International Journal of Pediatrics and Genetics
Vol. 3 No. 10 (2025)
ISSN: 2995-5483

Single Nucleotide Polymorphisms (SNPs) and Their Role in Reproductive Health: A Genetic Analysis

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Abstract: Infertility is considered a disease with a high prevalence and a primary genetic factor, as single-nucleotide polymorphisms (SNPs) in metabolic and hormonal genes impair fertility. Our current study aimed to determine the impact of single-nucleotide polymorphisms (SNPs) on reproductive health in a group of 68 infertile patients (34 couples). Based on the study's methodological design, a cross-sectional study was conducted on 68 patients in different hospitals in Iraq, during a follow-up period of 12 months. Clinical data were recorded, including semen parameters, hormonal profiles, history of pregnancy loss, and genotype distributions. The study results showed a strong association between the FSHR rs6166 polymorphism and decreased ovarian reserve. It was observed that female patients who received the G allele vaccine had lower AFC levels (6.3 ± 2.1 vs. 14.5 ± 4.2), which constituted 83.3% of women with poor ovarian response, while the MTHFR rs1801133 TT genotype was associated with lower semen quality and also lower sperm concentration (15.4 ± 12.1 vs. 38.2 ± 18.5 ml/ml) in males. Furthermore, the ESR1 rs2234693 TT genotype was associated with higher baseline FSH levels (9.2 ± 2.8 vs. 6.8 ± 1.5 mIU/mL, p=0.041), which may indicate a higher frequency of F5 Leiden (rs6025) mutation carriers in women with recurrent pregnancy loss (21.4% vs. 5.0%). This study concludes that single-nucleotide polymorphisms (SNPs) in FSHR and MTHFR are strongly associated with ovarian response and sperm quality in infertile patients, and that genetic testing for these variants could provide valuable diagnostic information.

Key words: Single Nucleotide Polymorphism (Snp); Infertility; Mthfr; Fshr; Ovarian Reserve; And Male Factor Infertility.

Introduction

The SNP was the most frequent type of polymorphism, constituted at least 90% of all genetic variations, which SNPs referred to one base pair substitutions that occur at specific loci in the genome and the less frequent allele had a prevalence of at least 1% in a given population, where most SNPs may go unnoticed, but a subset of SNPs may have a very important functional consequence;

the consequence may be alteration of gene expression, disrupting splicing, or changing the amino acid sequence of a protein and therefore affecting cellular and organismal functions. [1,2,3,4]

One compelling and quickly advancing new frontier in reproductive health was in the application of SNP studies, where reproduction got an intricate, multifactorial process requiring a fine balance shared in hormonal signaling during gametogenesis, fertilization, implantation, and successful gestation. [5,6]

Moreover, disruptions at any of these stages could lead to a spectrum of reproductive problems such as infertility, recurrent pregnancy loss (RPL), preeclampsia, and even preterm birth; thereby, implying a bit of polygenic or complex etiology where numerous genetic variants modulate complexity while exhibiting a low effect or interaction with environmental factors. [7,8]

In addition, SNPs were considered increasingly as orchestrators of potential reproductive success, while GWAS and candidate-gene analysis have located polymorphisms in genes playing a role in folliculogenesis, spermatogenesis, hormonal receptor function, endometrial receptivity, and immune tolerance at the maternal-fetal interface that are significantly implicated in reproductive pathologies, included the following: SNPs in genes such as FSHR and ESR1 are implicated in ovarian response, whereas variants of protamine genes affect sperm quality, as well as other thrombophilic SNPs, such as Factor V Leiden, influence placentation and pregnancy maintenance. [9,10,11]

Materials and Methods

Study Design:

A cross-sectional study was conducted to investigate the impact of single-nucleotide polymorphisms (SNPs) on the reproductive health of 68 patients (34 females and 34 males) who presented to a reproductive medicine center for infertility evaluation at different hospitals in Iraq, during the follow-up period of January 2024–January 2025. This study documented the patients' conditions and determined their impact. All patients were examined, and 47.1% (n = 32) were diagnosed with primary infertility, and 52.9% (n = 36) with secondary infertility. 41.2% (n = 14) had a history of recurrent miscarriage. The mean sperm concentration of the male partners was 28.5 ± 21.3 million/ml.

Determining the biological basis for selecting single-nucleotide polymorphisms (SNPs):

Genotyping tests and their impact on reproductive physiology were determined by selecting five single-nucleotide polymorphisms (SNPs) for genotyping, as well as two other polymorphisms in the MTHFR gene (rs1801133 and rs1801131), due to their impact on folic acid metabolism and homocysteine levels, to determine egg quality, sperm formation, and early embryonic development. In addition, to determine the primary involvement in hormonal response and ovarian follicle development, a single polymorphism (SNP) of rs2234693 in the estrogen receptor 1 (ESR1) gene was selected, as the RS6166 SNP in the follicle-stimulating hormone receptor (FSHR) gene modifies the ovarian response to FSH stimulation. Another SNP of rs6025 in the F5 (factor V Leiden) gene was selected, as it is associated with an increased risk of thrombosis and recurrent miscarriage.

Data Collection and Clinical Evaluations:

A standard infertility screening test was performed using transvaginal ultrasound on day 3 of the cycle to determine the antral follicle count (AFC). Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2) were also measured on day 3 of the cycle. Anti-Müllerian hormone (AMH) levels were also assessed as an indicator of ovarian reserve, with patients classified as having poor ovarian responsiveness if their antral follicle count was less than 5. Furthermore, the five single-nucleotide polymorphisms (SNPs) in which the patients experienced infertility were genotyped (MTHFR rs1801133, MTHFR rs1801131, ESR1 rs2234693, FSHR rs6166, and F5 rs6025) using polymerase chain reaction (PCR), followed by fragment length polymorphism analysis. Restriction PCR-RFLP

Statistical Analysis

Allele and genotype frequencies were calculated by direct counting. Continuous variables such as age, hormone levels, and semen parameters were expressed as mean ± standard deviation (SD). Odds ratios (OR) and 95% confidence intervals (CI) were calculated to assess the risk of certain genotypes in RPL. A two-tailed p-value lower than 0.05 implied statistical significance. All analysis was carried out using the SPSS Statistics software with version 24.0.

Results

Table 1: Enroll the demographics and clinical features at patients.

Characteristic	Female Patients (n=34)	Male Patients (n=34)	Total Cohort
Mean Age (years ± SD)	35.2 ± 4.1	37.1 ± 5.3	36.2 ± 4.8
Primary Infertility (n, %)	16 (47.1%)	16 (47.1%)	32 (47.1%)
Secondary Infertility (n, %)	18 (52.9%)	18 (52.9%)	36 (52.9%)
History of Pregnancy Loss (>1)	14 (41.2%)	N/A	14 (41.2%)
Mean Sperm Concentration (mil/mL)	N/A	28.5 ± 21.3	N/A
Poor Ovarian Responders (AFC < 5)	9 (26.5%)	N/A	9 (26.5%)

Table 2: Genotyped SNPs and Their Biological Functions.

Gene	SNP ID	Major/Minor Allele
MTHFR	rs1801133	C/T
MTHFR	rs1801131	A/C
ESR1	rs2234693	C/T
FSHR	rs6166	A/G
F5	rs6025	G/A

Table 3: Overall Allele and Genotype Frequencies in the Study Cohort (n=68 patients).

SNP ID	Major Allele Freq.	Minor Allele Freq.	Homozygous Major	Heterozygous	Homozygous Minor
MTHFR rs1801133	0.68	0.32	30 (44.1%)	31 (45.6%)	7 (10.3%)
MTHFR rs1801131	0.74	0.26	38 (55.9%)	25 (36.8%)	5 (7.3%)
ESR1 rs2234693	0.59	0.41	22 (32.4%)	35 (51.5%)	11 (16.1%)
FSHR rs6166	0.63	0.37	26 (38.2%)	33 (48.5%)	9 (13.3%)
F5 rs6025	0.96	0.04	63 (92.6%)	5 (7.4%)	0 (0%)

Table 4: Association of *MTHFR* rs1801133 Genotype with Semen Parameters in Male Patients (n=34).

Semen Parameters	CC (n=14)	CT (n=16)	TT (n=4)	p-value
Sperm Concentration (mil/mL, mean ± SD)	38.2 ± 18.5	25.1 ± 20.3	15.4 ± 12.1	0.048
Total Motility (%, mean ± SD)	52.1 ± 11.2	45.3 ± 14.6	38.5 ± 10.8	0.087
Normal Morphology (%, mean ± SD)	7.1 ± 2.5	5.8 ± 3.1	4.2 ± 2.0	0.039

Table 5: Association of *FSHR* rs6166 Genotype with Ovarian Response in Female Patients (n=34).

Ovarian Response indicator	AA (n=12)	AG (n=16)	GG (n=6)	p-value
Antral Follicle Count (AFC, mean ± SD)	14.5 ± 4.2	10.1 ± 5.0	6.3 ± 2.1	0.005

AMH level (ng/mL, mean ± SD)	3.8 ± 1.9	2.1 ± 1.5	1.0 ± 0.6	0.002
Poor Responders (AFC <5, n, %)	0 (0%)	4 (25.0%)	5 (83.3%)	<0.001

Table 6: Association of *ESR1* rs2234693 and *F5* rs6025 with Recurrent Pregnancy Loss (RPL) in Female Patients.

Genotype	Patients with RPL (n=14)	Patients without RPL (n=20)	Odds Ratio (95% CI)	p-value
ESR1 rs2234693 (TT vs. CC/CT)	5 (35.7%)	3 (15.0%)	3.18 (0.61-16.5)	0.16
F5 rs6025 (GA vs. GG)	3 (21.4%)	1 (5.0%)	5.25 (0.50-55.2)	0.11

Table 7: Combined Genotype Analysis: *MTHFR* rs1801133 and rs1801131.

Compound Genotype	Patients (n=68)	Prevalence
Any two variant alleles (across both SNPs)	18	26.5%
Homozygous variant for at least one SNP	10	14.7%
No variant alleles (homozygous major for both)	22	32.4%

Table 8: Hormonal Profile by *ESR1* Genotype in Female Patients (n=34).

Hormone (Day 3)	CC (n=8)	CT (n=19)	TT (n=7)	p-value
FSH (mIU/mL, mean ± SD)	6.8 ± 1.5	7.9 ± 2.1	9.2 ± 2.8	0.041
LH (mIU/mL, mean ± SD)	5.1 ± 1.2	5.5 ± 1.8	6.0 ± 2.0	0.52
Estradiol (pg/mL, mean ± SD)	35.2 ± 10.1	42.5 ± 15.3	48.8 ± 12.7	0.09

Table 9: Prevalence of Thrombophilic Mutation (*F5* rs6025) in the patients.

Patients Subgroup	n	Heterozygous (GA) Carriers	Prevalence
All Patients	68	5	7.4%
Females with RPL	14	3	21.4%
Females without RPL	20	1	5.0%
All Male Patients	34	1	2.9%

Discussion

This study analyzed the influence of some Single single-nucleotide polymorphisms (SNPs) of the key biologic pathways—folate metabolism, hormonal signaling, and coagulation—on reproductive health outcomes in a cohort of 68 infertile patients, where among the most striking findings was the strong association of the FSHR rs6166 (c.2039A>G) polymorphism with ovarian reserve in female patients.

Accorrding to the assocaition between gene-dose effects, the GG genotype had carried significantly lower antral follicle count (AFC), lower AMH levels, and a significantly greater incidence of poor ovarian response (83.3%) compared with the AA genotype, which some literature [12,13] implicated this non-synonymous SNP as a key regulator of FSH receptor sensitivity.

Also, Welsh study [14] has found that the G allele is often related to high basal FSH requirements and poor ovarian response to controlled ovarian stimulation, where our outcomes noticed MTHFR rs1801133 (C677T) polymorphism significantly had an adverse impact on semen parameters in male infertility.

Furthermore, men with TT genotype showed significantly lower sperm concentration and percentage of normal sperm morphology compared to CC genotype men, where hypothesis impaired folate metabolism, leading to hyperhomocysteinemia and aberrant DNA methylation/synthesis, can be harmful to spermatogenesis, while the association of MTHFR polymorphisms with male infertility has been reported in Chinese studies [15,16,17] with significant associations and others suggesting population-specific effects.

Our study got a clear stepwise reduction in semen quality from CC to TT genotypes, adds weight to the argument for a causal association, at least in our patient group, that the trend towards reduced motility (p=0.087) also suggests an overall negative impact on sperm function as well as ESR1 rs2234693 (PvuII) TT genotype was more frequent in RPL women (35.7% vs. 15.0%) with an Odds Ratio of 3.18.

Studies conducted in the USA [18,19,20] speculated that estrogen receptor alpha polymorphisms can influence endometrial receptivity and placental development, whereby the Factor V Leiden mutation (F5 rs6025) was much more prevalent in the RPL cohort (21.4% vs. 5.0%), with an OR of 5.25. The failure to achieve statistical significance (p=0.16 and p=0.11, respectively) is likely a result of our limited sample size (n=14 RPL patients), a common limitation of single-center RPL investigations, alongside elevated basal FSH levels in women with the ESR1 TT genotype also provide additional support for the idea of a more generalized impact on reproductive endocrine function, with the potential for a less competent ovarian environment.

Conclusion

Our study shows that almost all gene polymorphisms involving with hormone response, metabolism, and coagulation, namely FSHR, MTHFR, and F5, are significantly related to phenotypic markers of reproductive health, which are associated with ovarian response. Semen parameters appeared much stronger, opening the possibility of preemptive genetic screening to help optimize infertility treatment. Further to Genetic testing in incorporated into routine reproductive medicine nowadays, it might really herald the emergence of a more personalized, predictive, and efficacious care grasp for infertile couples.

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Vol. 3 No. 10 (2025)
ISSN: 2995-5483

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