Identification of ITGB3 Polymorphisms with implantation outcome during in vitro fertilization program

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Abstract
Successful embryo implantation requires a competent embryo, a receptive endometrium, and synchronized communication between them. In the field of assisted reproductive technologies, determining the embryos with the best opportunity for implantation remains difficult. ITGB3 has been shown to play an important role in implantation outcomes after in vitro fertilization (IVF). In this study, the frequencies of rs4642 and rs4634 polymorphisms were investigated in 50 infertile females under IVF protocol (divided into successful implantation and failure implantation) and compared them with 25 healthy fertile females. the results of current study demonstrated statistically significant associations between the heterogenotypes rs4642 and rs4634 polymorphisms and IVF implantation failure (odds ratio [OR] 0.36, 95% confidence interval [CI]: 0.13 to 1.04, p: 0.04; and OR 0.14, 95% CI: 0.03 to 0.57, p:0.003, respectively) this indicates that the two heterogenotypes for two SNPs act as a protective factor. In conclusion, in our pilot study, a strong association between rs4642 and rs4634 polymorphisms and IVF implantation failure in Iraqi female has been detected. Female who carries the homogenotype of SNPs rs4642 and rs4634 may become susceptible to implantation failure.

1. Introduction

Embryo implantation failure is considered the leading cause of infertility following natural conception and assisted reproductive technology (ART). Despite the major advances in reproductive medicine over the last few decades, especially in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI)-embryo transfer (ET), implantation failure is still a rate-limiting process for a successful pregnancy [1].

Successful embryo implantation requires an intimate dialogue between a blastocyst and a receptive endometrium during a brief period of time known as the window of implantation [2,3]. This period is characterized by the control of the endometrium by progesterone in preparation for implantation. The imposition of progesterone over estrogen during (days 20–24) from the secretory phase [4,5]. During the window of implantation, the blastocyst can attach to the endometrial epithelial cells and invade the endometrial stroma and vasculature. This process can only occur when the endometrium is receptive [6]. Selecting high-quality embryos for transfer and elucidating the molecular mechanisms underlying embryo implantation may aid in improving pregnancy outcomes. Embryos enter the uterine cavity at embryonic day (D)4, approximately 48 or 72 h before implanting [7]. Human implantation is a process that requires essential events such as apposition, adhesion/attachment, invasion, and immune regulation [8]. during this period, in order to prepare for implantation, the preimplantation embryos interact with the endometrium via soluble substances such as cytokines and growth factors[9]. Similarly, endometrium-secreted molecules internalized by the trophectoderm can lead to the expression of genes involved in embryo adhesion [10], one of these molecules, along with cytokines, growth factors, and others, is cellular adhesion molecules (CAM). Cellular Adhesion Molecules are a family that includes members such as integrins, cadherins, and selectins [11].

2. Integrins

Integrins are a family of transmembrane heterodimeric glycoproteins that facilitate cell-extracellular matrix adhesion [12]. The integrin family includes 18 alpha (α) and 8 beta (β) subunits that form 24 distinct αβ heterodimers. Each integrin heterodimer consists of a large extracellular domain region, two single-pass transmembrane helices (one in each subunit), and short cytoplasmic tails [13]. During the menstrual cycle, they undergo dynamic spatial and temporal modifications in the endometrium. [14]. They are also expressed in the endometrium during the time of implantation [15]. Their major roles are focused on differentiation, apoptosis, motility, and attachment [16]. During implantation, integrins play a role in the attachment of the cells to the ECM and initiate signaling transduction from the embryo to the ECM to initiate the translation of genes involved in the implantation process [17]. integrin activates FAK, which in turn can switch on the VAV-RAC1 signaling axis to bring the endometrial epithelial cell receptivity for blastocyst attachment. Furthermore, integrin directly interacts with the implanting blastocyst [18]. Reduced

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expression of integrin in the endometrial may contribute to unexplained infertility and this gene could account as the potential molecular markers of infertility [19].in this study, ITGB3 was selected to investigate its polymorphisms' role in embryonic implantation.

ITGB3 is a subunit of both the GpIIIb/ IIIa receptor (integrin α I I b β 3) which has a key role in platelet aggregation, clot retraction, and stabilization of thrombi by binding fibrinogen and von Willebrand factor [20]. The implication of inherited and acquired thrombophilia in IVF-ET failure has been proposed, probably by impairing the initial vascularisation process occurring at implantation [21]. Therefore, any variation in ITGB3 gene could be led to changes either in the vascular supply in the primary intervillous space during the first days of gestation or in implantation events.

However, due to the complexity of human embryo implantation, as well as ethical concerns, the intricate physiological and molecular processes remain unclear. The objective of this study was to evaluate the role of ITGB3 polymorphisms in the risk of unexplained IVF implantation failure.

3. Supjects, Material, and Methods

This study is carried out between March 2021 to April 2022 to determine the association of some biomarkers and ITGB3 polymorphisms with implantation failure in females attending General Hospitals in Baghdad, Iraq.

Subjects

All females included in this study were within the reproductive age range (20- 43 years) without any endocrine diseases were included; while those with endocrine diseases or polycystic ovary syndrome or male infertility were excluded; 71 infertile females under IVF protocol (divided into two subgroups , 29 success implantation and 42 failure implantation after follow up all these cases to determine the results of implantation after blastocytes implantation process within IVF program)and 50 fertile females as control were involved in the study, each fertile female have, at least, one previous birth.

Blood sampling

Venous blood samples (3ml) were collected from each female. Each blood was drawn in EDTA tube and stored at -18°C until DNA extraction for ITGB3 gene variations determination.

Anthropometric measurements

These measurements included: age, age of menarche by years, Weight (kg), height (m), and body mass index (BMI) was calculated by dividing the body weight by the square of the height according to the following equation: BMI = Weight (Kg) / [height (cm)]2. information has been set for each female in a special questionnaire list.

Genetic Analysis

Genomic DNA was extracted from peripheral whole blood samples of each subject using an EasyPure®Genomic DNA Kit (TransGen, biotech. EE101-01). After the polymerase chain reaction (PCR) products were obtained, all samples were directly sequenced, Sanger sequencing was performed on the amplified PCR fragments using an ABI3730XL automated DNA sequencer (Macrogen Corporation, Korea) to determine the SNPs in ITGB3. genotypes detected after aligning with a reference sequence in NCBI, Primers required for PCR amplification and sequencing were displayed in table 1. NCBI was used to match the primer sequences.

Table 1: the primer pairs used to amplify exon 10 at ITGB3 gene.

Forward primer (5 to 3)	Reverse primer (5 to 3)	Amplified region	Length (bp)	reference
CAGGGCAGGGAACAACTT	GGATTGGTCCTTATACTCAAAA	47292050-47292720	670	[22]

4. Statistical Analysis

Statistical analysis of data was performed using SPSS (Statistical Package for the Social Sciences - version 26). One-way ANOVA and the least significant difference (LSD) test were performed to assess significant differences among means. P< 0.05 was considered statistically significant. SNP statistical analysis and the Odds ratios along with the confidence intervals were calculated by using the WINPEPI computer programs for epidemiologists-version 11.65. Allele frequencies of genes were calculated by direct gene counting methods, while a departure from Hardy-Weinberg (H-W) equilibrium was estimated using H-W calculator for two alleles, which is available online at https://gene-calc.pl/hardy-weinberg-page.

5. Results and Discussion

This study examined some stress biomarkers and ITGB3 polymorphism in fertile and infertile females who are under IVF protocol divided between the success of implantation and failure of it.

The demographic and gynecological characteristics are presented in Table 2.

Table 2: Comparison among study groups in Anthropometric measurements

Parameters	Mean ± ES			LSD	P-value 30.57
	Success	Failure	Fertile		
Age	30.57±0.77 a	30.30 ±0.92a	25.56±0.89 b	2.581 **	0.0001
BMI	28.45±0.78	26.98 ±0.59	27.68 ±0.54	1.790 NS	0.306
AOM	12.63±0.19 ab	13.17 ±0.21 a	12.20 ±0.16 b	0.546 **	0.0011
DMC	32.96 ± 0.96 a	30.10 ± 0.81 b	29.52 ± 0.13 b	1.812 **	0.0010
DMF	5.03 ± 0.29	5.10 ± 0.19	5.14 ± 0. 16	0.595 NS	0.940

BMI (body mass index), AOM (age of menarche), DMC (Duration of the menstrual cycle by days), DMF (Duration of the menstrual flow by days). Mean having the different letters in the same column differed significantly. ** (P ≤ 0.01). NC (Non-significant)

The infertile female in the success group had a mean age of 30.57 ±0.92 years, females in the failure group had a mean age of 30.30±0.92 years, and the fertile females have a mean age of 25.56 ±0.89 years. These results show that age was significantly higher ($p<0.01$) in infertile females in the success and failure groups (30.57, 30.30 years) than in the fertile female in the control group (25.56 years). A previous study showed similar results to what was obtained in the current study [23]. On the other hand, other Iraqi studies showed that there are no significant differences in age between infertile and fertile females [24;25]. In previous studies, the mean age of menarche (AOM) amounted to 12.86 years [26,27], Thus the age at menarche in the present sample lies within the normal age range (12 -14 years). For 8 females (11.4%) the first menstrual bleeding occurred relatively early i.e., before the 12th year, 55 females (78.6%) experienced menarche between the 12th and 14th year, with 19 females (10%) the menarche occurred after the age of 14.

The table above shows that there are highly significant differences ($p<0.01$) between the failure and control groups (13.17 years versus 12.20 years). So maybe there is a correlation between the age of menarche and infertility.

Menarche that happens too early or late, or not at all is particularly concerning because these situations can have negative effects in the future. Menarche is regarded as early if it happens before or at the age of 10, and late if it happens at or beyond the age of fifteen [28]. An earlier menarche history has been associated with a higher risk of preterm birth [29]. Another study looks into how nulliparous ladies' menarche ages correlate with their obstetric outcomes [30]. The onset of menarche on fecundity is not well known since the few existing results are conflicting.

The infertile female in the success and in failure groups had a mean BMI (of 28.45, and 26.98) kg/m2 respectively, while the fertile females in the control group had a mean BMI of 27.68 kg/m2. Thus, there are no significant differences between the groups. These results were consistent with what Mirza and Rana discovered in their studies [31,32].

However, the duration of menstrual cycles was significantly higher ($p<0.01$) in infertile females, however, in current study the success group has longer cycle than the infertile females in the failure group, at the same time, both infertile groups recorded longer cycle days than the fertile females' group (32.96 days versus 30.10 and 29.52 days respectively).

While the mean duration of menstrual flow of the infertile females was not statistically different from those of the fertile females ($p>0.05$).

Genotyping results

Genomic DNA was extracted from blood samples of infertile and fertile groups by using Easy Pure Genomic DNA kit. The DNA integrity was checked through agarose gel electrophoresis. DNA must be displayed as a single sharp band when visualized under UV light next to an ethidium bromide stain.

In the present study, PCR reaction was performed for the samples of healthy controls and infertile patients' groups, to amplification ITGB3 gene at exon 10 and agarose gel electrophoresis was used to confirm this amplification for this region, as shown in figure 1 Which demonstrates of agarose gel electrophoresis of ITGB3 gene PCR amplified products using the ethidium bromide stain showed the fragment size 670 bp of all samples were amplified successfully with a single band.

Figure (1): Electrophoresis of the ITGB3 gene PCR reaction product (670 bp PCR product size). A DNA marker ladder (100–1500bp) and a 2% agarose gel at 70 Volts for 90 min. (1-4) success, (5-8) failure, (8-10) control .

Direct sequencing (Sanger's sequencing) was performed on samples of amplified PCR products for exon 10 on ITGB3 gene from infertile females and fertile control, using an ABI3730XL automated DNA sequencer (Macrogen Company/Korea) to pinpoint mutations within these sequences responsible for a gain of function mutation. The sequences were matched to a reference sequence of ITGB3 gene in the National Center for Biotechnology Information (NCBI) Gene Bank (www.ncbi.nlm.nih.gov) The nucleotide numbers from the gene bank resulted in Sequence ID NG_008332.2, which has a bit score of (1014), identities 100%, and gap 0%, as appeared in Table 3.

Table (3) Sequencing ID at gene bank, score, expects and compatibility of DNA sequences obtained. Homo sapiens Integrin Beta-3 (ITGB3)

Accession	Identities	Gaps	Score	Expect	Range	Strand
NG_008332.2	549/549 100%	0/549 0%	1014 bits (549)	0.0	43289 to 43837	Plus – Plus

Expected values can be considered as the possibility of observing such results by accident. A number close to zero is returned by BLAST when two identical sequences are compared. due to the existence of two identical sequences. Higher quality

in the search, alignment, or comparison is indicated by a lower e-value. 50 is typically a significant bit score. In searches of protein databases with fewer than 7000 entries, a bit score of 40 is only significant if (e- value 0.001). In a database with fewer than 7 million entries, 50 bits would be valuable due to the fact that increasing the score by 10 bits enhances the significance by 210 = 1000-fold. As a result, the NCBI Blast website uses a color-code system, with blue denoting alignment with scores of 40–50 bits and green representing alignment with scores of 50–80 bits. Because e-value is less sensitive to sequence length than score value, e-value is favored over score value. When this happens, you should always look at the e-value because it will provide you more details about the quality of your alignment and search. For inferring homology, e-values and bit scores (bits > 50) are much more sensitive and accurate than percent identity [33].

integrin beta 3 is involved in human endometrial and trophoblast cells. in current study, two SNPs (rs4642 and rs4634) in Integrin β3 gene, were investigated for their potential associations with risk of implantation failure. which is a major contributor to infertility, which is one of the leading causes of infertility.

1. Association between rs 4642 polymorphism and implantation of embryos:

Position	chr17:47292411 (GRCh38.p13)
Gene	ITGB3
Alleles	A>C / A>G / A>T
Consequence	Missense Variant

Figure (2): A representative sequence alignment of ITGB3 at exon 10 amplification results with NCBI Blast. Red arrow is for mutant rs4642(A/G) from infertile sample

The Sequence alignment showed that at genomic location 43570 there is a transition in some samples so that the normal genotype AA is transformed into the AG genotype shown in Figure 2. as a result, this genomic location shows three genotypes AA, AG, and GG.

The findings of genotyping and allele frequency analyses for the rs4642 in infertile females compared to control are shown in Table 4. With a total frequency of 64 % (32/70), 32 were confirmed to be homogenotype. With a frequency of 22% (11/70), 11 females were heterogenotype. Only 7 females were found to be homozygous mutants, representing a frequency of 14% (7/70). The frequencies of genotypes AA and GG did not differ significantly from the control group (11/50, 44 %), and (3/50, 12 %), respectively, while genotype AG was significantly higher in fertile (11(44%)) than infertile (11(22%)) P= 0.046 , therefore AG genotype considers as protective factor (Prevented fraction 64.1 %, The OR 0.36) . Allele A tends to be an etiological allele (odds ratio 1.55) with association with infertility, and the genotype AA shows the highest odds ratio (2.26) in infertile. Even though G allele tend to be protective allele with odds ratio 0.65, GG genotype record less frequency in fertile female (12%) therefore GG genotype act as a risk factor (table 4).

Table 5 represents Genotypes and alleles of ITGB3, rs4642 polymorphism between failure group and success group. these results show that There are no statistically significant differences in the frequency of genotypes and alleles between failure and success. GG genotype appears as a risk factor (28.4 %). while in AA genotype, the proportion exposed to the risk/protective factor is the same in groups success and failure.on the other hand AG genotype and A allele act as protective agent assist in success of implantation with odds ratio 0.79 and 0.90 respectively.

As a result, rs4642, according to its genotypes, may affect positively or negatively the success of embryo implantation. this was demonstrated by other Iraqi study, which revealed that the variation in genes, related to implantation such as LIF. the LIF gene polymorphisms and its gene expression were associated with implantation outcome (success or failure) [34]. The observed genotypes Distribution in all groups was consistent with Hardy Weinberg's law (table 6). P-value was (0.231, 0.0559, 0.995) for success, failure and control respectively. In a previous study, it was shown that genotypes Distribution for rs4642 matches that of Hardy Weinberg's law (P-value = 0.2) [35]. The minor allele frequency (MAF) is the frequency at which the second most common allele occurs in a given population. The HapMap project focuses on single nucleotide polymorphisms (SNPs) with minor allele frequencies of 0.05 (5%) or above. If the MAF is low < 0.05 (5%) it might imply that the major allele for the SNP is conserved and more or less fixed. This measure gives an idea about the variation of genotypes for a given SNP in a given population, in other words, it gives an idea about how common that SNP is [36]. from table 4, a notice shows that G allele frequency is 25 in infertile and 17 in fertile, the total frequency for G allele is 42 from 150 alleles, therefore, MAF =42/150 =0.28 (28%) [37]. Hence, rs4642 is considered as a common variant depending on the HapMap project. A previous study revealed that MAF of rs4642 was 0.331, 0.31, 0.283, and 0.306 for Chinese, Asian, European-ancestry, and African respectively. and also demonstrates that rs4642 was correlated with thrombin time and fibrinogen [22].

Table(4): Genotypes and alleles frequencies of ITGB3 gene polymorphism rs4642 between patient group and control group.							
Genotypes	Study groups		Odds ratio	CI 95%	Fisher exact probability	Attributable fraction	Prevented fraction
	Infertile	Fertile					
AA	32(64%)	11(44%)	2.26	0.84 to6.12	0.112	55.8 %	
AG	11(22%)	11(44%)	0.36	0.13 to1.04	0.046		11(22%)
GG	7(14%)	3(12%)	1.19	0.28 to6.16	0.861	16.2 %	
Total 50 25							
Alleles distribution							
A	75(75%)	33(66%)	1.55	0.73to3.24	0.215	35.3%	
G	25(25%)	17(34%)	0.65	0.31to1.38	0.215		35.3%

Table (5): Genotypes and alleles frequencies of ITGB3 gene polymorphism rs4642 between failure and success groups.							
Genotypes	Study groups		Odds ratio	CI 95%	Fisher exact probability	Attributable fraction	Prevented fraction
	Failure	success					
AA	16(64%)	16(64%)	1.00	0.31 to3.26	0.769	50 %	50 %
AG	5(20%)	6(24%)	0.79	0.19 to3.17	0.748		20.8%
GG	4(16%)	3(12%)	1.40	0.26 to8.24	0.709	28.4%	
Total 25 25							
Alleles distribution							
A	37(74%)	38(76%)	0.90	0.36 to2.26	0.822		37(74%)
G	13(26%)	12(24%)	1.11	0.44to2.80	0.822	10.1%	

Table (6) Expected frequencies of ITGB3 rs4642 genotypes using Hardy-Weinberg Equilibrium.						
Groups		AA	AG	GG	X2	P-value
Success genotypes	Observed	16	6	3	2.92	0.231 C
	Expected	14.44	9.12	1.44		
Failure genotypes	Observed	16	5	4	5.765	0.0559 C
	Expected	13.69	9.62	1.69		
Control genotypes	Observed	11	11	3	0.0096	0.995 C
	Expected	10.89	11.22	2.89		
Total observed		43	22	10		
Distribution consistent (C) with Hardy Weinberg's law at the level of significance: 0.05 if P-value > 0.05 and $X^2 < 3.84$, distribution does not consistent with Hardy Weinberg's law at the level of significance:0.05 if P-value < 0.05 and $X^2 > 3.84$.						

2. Association between rs 4634 polymorphism and implantation of embryos:

Position chr17:47292423 (GRCh38.p13)
Gene: ITGB3
Alleles G>A
Consequence Synonymous Variant

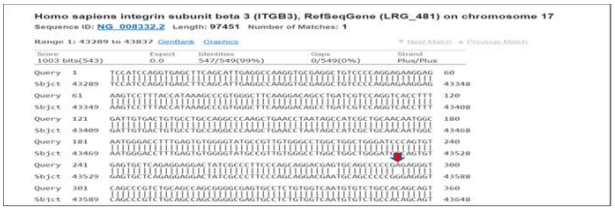

Figure (3) : A representative sequence alignment of ITGB3 at exon 10 amplification results with NCBI Blast. Red arrow is for mutant-type rs4634 (G/A) from infertile sample.

The second variation in ITGB3 was rs4634 at the genomic location 43582 which revealed the genotype GA and AA in a population of the study (cases and control) instead of GG in accordance to the blast of the NCBI (figure 3). The later variation is considered a silent mutation because the final result is Arginine > Arginine.

Table 7 displays the genotypes distribution and alleles frequencies of ITGB3 rs4634. There were no significant differences in the GG and AA genotypes' distribution and allele frequency between infertile and fertile groups and the tow genotypes have an odds ratio > 1 thus it acts as a risk factor. but GA genotype is significantly different between the two groups. and odds ratio was 0.14 therefore GA genotype act as a protective factor. the odds ratio for the G allele was 1.30 while the odds ratio for the A allele was 0.77 indicating that the G allele could have the susceptibility to association with the disease, but T allele decreases this susceptibility by acting as a protective agent. On the other hand when a comparison between failure and success, as in table 8, which shows that there is no significant differences in genotypes and alleles between groups. The rest of the results were similar to what we obtained when comparing infertile and fertile females, as the two genotypes GG and AA and allele G are considered risk factors, but genotype GA and allele A act as protective factors.

The results in success and failure groups disagree with the Hardy-Weinberg Equilibrium, the observed genotypes frequencies had significant differences from those predicted. That means the infertile may affect the population leading to deviating from HWE.these results were not consistent with the

China study by Xiang, which revealed that the observed genotype of this SNP is consistent with HWE [22]. This deviation from HWE may point to the fact that in Iraqi population, there is selection toward GG genotype. From the total observed results are shown in Table 9, GG genotype may be considered a common genotype in Iraqi infertile females population because the total observed record was highly in both infertile groups (19+18) =37 while the other genotypes GA were (2+1)=3 and AA were (4+6)=10.

The present study emphasizes in table 7, that A allele frequency is 23 in infertile and 14 in fertile, the total frequency for A allele is 37 from 150 alleles, therefore, MAF =37/150 =0.25 (25%). Hence, rs4634 is considered a common variant depending on the HapMap project. A previous srudy revealed that MAF for this variant was 0.33, 0.35, 0.28, and 0.33 for Chinese, Asian, European-ancestry, and African respectively, and demonstrated that rs4634 is associated with induction of platelets aggregation [22]. This may be the reason why it is considered a risk factor for implantation failure. Implication of inherited and acquired thrombophilia in IVF-ET failure has been proposed, probably by impairing the initial vascularisation process occurring at implantation [38].

Table (7): Genotypes and alleles frequencies of ITGB3 gene polymorphism rs4634 between infertile groups and control group.

Genotypes	Study groups		Odds ratio	CI 95%	Fisher exact probability	Attributable fraction	Prevented fraction
	Infertile	Fertile					
GG	37(74%)	14(56%)	2.24	0.79 to6.22	0.095	55.3%	
GA	3(6%)	8(32%)	0.14	0.03 to0.57	0.003		86.4%
AA	10(20%)	3(12%)	1.83	0.47 to8.97	0.431	45.5%	
Total			50	25			
Alleles distribution							
G	77(77%)	36(66%)	1.30	0.59to2.82	0.486	23.2%	
A	23(23%)	14(28%)	0.77	0.35to1.70	0.486		23.2%

Table (8): Genotype and allele frequencies of ITGB3 gene polymorphism rs4634 between success and failure groups.

Genotypes	Study groups		Odds ratio	CI 95%	Fisher exact probability	Attributable fraction	Prevented fraction
	Failure	Success					
GG	19(76%)	18(72%)	1.23	0.33 to 4.60	0.760	18.8%	
GA	2(8%)	1(4%)	2.09	0.15 to 63.9	0.617	52.1%	
AA	4(16%)	6(24%)	0.61	0.16 to2.42	0.507		39.7%
Total			25	25			
Alleles distribution							
G	40(80%)	37(74%)	1.41	0.54 to 3.68	0.489	28.8%	
A	10(20%)	13(26%)	0.71	0.27 to 1.84	0.489		28.8%

Table (9): Expected Frequencies of rs4634 at ITGB3 Using Hardy-Weinberg Equilibrium for the expected frequencies of genotypes.

Groups		GG	GA	AA	X^2	P-value
Success genotypes	Observed	18	1	6	20.07	0.00004 NC
	Expected	13.9	9.62	1.69		
Failure genotypes	Observed	19	2	4	14.06	0.00088 NC
	Expected	16	8	1		
Control genotypes	Observed	14	8	3	1.064	0.587 C
	Expected	12.96	10.08	1.96		
Total observed		51	11	13		

Distribution consistent (C) with Hardy Weinberg's law at the level of significance: 0.05 if P-value > 0.05 and $X^2 < 3.84$, distribution does not consistent (NC) with Hardy Weinberg's law at the level of significance:0.05 if P-value < 0.05 and $X^2 > 3.84$.

6. Conclusion

In the current study, two SNPs were discussed to assess their relationship to implantation failure. The distribution of genotypes for the second studied SNP showed fluctuation according to different comparison but allele A still behave as a protective allele and females who carry allele A tend to be fertile, while the distribution for the first SNP showed that heterogenotype act as a protective factor. The clinical significance of the abovementioned SNPs in disease susceptibility will need to be further evaluated, in particular. The roles of ITGB3 SNPs in ethnic variances should receive further attention in future research.

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