results in the change from asparagine (Asn) to serine (Ser) in amino acid position 680 and reduced receptor sensitivity (4, 5). The -29G>A (rs1394205) at the 5'-untranslated core promoter region results in a change from guanine (G) to adenine (A) in position -29. The A allele in position -29 could lead to the loss of one specific transcription factor binding site (6, 7). The -211G>T (rs10835638) in the FSH β subunit promoter falls in a binding element for the LHX3 homeodomain transcription factor, capable of influencing gene transcription. The evolutionarily more recent SNPs, both in the *FSHR* and in the *FSHB* genes are associated *in vitro* with changes either in signal transduction or in transcriptional activity resulting in an overall reduced FSH

action (8). These have been identified in European and Asian populations and have been shown to influence receptor function and clinical outcomes (9). These polymorphisms may contribute to poor ovarian response (POR) during ART procedures, including reduced oocyte yield and suboptimal follicular development. Therefore, understanding the presence and effects of these genetic variants is essential for optimizing individualized ovarian stimulation protocols. The previous research demonstrated that common genetic variants in the *FSHR* and *FSHB* genes influenced individual serum hormone levels. However, the clinical significance of these polymorphisms in gonadotropin treatment remains unclear and there is need to be studied in other ethnicities to support practical pharmacogenetic recommendations for the clinical use of FSH (10-14).

This study aimed to investigate the presence of *FSHR* and *FSHB* gene polymorphisms in Thai women with infertility undergoing treatment at the Center of Excellence for Infertility Treatment, Srinagarind Hospital, Khon Kaen University. Specifically, it assessed the association of the SNPs Asn680Ser, -29G>A, and -211G>T with ovarian stimulation outcomes, oocyte maturation, and embryo quality. The findings may contribute to the development of predictive models integrating these genetic markers to assist gynecologists in identifying patients at risk of poor ovarian response. This approach supports the advancement of personalized infertility treatment, aiming to improve efficacy and clinical outcomes.

Patients and Methods

Patients. This study was designed as a prospective cohort study conducted at the Srinagarind Hospital Center of Excellence for Infertility Treatment, Khon Kaen University, Thailand from June 2024 to May 2025. During this time, 100

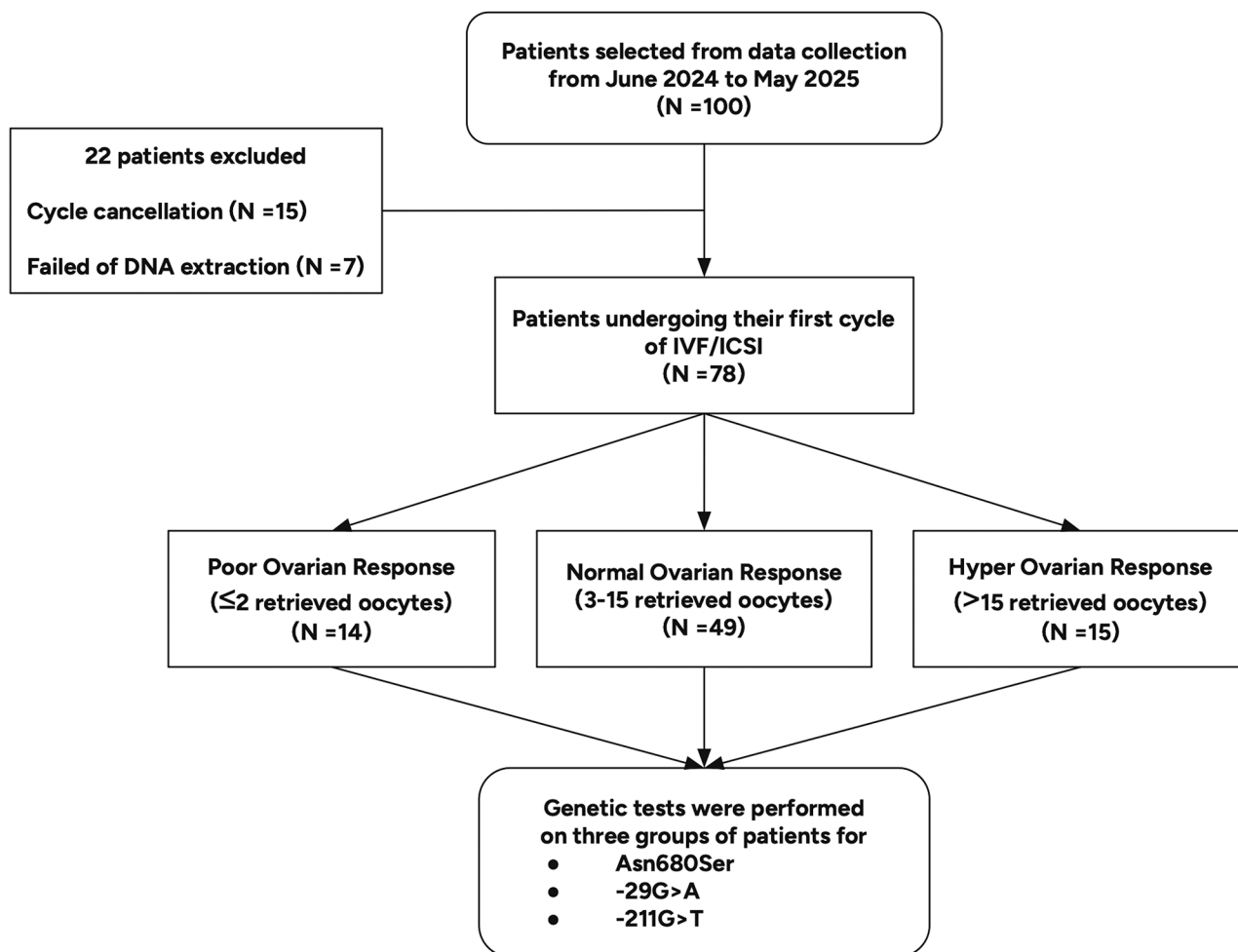


Figure 1. Study flow diagram. IVF/ICSI: In vitro fertilization/intracytoplasmic sperm injection; Asn: asparagine; Ser: serine.

ART treatments were performed using *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) as insemination methods. The age of all patients recruited in the study ranged from 26-47 years old and had at least 1 year of infertility, either primary or secondary with first IVF/ICSI treatment cycle. Patients who had cycle cancellation and DNA extraction failed were excluded from the analysis. From total of 100, 78 patients were included in the study as shown in Figure 1. This research was approved by the Khon Kaen University Ethics Committee for Human Research based on the Declaration of Helsinki and the ICH Good Clinical Practice Guidelines. (Reference No. HE671280)

Stimulation and prediction of the ovarian response. Patients undergoing ART treatment underwent Anti-Müllerian Hormone (AMH) testing to evaluate ovarian reserve and FSH, Luteinizing Hormone (LH) and Estradiol (E2) analysis. The measurement was conducted using electrochemiluminescence (ECL) technology (Cobas e 801 analyzer; Roche Diagnostics, Basel, Switzerland). Antral follicle count (AFC) assessments were performed *via* transvaginal ultrasound on day 2 or 3 of menstrual cycle. To maintain consistency, all ultrasounds were conducted by the same doctor. Controlled ovarian stimulation (COS) was initiated using a gonadotropin-

releasing hormone antagonist (GnRH-ant) protocol. rFSH administration began on the second day of menstruation and was continued daily alongside the GnRH antagonist until the administration of human chorionic gonadotropin (HCG). Treatment cycles were closely monitored through transvaginal ultrasonography, which assessed follicular development and endometrial thickness. Ultrasound evaluations were repeated on the eighth day of stimulation and then every 1-3 days, depending on clinical indications until the criteria for HCG triggering were met. Oocyte retrieval was scheduled 36 h after HCG administration. Ovarian response was classified as poor (≤ 2 oocytes), normal (3-15 oocytes) and hyper response (>15 oocytes) based on the number of retrieved oocytes, according to previous studies (13-16).

DNA isolation from whole blood. DNA was extracted from EDTA blood using the FlexiGene® DNA kit (QIAGEN, Hilden, Germany). A spectrophotometer was used to measure absorbance at 260 and 280 nm for each sample to verify its purity. DNA samples were deemed clean enough for analysis if their A260/A280 ratio fell between 1.8 and 2.0. Before being used, the extracted DNA was stored at -20°C .

Analysis of polymorphisms. The polymerase chain reaction (PCR) amplification was carried out with a Veriti 96-Well Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA). Gel electrophoresis was performed twice, first to confirm successful PCR amplification, and again after enzyme digestion to identify the restriction fragments. The Asn680Ser variant was analyzed using the PCR-restriction fragments length polymorphism (PCR-RFLP) method. The primers used in the reaction were: forward 5'-TTT GTG GTC ATC TGT GGC TGC-3' and reverse 5'-CAA AGG CAA GGA CTG AAT TAT CAT T-3'. A 520 bp DNA fragment was produced under the following PCR conditions: 35 cycles of denaturation at 95°C for 30 s, annealing at 61°C for 30 s, and extension at 72°C for 45 s. The amplified products were then digested with *BsrI* (New England Biolabs, Ipswich, MA, USA) and separated

on a 2.8% agarose gel containing 0.4 $\mu\text{g}/\text{ml}$ ethidium bromide. Digestion resulted in two fragments of 413 bp and 107 bp as shown in Figure 2A.

The $-29\text{G}>\text{A}$ variant, a set of primers (forward 5'-TCT TCT CAT AAG GGC ACT GTG T-3' and reverse 5'-ATC TCT GTC ACC TTG CTC TCT T-3') was used in the reaction. A 212 bp DNA fragment was amplified with 40 cycles of denaturation at 95°C for 1 min, annealing at 57°C for 50 s, and extension at 72°C for 30 s. The fragment was digested by *MboII* (New England Biolabs, Ipswich, MA, USA) and separated on a 2.8% agarose electrophoresis gel containing 0.4 $\mu\text{g}/\text{ml}$ ethidium bromide. The product was digested into 135 and 77 bp fragments as shown in Figure 2B.

To ensure accuracy, representative samples from each of the three genotypes were randomly selected and confirmed by Sanger sequencing of the PCR/RFLP products. For the third polymorphism ($-211\text{G}>\text{T}$), Sanger sequencing was performed on all 78 samples to determine the variant genotypes as shown in Figure 2C.

Statistical analysis. Statistical analyses were conducted using SPSS Statistics software (version 29.0.2.0; IBM, Armonk, NY, USA). Descriptive statistics are presented as mean \pm standard deviation (SD), median (IQR), and n (%). Data normality was assessed using the Kolmogorov-Smirnov test. For group comparisons, the independent samples *t*-test and ANOVA were used for normally distributed data, while the Mann-Whitney U test and Kruskal-Wallis test were applied for non-normally distributed data. Differences in proportions between categorical variables were analyzed using the chi-square test. Associations between variables were evaluated using binary logistic regression analysis. A *p*-value <0.05 was considered statistically significant.

Results

Distribution of Asn680Ser, $-29\text{G}>\text{A}$, $-211\text{G}>\text{T}$ genotype and allele frequency. The distribution of the three polymorphisms is presented in Table I. For the Asn680Ser variant, the

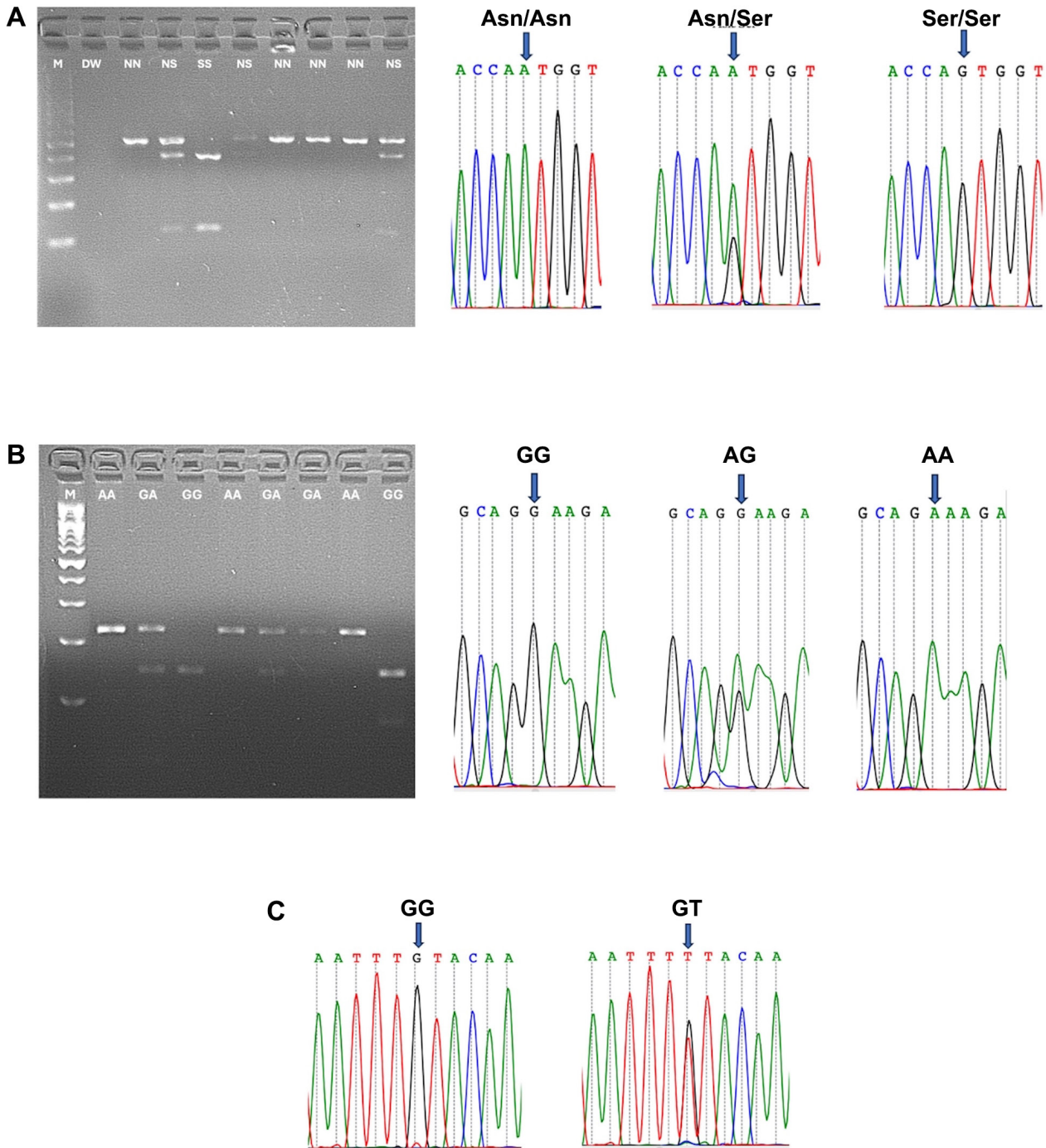


Figure 2. Representative electrophoretic analysis of PCR-RFLP products and corresponding Sanger sequencing chromatograms for the analyzed polymorphisms. (A) Asn680Ser polymorphism. Agarose gel electrophoresis of restriction fragments and representative sequencing chromatograms showing the Asn/Asn, Asn/Ser, and Ser/Ser genotypes. N indicates the Asn (normal) allele; S indicates the Ser (variant) allele. (B) -29G>A polymorphism. Electrophoretic patterns and sequencing chromatograms illustrating GG, GA, and AA genotypes. G represents the normal allele; A represents the variant allele. (C) -211G>T polymorphism. Representative sequencing chromatograms demonstrating GG and GT genotypes. G denotes the normal allele; T denotes the variant allele. M: DNA fragment size marker (100–1000 bp ladder); PCR-RFLP: polymerase chain reaction–restriction fragment length polymorphism.

Table I. Genotype and allele frequencies of the Asn680Ser, -29G>A, and -211G>T polymorphisms.

SNPs	Genotype	Genotype n (%)	Allele	Allele n (%)
Asn680Ser	Asn/Asn	41 (52.56)	N	112 (0.72)
	Asn/Ser	30 (38.46)	S	44 (0.28)
	Ser/Ser	7 (8.98)		
-29G>A	GG	24 (30.77)	G	89 (0.57)
	GA	41 (52.56)	A	67 (0.43)
	AA	13 (16.67)		
-211G>T	GG	74 (94.87)	G	152 (97.44)
	GT	4 (5.13)	T	4 (2.56)
	TT	-		

SNPs: Single nucleotide polymorphisms; Asn: asparagine; Ser: serine.

frequencies of the Asn/Asn, Asn/Ser, and Ser/Ser genotypes were 52.56%, 38.46%, and 8.98%, respectively. For the -29G>A variant, the GG, GA, and AA genotypes accounted for 30.77%, 52.56%, and 16.67%, respectively. In the case of the -211G>T variant, the GG and GT genotypes were observed at frequencies of 94.87% and 5.13%, while the TT genotype was not detected in this study. Overall, these distributions were similar to those in other Asian countries (9).

Baseline characteristics in the poor-, normal- and hyper-response group. According to Table II, the poor-, normal- and hyper-response groups differed significantly across several clinical parameters. The poor response group had a higher mean age (40.00±3.68 vs. 37.59±4.22 vs. 33.87±4.26, $p<0.001$) and demonstrated markedly lower AFC (3.00 vs. 5.50 vs. 7.00, $p<0.001$). Serum AMH concentrations were markedly lower in the poor-response group than in the normal-response and hyper-response groups. (0.70 vs. 1.01 vs. 3.73, $p<0.001$). The hyper-response group showed higher in LH more than poor- and normal-response group (7.44 vs. 6.62 vs. 9.05, $p=0.038$). In addition, the number of retrieved oocytes (1.00 vs. 7.00 vs. 19.00, $p<0.001$) and the number of mature oocytes, defined as metaphase II (MII) oocytes, (0.00 vs. 4.00 vs. 12.00, $p<0.001$) were substantially lower in women classified as poor responders.

Association of polymorphisms with ovarian response. As presented in Table III, the analysis of the Asn680Ser polymorphism revealed that the Asn/Asn genotype was more prevalent in the hyper-response group (66.67%), whereas the Asn/Ser genotype was more frequent among poor responders (71.43%), which was statistically significant ($p=0.038$). When the Asn/Asn genotype was compared with S-carriers (Asn/Ser + Ser/Ser), the proportion of S-carriers was significantly higher in the poor-response group (78.57%, $p=0.029$).

For the -29G>A polymorphism, the GG genotype was observed more frequently in the hyper-response group (33.33%), whereas the GA genotype predominated among poor responders (64.29%). When GA and AA genotypes were grouped as A-carriers, their prevalence was higher in the poor-response group (71.43%). However, unlike Asn680Ser, the observed difference for -29G>A did not reach statistical significance. The -211G>T polymorphism showed that the GG genotype was more associated with a hyper response (100.00%), while the GT genotype was more common in poor responders (14.29%). However, this difference was not statistically significant.

As shown in Table IV, logistic regression analysis was conducted to examine the odds ratios of Asn680Ser polymorphism between the reference (Asn/Asn) and variant groups. The analysis indicated that both the Asn/Ser genotype and the combined Asn/Ser + Ser/Ser genotypes functioned as significant predictive factors for poor ovarian response. The Asn/Ser genotype exhibited a crude odds ratio (OR) of 6.33 [95% confidence interval (CI)=1.56-25.66], while the S-carriers group demonstrated a crude OR of 5.36 (95% CI=1.36-21.10), suggesting that carriers of these genotypes had substantially higher odds of poor response compared with the reference group. After adjusting for patient age to minimize potential confounding, the association remained robust, with adjusted ORs of 7.71 (95% CI=1.73-34.45) for the Asn/Ser genotype and 6.78 (95% CI=1.57-29.29) for the Asn/Ser + Ser/Ser genotype, as shown in Figure 3. These findings provide strong statistical evidence that the Asn680Ser polymorphism exerts a genuine and independent influence on poor ovarian response.

Table II. Baseline characteristics in poor, normal, and hyper responders of ovarian stimulation.

Baseline characteristics	Poor response (n=14)	Normal response (n=49)	Hyper response (n=15)	p-Value
Age, years, mean±SD	40.00±3.68	37.59±4.22	33.87±4.26	<0.001
BMI*, kg/m ²	21.95 (20.58-23.74)	22.23 (20.43-25.13)	21.60 (19.45-23.53)	0.431
AFC*	3.00 (1.75-3.50)	5.50 (3.75-7.00)	7.00 (5.00-12.50)	<0.001
AMH* (ng/ml)	0.70 (0.24-1.30)	1.01 (0.42-1.56)	3.73 (2.47-6.10)	<0.001
LH* (mIU/ml)	7.44 (4.51-11.67)	6.62 (3.88-8.49)	9.05 (6.74-26.18)	0.038
Basal FSH* (mIU/ml)	10.30 (7.67-17.77)	8.53 (7.26-10.97)	8.18 (6.65-9.67)	0.065
Estradiol* (E2) (pg/ml)	67.06 (30.79-165.30)	38.70 (28.77-60.21)	44.70 (37.45-671.87)	0.666
Days of FSH administration*	8.50 (5.00-10.25)	9.00 (7.00-10.00)	10.00 (9.50-10.00)	0.114
Total dose of FSH administration*, IU	2062.50 (1500.00-3075.00)	2700.00 (1987.50-3000.00)	2250.00 (1500.00-3037.60)	0.391
Number of oocytes retrieved*	1.00 (0.00-2.00)	7.00 (5.00-8.50)	19.00 (18.50-27.00)	<0.001
Number of MII oocytes*	0.00 (0.00-2.00)	4.00 (1.50-7.00)	12.00 (9.50-16.00)	<0.001

*Data shown as median (IQR). Statistically significant p-Values are shown in bold. BMI: Body mass index; AFC: antral follicle count; AMH: anti-müllerian hormone; LH: luteinizing hormone; FSH: follicle-stimulating hormone; Poor response: ≤2 retrieved oocytes; Normal response: 3-15 retrieved oocytes; Hyper response: >15 retrieved oocytes; SD: standard deviation.

Association of polymorphisms with oocyte maturation. In Table V, the percentage of MII oocytes was calculated as the ratio of MII to the total number of oocytes retrieved. The low MII ratio (≤70% MII oocytes/oocytes retrieved) and high MII ratio (>70% MII oocytes/oocytes retrieved) categorized by the median of MII ratio in this study. The box plots of the number of MII oocytes and Asn680Ser, -29G>A and -211G>T are shown in Figure S1. No significant differences were observed for Asn680Ser, -29G>A and -211G>T variants. These findings suggest that, although some variation in MII ratios was present among genotypes, the differences were not strong enough to indicate a meaningful genetic effect in this study population.

Association of polymorphisms with embryos quality. Blastocyst grading system is composed of four categories (17). However, in the present study, embryo quality was classified into two groups. Good-quality embryos included grades 3AA, 3-6AB, 3-6BA, 1-2AA, 3-6BB, 3-6AC, 3-6CA,

Table III. Distribution of polymorphism genotypes among poor, normal, and hyper response groups.

Variable	Poor response (n=14)	Normal response (n=49)	Hyper response (n=15)	p-Value
Asn680Ser, n (%)				
Asn/Asn	3 (21.43)	28 (57.14)	10 (66.67)	0.038
Asn/Ser	10 (71.43)	15 (30.61)	5 (33.33)	
Ser/Ser	1 (7.14)	6 (12.25)	0	
Asn/Asn	3 (21.43)	28 (57.14)	10 (66.67)	0.029
Asn/Ser*Ser/Ser	11 (78.57)	21 (42.86)	5 (33.33)	
-29G>A, n (%)				
GG	4 (28.57)	15 (30.61)	5 (33.33)	0.770
GA	9 (64.29)	24 (48.98)	8 (53.34)	
AA	1 (7.14)	10 (20.41)	2 (13.33)	
GG	4 (28.57)	15 (30.61)	5 (33.33)	0.961
GA+AA	10 (71.43)	34 (69.39)	10 (66.67)	
-211G>T, n (%)				
GG	12 (85.71)	47 (95.92)	15 (100.00)	0.189
GT	2 (14.29)	2 (4.08)	0	
TT	-	-	-	

Statistically significant p-values are shown in bold. Poor response: ≤2 retrieved oocytes; Normal response: 3-15 retrieved oocytes; Hyper response: >15 retrieved oocytes; Asn: asparagine; Ser: serine.

Table IV. Binary logistic regression analysis of Asn680Ser polymorphism and risk of poor ovarian response.

Variable	Genotype	Crude OR	p-Value	(95% CI)
Asn680Ser	Asn/Ser	6.33	0.010	1.56 to 25.66
	Asn/Ser+Ser/Ser	5.36	0.016	1.36 to 21.10
Variable	Genotype	Adjusted OR	p-Value	(95%CI)
Asn680Ser	Asn/Ser	7.71	0.007	1.73 to 34.45
	Asn/Ser+Ser/Ser	6.78	0.010	1.57 to 29.29

Reference group: Asn/Asn. OR: odds ratio; CI: confidence interval; Asn: asparagine; Ser: serine.

1-2AB, and 1-2BA. Poor-quality embryos comprised grades 1-6BC, 1-6CB, 1-6CC, and 1-2BB. In this grading system, the first number represented the blastocyst expansion stage according to the Gardner criteria, the first letter indicated the inner cell mass quality, and the second letter indicated the trophectoderm quality. As presented in Table VI, the proportion of good-quality embryos was categorized using the median value into a low group ($\leq 11.11\%$ good-quality embryos) and a high group ($> 11.11\%$ good-quality embryos). The analysis showed no significant association between the Asn680Ser, -29G>A, or -211G>T polymorphisms and embryo quality.

Discussion

Ovarian response was classified as poor, normal, or hyper according to the 2011 ESHRE consensus on POR (18). POR is defined by the presence of at least two of the following criteria: advanced maternal age (≥ 40 years) or other risk factors; a previous poor response, indicated by cycle cancellation or retrieval of three or fewer oocytes with a conventional protocol; and an abnormal ovarian reserve test, reflected by an antral follicle count of 5-7 follicles or anti-Müllerian hormone levels of 0.5-1.1 ng/ml. Equal or less than 3 retrieved oocytes were considered a poor response, in line with the most commonly used definition at the time. We also analyzed the data according to the ESHRE 2011 consensus, using a cutoff of ≤ 3 retrieved oocytes. The results were consistent with the baseline characteristics and were similar

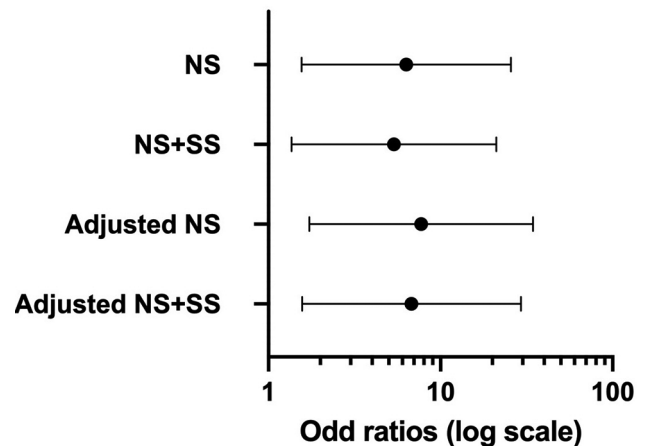


Figure 3. Odds ratios (Ors) with 95% confidence intervals demonstrating the association between Asn680Ser polymorphism and poor ovarian response. NN: Asn/Asn; NS: Asn/Ser; SS: Ser/Ser, Asn: asparagine; Ser: serine.

to those obtained using the ≤ 2 oocyte criterion, except for basal FSH levels, which were highest in the poor-response group compared with the normal- and hyper-response groups (10.30 vs. 8.28 vs. 7.46, $p=0.019$). No association was found between the Asn680Ser, -29G>A, or -211G>T polymorphisms and the three ovarian response groups, as shown in Table SI and Table SII.

Poor ovarian response had been defined differently across studies (19, 20). Some authors considered fewer than three follicles as a poor response, while others used a threshold of fewer than five. A Swedish study in 2004 defined poor response, from a clinical management perspective, as the retrieval of one to two oocytes. Although couples were generally encouraged to continue treatment regardless of the initial outcome, the likelihood of success was considered very low when only one to two oocytes were retrieved in the first cycle and four or fewer in the second (15). In the United Kingdom, poor response to FSH in ART cycles was defined as the retrieval of two oocytes, corresponding to two standard deviations below the mean number collected (21). A Korean study in 2015 validated the Bologna criteria and proposed a refined definition of poor ovarian response based on statistical and prognostic analyses, defining POR as total oocytes ≤ 2 or mature oocytes ≤ 1 (16).

Table V. The MII ratio according to Asn680Ser, -29G>A, and -211G>T gene polymorphisms.

Variable	Asn680Ser			-29G>A			-211G>T		
	Asn/Asn (n=40)	Asn/Ser* Ser/Ser (n=33)	p-Value	GG (n=22)	GA+AA (n=51)	p-Value	GG (n=70)	GT (n=3)	p-Value
MI I ratio									
Low, n(%)	20 (50.00)	14 (42.42)	0.518	14 (63.64)	20 (39.22)	0.055	33 (47.14)	1 (33.33)	0.639
High, n(%)	20 (50.00)	19 (57.58)		8 (36.36)	31 (60.78)		37 (52.86)	2 (66.67)	

Low MII ratio: ≤70% MII oocytes/oocytes retrieved; High MII ratio: >70% MII oocytes/oocytes retrieved) categorized by the median of MII ratio in this study. Asn: Asparagine; Ser: serine; MII: mature oocytes (metaphase II).

Table VI. Association between Asn680Ser, -29G>A, and -211G>T polymorphisms and embryo quality.

Variable	N680S			-29G>A			-211G>T		
	Asn/Asn (n=32)	Asn/Ser* Ser/Ser (n=22)	p-Value	GG (n=15)	GA+AA (n=39)	p-Value	GG (n=52)	GT (n=2)	p-Value
Embryo quality									
Low, n(%)	18 (56.25)	11 (50.00)	0.651	11 (73.30)	18 (46.20)	0.073	28 (53.80)	1 (50.00)	0.915
High, n(%)	14 (43.75)	11 (50.00)		4 (26.70)	21 (53.80)		24 (46.20)	1 (50.00)	

Low embryo quality: ≤11.11% good embryos; High embryo quality: >11.11% good embryos (categorized according to the median percentage of good embryos). Blastocyst criteria: Good (3AA, 3-6AB, 3-6BA, 1-2AA, 3-6BB, 3-6AC, 3-6CA, 1-2AB, 1-2BA); Poor (1-6BC, 1-6CB, 1-6CC, 1-2BB).

Therefore, the percentiles for the 5th and 10th which commonly used as the critical value in this study were 0 and 2 oocytes, respectively. Consequently, the 10th percentile was chosen as the cutoff value for this investigation. Following other studies (20, 22, 23), subjects were divided into three groups: poor (≤2 oocytes retrieved), normal (3-15 oocytes retrieved), and hyper response (>15 oocytes retrieved).

A cross-sectional study conducted in Thailand, 2014 (24) examined the association between *FSHR* gene polymorphisms and chronic anovulation in women with and without polycystic ovary syndrome. To the best of our knowledge, this was the first study in Thailand to examine whether *FSHR* variants, particularly Asn680Ser, which impairs FSH receptor function, and -29G>A, which reduces receptor expression, as well as *FSHB*, the gene encoding the β-subunit responsible for FSH biological activity, are associated with ovarian stimulation outcomes, oocyte maturation, and embryo quality.

Regarding the Asn680Ser variant, S-carriers (Asn/Ser and Ser/Ser genotypes) showed higher basal FSH levels compared with Asn/Asn individuals, consistent with previous findings (25). Notably, the Ser/Ser genotype was significantly associated with a lower number MII oocytes than the Asn/Asn genotype, indicating a poorer ovarian response among Ser/Ser homozygotes. In line with this, the frequency of S-carriers was higher in the poor-response group than in the normal- and hyper-response group, So, S-carrier status was linked to poor ovarian response.

Regarding the -29G>A variant, patients with the AA genotype did not show a tendency toward poor ovarian response in the present study. However, earlier research reported (26) a possible association with reduced responsiveness to gonadotropin treatment based on endocrine and clinical parameters, although this finding did not reach statistical significance, likely due to a small sample size. Similarly, the -211G>T polymorphism showed a non-significant trend toward higher basal FSH levels,

lower antral follicle counts, and fewer retrieved and MII oocytes in GT heterozygotes compared with GG wild-type individuals, consistent with a previous report (23).

Recent research examining different FSH types and the Asn680Ser variant suggests that rFSH works better for people with the Asn/Asn genotype, while urinary FSH (uFSH) may be a better for S-carriers. This raises the possibility of choosing hormone treatment based on a patient's *FSHR* Asn680Ser genotype. Furthermore, the use of genotype-matched hormone therapy was associated with a 38% increase in the likelihood of achieving a live birth in the first IVF cycle (27). The authors therefore recommended further research to examine whether the same approach applies to Thai women. Beyond the clinical angle, this work highlights how pharmacogenetics could help tailor ovarian stimulation to improve outcomes, decrease the need for repeat IVF/ICSI cycles, and reduce both health and financial burdens through more personalized and safer ART care.

However, our study has a limitation in that it included only 78 samples. Therefore, further studies involving larger populations are needed to validate these findings.

Conclusion

FSHR gene polymorphisms, particularly Asn680Ser, are associated with poor ovarian stimulation. Although previous studies have been conducted, this is the first study in a Thai population. As these associations also depend on ethnicity, our study confirms the association of polymorphisms with poor ovarian stimulation across ethnicities. We recommend that ART centers consider genetic screening for *FSHR* Asn680Ser variants prior to initiating gonadotropin protocols, as this may help determine the most suitable dose and type of FSH for each patient. Such investigations will contribute to the development of more personalized treatment strategies, ultimately improving the success rates of ART.

Supplementary Material

Figure S1 is available at: <https://figshare.com/s/f4a56987e105a061dc4f>

Table SI is available at: <https://figshare.com/s/0d2fb19fefeacdf3798f>

Table SII is available at: <https://figshare.com/s/50391e11612eec042f47>

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

Research concept: NB, SH, LS, SP, PM, JD, TP and SS; Execution of the experiments: NB; patient collection: NB, LS; TP and SS; analysis of data: NB, SH; contribution to the writing of manuscript: NB, SH; validation, writing-review & editing, supervision and funding acquisition: SP, PM and JD; All Authors have read and agreed to the published version of the manuscript.

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Artificial Intelligence (AI) Disclosure

No artificial intelligence (AI) tools, including large language models or machine learning software, were used in the preparation, analysis, or presentation of this manuscript.

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