History of Medicine, 2023, 9(1): 2195–2205
DOI: 10.17720/2409-5834.v9.1.2023.284

Polymorphism of Endothelin-1 Gene in Iraqi Infertile Females under In Vitro Fertilization Program

Zainab Jalil Abdulkareem[1], Asmaa M. Salih Almohaidi[2]

[1] *Al-Sadreen high school for distinguishing students, Ministry of Education, Baghdad, Iraq.*
EM: zainab.jaleel1102a@csw.uobaghdad.edu.iq
[2]*Biology Department/ College of Science for Women, University of Baghdad, Baghdad, Iraq*
EM: asmaams_bio@csw.uobaghdad.edu.iq

*Corresponding author: Zainab Jalil Abdulkareem (zainab.jaleel1102a@csw.uobaghdad.edu.iq)

Received: 20 January 2023 **Accepted:** 15 April 2023

Citation: Abdulkareem ZJ, AlmohaidiAMS (2023) Polymorphism of Endothelin-1 Gene in Iraqi Infertile Females under In Vitro Fertilization Program. History of Medicine 9(1): 2195–2205. https://doi.org/10.17720/2409-5834.v9.1.2023.284

Abstract

Embryo implantation is an important step in establishing pregnancy and a major concern in the management of infertility. Failure at this step greatly limits the success of IVF treatments. Endothelin-1 is released by many different tissues, including uterine smooth muscle, and is able to regulate myometrial functions, EDN1-mediated cellular functions play a critical role in normal pregnancy. This comparative study was designed for Iraqi women who underwent the IVF program to understand the role of the EDN1 gene in embryo implanting. Blood samples were collected an hour after embryo transfer after approval from 60 females. The samples were divided into two groups according to the embryo implantation outcomes, the Implantation Failure group included 35 females, and the Implantation Success group included 25 females. PCR was performed by using a specific primer. The sequencing exposed polymorphisms in three positions rs1800543, rs5369, and a new locus No. 8566 A>C showed a significant difference in the heterozygous genotype AC between groups. The findings suggest that polymorphism No.8566 A>C associated with infertility and the AC genotype may be related with increase susceptibility to infertility in Iraqi females.

Keywords
Endotheli-1, EDN1, IVF program, Infertility, Implantation, Iraqi women.

In vitro fertilization (IVF) is a clinical technique that has revolutionized infertility treatment. The process involves fertilizing the egg in a laboratory and replacing the resulting embryo into the uterus [1]. Implantation is an important step in establishing pregnancy and is of major concern in the management of infertility. Failure at this step greatly limits the success of IVF treatments [2]. Implantation of the embryo is a process of mutual communication between the uterus and the blastocyst primarily under the direction of ovarian estrogen and progestin [3], is an excessively regulated local tissue remodeling step involving a complex sequence of genetic and cellular interactions executed within an optimal time frame [4].

Endothelin-1 is important in various reproduction phases and is a potent vasoconstrictor peptide family produced basically by vascular endothelial cells and two distinct cell types in human endometrial tissue. EDNl derived from endometrial stromal cells may act on the adventitial surface of contiguous spiral

arterioles of the endometrium to modulate endometrial blood flow [5]. EDN1 is expressed by human luteal cells and reduces basal and human chorionic gonadotropin-induced progesterone synthesis through the endothelin A receptor (EDNAR) receptors and the protein kinase C pathway [6]. Trophoblast produces EDN1, which is involved in the invasion and differentiation of trophoblast cells isolated from first-trimester placentas [7]. EDN1 plays a role in fetoplacental circulation, at all levels of the foeto-placental vascular system including the stem villus resistance vessels. It is important in maintaining foeto-plancental vascular resistance at the low oxygen tension which exists in this vascular system in utero [8]. In pregnant women the highest EDN1 concentrations are found in amniotic fluid, followed in decreasing order by the umbilical vein, artery retroplacental blood, uterine and brachial vein [9].

EDN1 consists of three main isopeptides, EDN1, EDN2, and EDN3, produced from biologically inactive precursors via proteolytic processing [10]. The human EDN1 was mapped to 6p24.1. It contains 5 exons, coding for a 2026-nucleotide [11,12]. EDN1 is synthesized as a 212-amino acid pre pro-EDN1 that is cleaved by a specific endopeptidase into a 38-amino acid big EDN1. Big EDN1 is subsequently cleaved into mature 21 amino acids EDN1 by an EDN-converting enzyme [13]. EDN1 acts through EDNA and EDNB receptors and is implicated in a wide range of biological and pathological functions, such as insulin resistance, also able to regulate myometrial functions, EDN1-mediated cellular functions play a critical role in normal pregnancy, preterm birth and uterine leiomyoma [14,15]. The aim of the present study we have to attempt to better understand the role of the EDN1 gene in embryo implanting during the window of implantation period in infertility women under IVF program.

Materials and methods

This comparative study was designed for Iraqi women who underwent an In Vitro Fertilization program (IVF). Blood samples were collected after approval from 60 females in age between 20-45 years at Al Nada Medical Center, Al Farah Specialized Center for Infertility and IVF, and Rooh Alhayat Center for the treatment of infertility & IVF from Dr. Ahmed Al-Salihi Medical Group, during the period from February 2022 to August 2022. The peripheral blood samples were collected an hour after embryo transfer by disposable syringe and stored in a 2ml EDTA tube for molecular study, then stored at -20ºC. The studied samples were divided into two groups according to the embryo implantation outcomes, the Implantation Failure group, which included 35 females, and the Implantation Success group, which included 25 females.

DNA extraction and PCR primer

DNA extraction kits (Geneaid, Taiwan) were used to extract total genomic DNA from the whole b,lood and extracted DNA samples were stored at -20°C for further use. The polymerase chain reaction (PCR) was performed in a 25µl reaction mixture, pre-mix 5µl (Bioneer, Korea), using a specific primer designed by the second author for EDN1 gene (gene ID: 1906) Ref Seq Gene on chromosome 6, sequence ID: NG_016196.1, the region from gene position 8469 to 9331 (end of intron 2, exon3, intron3, and exon4) is shown in (Table 1), (Fig.1) used NCBI/ Primer designing tool. The program of the PCR reaction is shown in (Table 2). The ladder marker and PCR products were dissolved by electrophoresis. The bands were pictured on the UV trains illuminator (Fig. 2). The PCR product Foreword and reverse were sent for Standard sequencing using ABI3730XL, an automated DNA sequence, by Macrogen Corporation – Korea. Then analyzed using Blast in NCBI and BioEdit sequence alignment editor computer program used for sequence analysis [16].

Table 1: Sequence of the EDN1 gene primer utilized in this study.

Primers	Sequences (5`→3`)	Product size

EDN1 Gene	F: 5`- TCAGGGCCATTGATGCACAG-3` R: 5`- ACAGAGGACATCGGTTTGCAT-3`	863bp

Reference: Designed by the second author

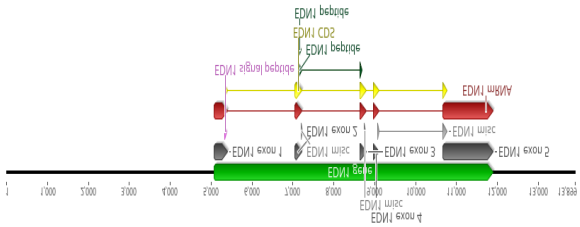

Figure 1: EDN1 gene on NCBI.

Table 2: PCR amplification program.

Steps	Temperature (ºC)	Time	NO. of cycles
Initial denaturation	95	5 min	1
Denaturation	94	30 second	35
Annealing	58	30 second	
Extension	72	1 min	
Final extension	72	5 min	1

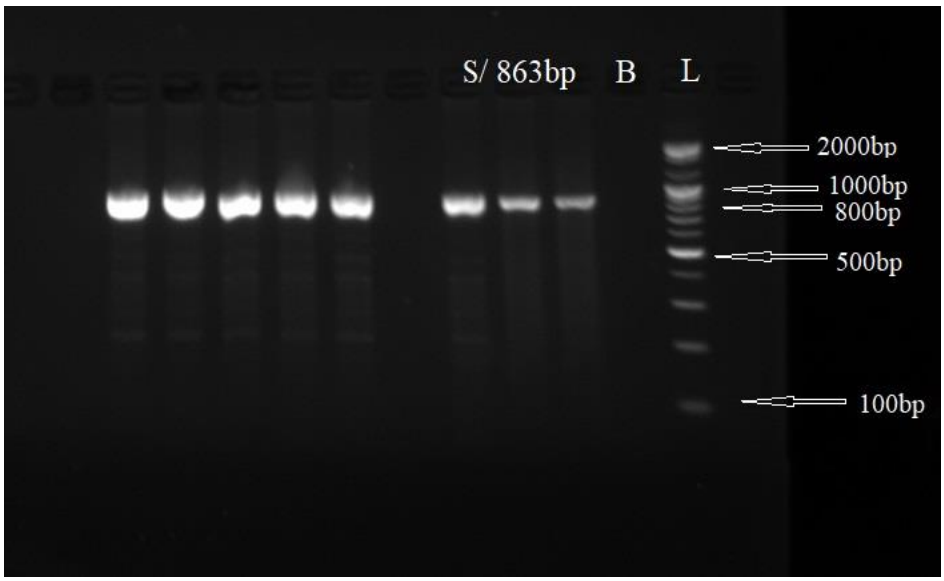

Figure 2: Gel electrophoresis for PCR product of EDN1 gene (863bp) with DNA ladder (100bp) on agarose gel (2%) in (100v, 1 hour). L: ladder, S: sample, B: blank.

Statistical Analysis

WINPEPI computer programs (version 11.65) were used to calculate the statistical significance of the P-value that was calculated with an Odd Ratio as well as Fisher s exact test. Hardy-Weinberg equilibrium (H.W.E) was tested by using the Gene-Calc- bioinformatic tool [17]. The Statistical Analysis System- SAS (2018) program was used to detect the effect of different factors on study parameters. A T-test was used to significantly compare between means. The Chi-square test was used to significantly compare between percentage (0.05 and 0.01 probability in this study [18].

Results

Characteristics of the study population

This comparative study was conducted on 60 infertile females with age between 20-45 years of undergoing the IVF program and divided into two groups. The data in (Table 3) illustrates that the mean age for the failure implantation group was

29.70 ±1.09, while the mean age of success implantation group was 27.92 ±1.12, and there were non-significant differences in age between groups under p<0.01. The BMI mean for the failure group was 27.25 ±0.85. While the BMI mean for the success group was 25.55 ±0.72, and there was a non-significant difference in BMI between groups under P<0.01. The duration of infertility for the failure group mean was 6.84 ±0.58. While the duration of infertility for the success group mean was 5.92 ±0.87, and there were non-significant differences in the duration of infertility between groups under P<0.01.

EDN1 gene polymorphisms

The region of the EDN1 gene was amplified by using a specific primer, the PCR was performed under optimum conditions. Sequencing was performed to determine the genetic variation in Iraqi infertile females under the IVF program. The alignment of the query and sbjct show Single nucleotide polymorphism SNP in three positions rs1800543 C>T, rs5369 A>G, and a new position in nucleotide No. 8566 A<C.

Table 3: Comparison between Failure and Success implantation groups in demographic characteristics

Characteristics		Groups	No (%)	Mean	T-test	P-value
Age (Year)	Less than 25 25-35 More than 35	Implantation Failure	9 (22.5) 25 (62.5) 6 (15)	29.70 ±1.09	3.292 NS	0.284
		Implantation Success	7 (28) 16 (64) 2 (8)	27.92 ±1.12		
BMI (kg/m²)	Less than 25 25-30 More than 30	Implantation Failure	12 (30) 16 (40) 12 (30)	27.25 ±0.85	2.404 NS	0.160
		Implantation Success	15 (60) 7 (28) 3 (12)	25.55 ±0.72		
Duration of infertility (year)	Less than 5 5-10 More than 10	Implantation Failure	12 (30) 21 (52.5) 7 (17.5)	6.84 ±0.58	2.015 NS	0.360
		Implantation Success	11 (44) 12 (48) 2 (8)	5.92 ±0.87		

NS: Non-Significant.

SNP rs1800543

Genetic polymorphism of the SNP rs1800543 C>T at a locus 8609 on the intron 2 variant of the EDN1 gene (Fig. 3) and chr6:12293904, was observed in three genotypes TT, TC, CC in groups of study, it is apparent from (Table 4), the common homozygote genotype TT showed a higher frequency in the implantation success group 64% compared to the implantation failure group 57%. The heterozygote genotype TC showed a higher frequency in the failure group 26% than success group 12%. While the other homozygote genotype CC showed a higher frequency in the success group 24% compare to the failure group 17%. With no significant difference between the two groups. The allele frequency of the T allele is 0.70% and the C allele frequency is 0.30% in both of the study groups. This result agreed with H.W.E at the level of significance P≤0.01. for the failure group, while in the success group, the distribution does not consistent with H.W.E at the level of significance P≤0.01, and was highly significantly different between expected and observed frequency may because it is under the procedure of hormonal control that may cause deviation from H.W.E.

The statistical analysis in (Table 5) found that the odd ratio (OR) of the TT genotype was 0.75 with a confidence interval (CI) value between 0.27 - 2.12 and show a Preventive fraction (PR) of 0.16. While the TC genotype frequency showed OR 2.54 with

a CI value between 0.63 - 10.28 and an etiological fraction (ET) of 0.156. another homozygote the CC genotype OR was 0.66 with a CI value between 0.19 - 2.28 and show a PR of 0.083. The T and C alleles record OR 1.00 with a CI value between 0.46 - 2.19 under 95%, and showed no significant difference under fishers' exact probability.

Table 4: Expected frequencies of genotypes and alleles of the rs1800543 T>C using Hardy- Weinberg equilibrium.

EDN1 rs1800543 T>C Genotypes		TT	TC	CC	T	C	P-value
Implantation Failure Group	Observed no (%).	20 (57%)	9 (26%)	6 (17%)	0.70	0.30	0.07199 NS
	Expected no (%).	17.15 (49%)	14.7 (42%)	3.15 (9%)			
Implantation Success Group	Observed no (%).	16 (64%)	3 (12%)	6 (24%)	0.70	0.30	0.0017 **
	Expected no (%).	12.25 (49%)	10.5 (42%)	2.25 (9%)			
P-value	--	0.505 NS	0.083 NS	1.00 NS	--		

** (P≤0.01), NS: Non-Significant. FIS for implantation success group: 0.7143

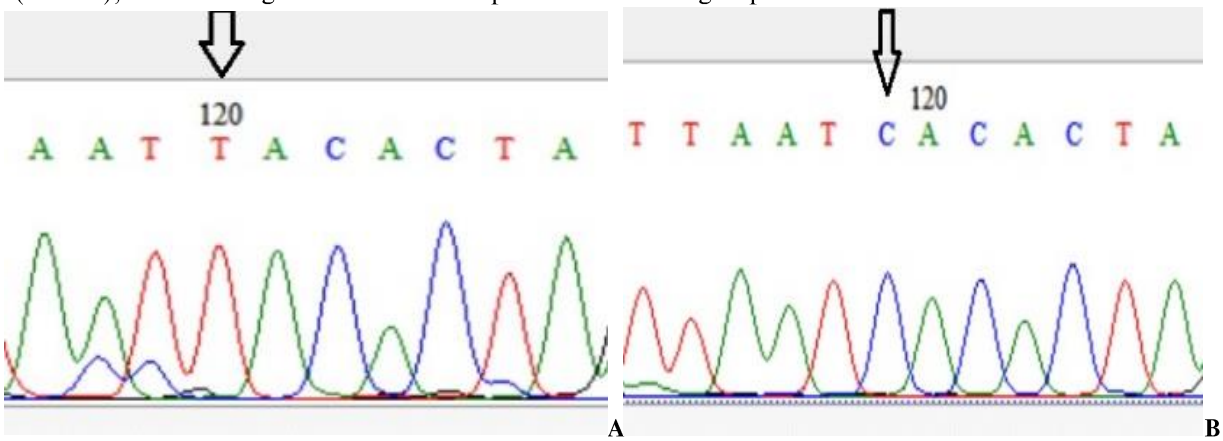

Figure 3: DNA sequencing chromatogram which shows the rs1800534 T>C located at genomic location 8609 on the intron 2 of the EDN1 gene chr6:12293904. A: the arrow points to the normal nucleotide T in the forward 5`-3`/ B: the arrow points to the substitution nucleotide T/C.

Table 5: The statistical comparison between groups of the rs1800543 T>C.

EDN1 rs1800543 T>C Genotypes	OR	ET Or PR	Fisher's exact probability	CI 95 %
TT	0.75	0.16	0.790 NS	0.27 - 2.12
TC	2.54	0.156	0.327 NS	0.63 -10.28
CC	0.66	0.083	0.532 NS	0.19 - 2.28
Allele distribution				
T	1.00	0.0	1.000 NS	0.46 - 2.19
C	1.00	0.0	1.000 NS	0.46 - 2.19

NS: Non-Significant

SNP rs5369

Genetic polymorphism of the SNP rs5369 A>G at a locus 8730 coding sequence variant on exon 3 of the EDN1 gene (Fig. 4) and chr6:12294025, was observed in three genotypes AA, AG, GG in the implantation failure group, but only two genotypes AA and GG in the implantation success group while the other genotype AG was absent. The results, as seen in (Table 6), indicate that the

wildtype homozygote genotype AA in the present study showed a higher frequency in success group 20% than in the failure group 14%. The heterozygote genotype AG showed a frequency of 9% in the failure group and don't observe in the success group. While the other homozygote genotype GG showed a higher frequency in the success group 80% than in the failure group 77%. The result was significantly different between expected and observed frequency in both the study groups and the distribution is not consistent with H.W.E at the level of P≤0.01.

The frequency of allele A in the failure and success groups was 0.19 and 0.2, respectively. Lower than the frequency of the G allele 0.2% and 0.8%, respectively. with no significant difference between groups. This distribution is not consistent with H.W.E at the level of significance under p<0.01. According to this result, a common allele in Iraqi females is the G allele which explains the high frequency of the GG genotype 78% in the study population higher than the other two genotypes AA and AG.

The statistical analysis in (Table 7) found that the OR of the AA genotype was 0.67 with a CI value between 0.17 - 2.54 and show a PR of 0.067. while the frequency of the AG genotype showed OR 6.49 with a CI value between 0.34 - 124.59 and show an ET of 0.096. The other homozygote GG genotype OR was 0.84 with a CI value between 0.25 - 2.90 and show a PR of 0.125. The A alleles record OR 0.91 with a CI value between 0.37 - 2.27 under 95%, and show PR 0f 0.018, while the G alleles record OR 1.10 with a CI value between 0.44 - 2.72 under 95%, and show ET 0.071, these results showed no significant difference under fishers' exact probability.

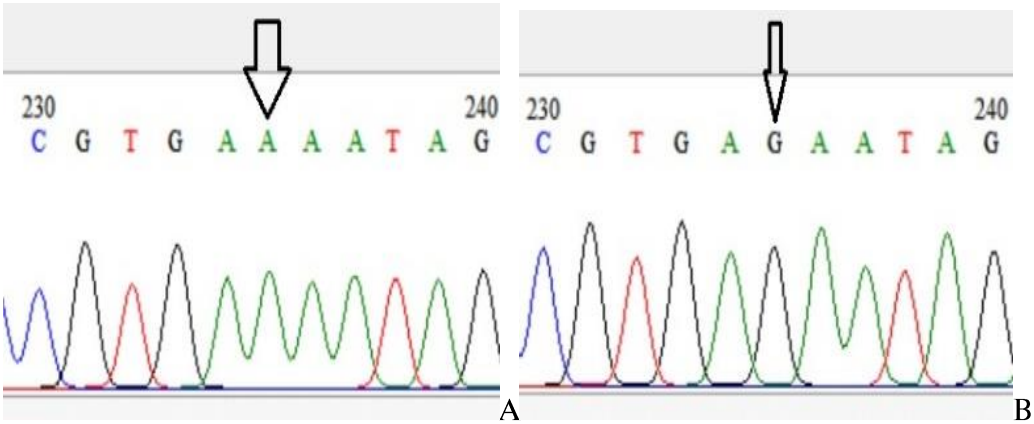

Figure 4: DNA sequencing chromatogram which shows the rs5369 A>G located at genomic location 8730 on the exon 3 of the EDN1 gene and chr6:12294025. A: the arrow points to the normal nucleotide A in the forward 5`-3`/ B: the arrow points to the substitution nucleotide A/G.

Table 6: Expected frequencies of genotypes and alleles of the rs5369 A>G using Hardy- Weinberg equilibrium.

EDN1 rs5369 / A>G Genotypes		AA	AG	GG	A	G	P-value
Implantation Failure Group	Observed no (%).	5 (14%)	3 (9%)	27 (77%)	0.19	0.81	0.00013 **
	Expected no (%).	1.21 (4%)	10.59 (30%)	23.21 (66%)			
Implantation Success Group	Observed no (%).	5 (20%)	0	20 (80%)	0.2	0.8	0.0001 **
	Expected no (%).	1 (4%)	8 (32%)	16 (64%)			
P-value	--	1.00 NS	0.085 NS	0.307 NS			--

** (P≤0.01), NS: Non-Significant. FIS for implantation failure group: 0.7166/ and for implantation success group: 1

Table 7: The statistical comparison between groups of the rs5369 A>G.

EDN1 rs5369 A>G Genotypes	OR	ET Or PR	Fisher's exact probability	CI 95 %
AA	0.67	0.067	0.728 NS	0.17 - 2.54
AG	6.49	0.096	0.242 NS	0.34 - 124.59
GG	0.84	0.125	1.000 NS	0.25 - 2.90
Allele distribution				
A	0.91	0.018	1.000 NS	0.37 - 2.27
G	1.10	0.071	1.000 NS	0.44 - 2.72

NS: Non-Significant.

Polymorphism No.8566 A>C

Genetic polymorphism at locus No.8566 A>C on intron 2 (Fig.5) and ch6:12293861, was observed as two genotypes (AA, AC) while the other genotype CC was absent in both study groups, it is apparent from (Table 8) that the homozygote genotype AA showed a high frequency of 92% in the success group higher than the frequency in the failure group of 69%. While the heterozygote genotype AC showed a higher frequency 31% in the failure compared to the success group 8% and this shows a significant difference between the two groups under P≤0.05. the frequency of the A allele in both groups was higher 0.84%, 0.96%, than the frequency of the C allele 0.16%, 0.0 4%, respectively. This distribution is consistent with H.W.E at the level of significance under P≤0.01. The statistical analysis shown in (Table 9) found that the OR of the AA genotype was 0.19 with a CI value between 0.04 - 0.92 and a PR of 0.745. While the AC genotype frequency showed OR 5.27 with a CI value between 1.08 - 25.61 and an ET of 0.255, and showed a significant difference under fishers' exact probability. The A alleles record OR 0.22 with a CI value between 0.05 - 1.04 under 95%, and show PR 0f 0.745, while the C alleles record OR 4.47 with a CI value between 0.96 - 20.86 under 95%, and show ET 0.122, showed no significant difference under fishers' exact probability.

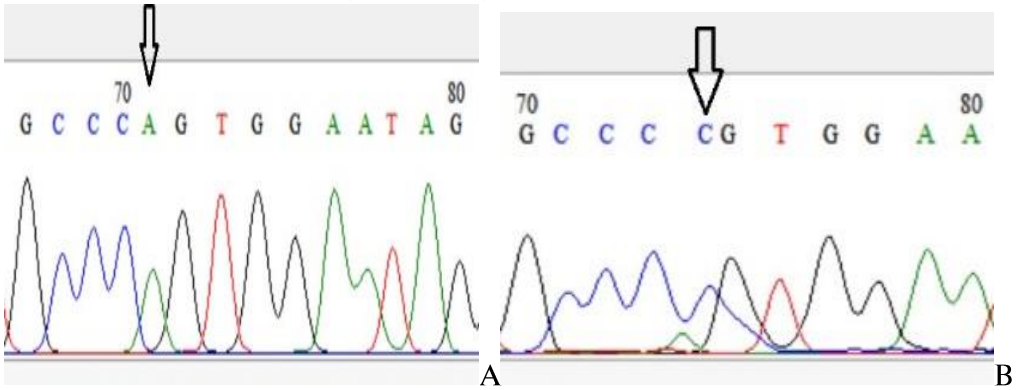

Figure 5: DNA sequencing chromatogram which shows the nucleotide in position No.8566 A>C located on the intron 2 of the EDN1 gene and ch6:12293861. A: the arrow points to the normal nucleotide A in the forward 5`-3`/ B: the arrow points to the substitution nucleotide A/C.

Table 8: Expected frequencies of genotypes and alleles of the SNP No.8566 A>C using Hardy-Weinberg equilibrium.

EDN1 No. 8566 A>C Genotypes		AA	AC	CC	A	C	P-value
Implantation Failure Group	Observed no (%).	24 (69%)	11 (31%)	0	0.84	0.16	0.54427 NS
	Expected no (%).	24.86 (71%)	9.27 (26%)	0.86 (3%)			

Implantation Success Group	Observed no (%).	23 (92%)	2 (8%)	0	0.96	0.04	0.97853 NS
	Expected no (%).	23.04 (92.16%)	1.92 (7.68%)	0.04 (0.16%)			
P-value	--	0.884 NS	0.013 *	1.00 NS	--		

* (P≤0.05), NS: Non-Significant.

Table 9: The statistical comparison between groups of SNP No.8566 A>C.

EDN1 No. 8566 A>C Genotypes	OR	ET Or PR	Fisher's exact probability	CI 95 %
AA	0.19	0.745	0.054*	0.04 - 0.92
AC	5.27	0.255	0.054*	1.08 - 25.61
Allele distribution				
A	0.22	0.745	0.071 NS	0.05 - 1.04
C	4.47	0.122	0.071 NS	0.96 - 20.86

* (P≤0.05), NS: Non-Significant.

5. Discussion

Infertility affects a substantial part of women and most of them resort to assisted reproductive techniques to get pregnant, this study examined some demographic characteristics of infertility females under the IVF program divided into two groups according to implantation outcome, and the results showed that there was no relationship between the age, BMI, and duration of infertility with the failure or success of embryo implantation in females under IVF programs. The findings in the present study are consistent with the findings of previous research that found no significant difference in these demographic characteristics between the failure implantation and success implantation groups in the Iraqi infertility females under the IVF program [19,20].

SNPs analysis of the EDN1 gene was performed on Iraqi infertile females under the IVF program, divided into two groups. The results of the SNP rs1800543 C>T showed no association with the failure or success implantation in infertility females, the results also showed the common allele in the Iraqi female is the T allele, which was recorded as the highest percentage in both groups of the study, this explains the high percentage of the homozygote genotype TT compared to the other two genotypes. The number of heterozygote TC genotype decreases and the homozygous TT and CC increases, there are possible explanations for this result may because the rate of inbreeding is high in the study population, and this may explain the deviate the frequency in the implantation success group from H.W.E. Findings in the present study are consistent with the findings of Kim et al., 2008, that suggest no significant differences in genotype or allele frequencies of the rs1800543 SNP in the EDN1 gene between preeclamptic and normotensive pregnancies in pregnant Korean women [21].

The result of SNP rs5369 illustrated that no association with the failure or success implantation in infertility females, the common genotype in the Iraqi females was the homozygous GG genotype, which records the highest frequency in the study population. While the heterozygous genotype AG records the least frequency in the implantation failure group and contrary to expectations disappeared in the implantation success group it is possible because the sample size was very limited because of many reasons related to culture and kind of medical problems. There are similarities in genotype distribution and allele frequency between the present study and those described by Vargas-

Alarcon, et al., 2014, which suggests some EDN1 polymorphism is involved in the risk of developing acute coronary syndromes in Mexican individuals [22].

The study recorded a new variant No.8566 A>C showed a significant difference in the heterozygous genotype AC that recorded a higher in the implantation failure group than the implantation success group and have an ET effect and which may have the risk of infertility compared to another genotype AA which shows statistical as PR effect and records high frequency in the group of females who had successful implantation and obtained a solution to their infertility problem, EDN1 have autocrine and/or paracrine function in human endometrial stromal cells and play a fundamental role in the control of uterine function in pregnancy. and the peptides are key determinants of placental blood flow by binding to vascular smooth muscle receptors in the placenta [23,24].

6. conclusion

The findings suggest that polymorphism No.8566 A>C associated with infertility and the AC genotype may be related with increase susceptibility to infertility in Iraqi females. EDN1 play important role in pregnancy variation in the EDN1 gene may affect the role of the EDN1 axis in embryo implantation and placentation.

Conflict of Interest

There is no conflict of interest

References

1. Afnan, M.A.M., Rudin, C., Conitzer, V., Savulescu, J., Mishra, A., Liu, Y. and Afnan, M., Ethical implementation of artificial intelligence to select embryos in vitro fertilization. In Proceedings of the 2021 AAAI/ACM Conference on AI, Ethics, and Society (2021) 316-326. https://doi.org/10.1145/ 3461702.3462589
2. Hertig, A.T., Rock, J. and Adams, E.C, A description of 34 human ova within the first 17 days of development. American Journal of Anatomy, 98(1956) 435-493. https://doi.org/10. 1002/aja.1000980306.
3. Nikas, G, Endometrial receptivity: changes in cell-surface morphology. In Seminars in reproductive medicine, Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA, 18 (2000) 229-236. DOI: 10.1055/s-2000-12561.
4. Griffith, O.W., Chavan, A.R., Protopapas, S., Maziarz, J., Romero, R. and Wagner, G.P., Embryo implantation evolved from an ancestral inflammatory attachment reaction. Proceedings of the National Academy of Sciences, 114(2017) E6566-E6575. https://doi.org/10.1073/pnas.1701129114.
5. Economos, K.A., MacDonald, P.C. and Casey, M.L., Endothelin-1 gene expression and protein biosynthesis in human endometrium: potential modulator of endometrial blood flow. The Journal of Clinical Endocrinology & Metabolism, 74(1992) 14-19. https://doi.org/10.1210/jcem.74.1. 1727813.
6. Apa, R., Miceli, F., Feo, D.D., Pierro, E., Ayala, G., Mancuso, S., Napolitano, M. and Lanzone, A., 1998. Endothelin-1: expression and role in human corpus luteum. American Journal of Reproductive Immunology, 40(5), pp.370-376. **https://doi.org/10.1111/j.1600-0897.1998.tb00068.x**
7. Wang, Y., August. Vascular biology of the placenta. In Colloquium Series on integrated systems physiology: from molecule to function. Morgan & Claypool Life Sciences, 2 (2010) 1-98). https://doi.org/10.4199/C00016ED1V01Y201008ISP009.
8. MacLean, M.R., Templeton, A.G. and McGrath, J.C., The influence of endothelin-1 on human foeto-placental blood vessels: a comparison with 5-

hydroxytryptamine. British journal of pharmacology, 106(1992) 937-941. https://doi.org/10.1111/ j.1476-5381.1992.tb14438.x.

9. Wolff, K., Nisell, H., Carlström, K., Kublickiene, K., Hemsén, A., Lunell, N.O. and Lindblom, B., Endothelin-1 and big endothelin-1 levels in normal term pregnancy and in preeclampsia. Regulatory peptides, 67(1996) 211-216. https:// doi.org/10.1016/S0167-0115(96)00122-X.
10. Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K. and Masaki, T., A novel potent vasoconstrictor peptide produced by vascular endothelial cells. nature, 332(1988) 411-415. DOI: 10.1038 /332411a0.
11. Arinami, T., Ishikawa, M., Inoue, A., Yanagisawa, M., Masaki, T., Yoshida, M.C. and Hamaguchi, H., Chromosomal assignments of the human endothelin family genes: the endothelin-1 gene (EDN1) to 6p23-p24, the endothelin-2 gene (EDN2) to 1p34, and the endothelin-3 gene (EDN3) to 20q13. 2-q13. 3. American journal of human genetics,48(1991)990.https://www.ncbi.nlm.nih.gov/
12. Inoue, A., Yanagisawa, M., Kimura, S., Kasuya, Y., Miyauchi, T., Goto, K. and Masaki, T., The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proceedings of the national academy of sciences, 86(1989) 2863-2867. https://doi.org/10.1073/pnas.86. 8.286.
13. Tanfin, Z., Robin, P., Oyeniran, C. and Breuiller-Fouché, M., Endothelin-1: physiological and pathological roles in myometrium. The international journal of biochemistry & cell biology, 43(2011) 299-302. https://doi.org/10.1016/j.biocel.2010.10.009.
14. Khidhir, S.M., Mahmud, A.M. and Maulood, I.M., Association of Endothelin-I and A symmetric Dimethylarginine Levels with Insulin Resistance in Type-2 Diabetes Mellitus Patients. Baghdad Science Journal, 19(2022) 0055-0055. DOI: http://dx.doi.org/10.21123/bsj.2022.19.1.0055.
15. Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis.
16. Bińkowski J., Miks S., Gene-Calc [Computer software]. Available from (2018, September): www.gene-calc.pl.
17. SAS, Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA (2018).
18. Hassab, Z.S., Al-Musawi, B.J. and Almohaidi, A.M.S., Evaluation Hormonal State in Iraqi Infertility Females Under In-Vitro Fertilization Program. Indian Journal of Forensic Medicine & Toxicology, 15(2021).
19. Yousif, R.A., Almohaidi, A.M.S. and Al-Musawi, B.J., Assessment of ITGB3 gene expression and hormonal status in infertile female undergoing an in vitro fertilization protocol. HIV Nursing, 23(2023) 770-778.
20. Kim, S.Y., Park, S.Y., Lim, J.H., Yang, J.H., Kim, M.Y., Park, H.Y., Lee, K.S. and Ryu, H.M., No association between endothelin-1 gene polymorphisms and preeclampsia in Korean population. Journal of Genetic Medicine, 5(2008) 34-40.
21. Vargas-Alarcon, G., Vallejo, M., Posadas-Romero, C., Juarez-Rojas, J.G., Martinez-Rios, M.A., Peña-Duque, M.A., Carrillo-Sanchez, S., Perez-Mendez, O. and Fragoso, J.M., The− 974C> A (rs3087459) gene polymorphism in the endothelin gene (EDN1) is associated with risk of developing acute coronary syndrome in Mexican patients. Gene, 542(2014) 258-262. https://doi.org/10.1016/j.gene.2013.09.003.
22. Kubota, T., Taguchi, M., Kamada, S., Imai,

T., Hirata, Y., Marumo, F. and Aso, T., Endothelin synthesis and receptors in human endometrium throughout the normal menstrual cycle. MHR: Basic science of reproductive medicine, 1(1995) 304-308. https://doi.org/10.1093/molehr/1.6.304.

23. Cameron, I.T., Bacon, C.R., Collett, G.P. and Davenport, A.P., Endothelin expression in the uterus. The Journal of steroid biochemistry and molecular biology, 53(1995) 209-214. https://doi.org/10.1016/0960-0760(95)00048-5.